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WESLANNY DE ANDRADE MORAIS

PROPRIEDADES FÍSICO-QUÍMICAS E ANTIBACTERIANAS DA INCORPORAÇÃO DE MICROPARTÍCULAS POLIMÉRICAS CARREGADAS COM CLOREXIDINA EM CIMENTO IONÔMERO DE VIDRO

FORTALEZA

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Dissertação apresentada ao Programa de Pós-graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como requisito parcial à obtenção do título de Mestre em Odontologia.
Área de concentração: Clínica Odontológica

Orientadora: Profa. Dra. Lidiany Karla Azevedo Rodrigues.
Co-orientadores: Profa. Dra. Monica Yamauti e Prof. Dr. Francisco Fábio Oliveira de Sousa.

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Aprovada em: ___ /___ /___.

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“Deleita-te também no Senhor, e te concederá os desejos do teu coração. Entrega o teu caminho ao Senhor; confia Nele e Ele o fará.”

(Salmo 37: 4-5)

RESUMO

A clorexidina (CLX) é o agente antimicrobiano que mais tem sido investigado no controle do biofilme e sua incorporação em cimento de ionômero de vidro (CIV) tem sido proposta para reduzir o número de micro-organismos em pacientes com alta atividade de cárie. Dessa forma, o objetivo deste estudo foi avaliar o efeito da adição de sais de CLX, em suas formas livres (diacetato - DA ou digluconato – DG) e incorporada em micropartículas poliméricas de Poli (ácido láctico-co-glicólico) (PLGA), nas propriedades físico-químicas e antibacterianas de um CIV ativado quimicamente. Materiais e métodos: Micropartículas de PLGA contendo diacetato (MPDA) ou digluconato de CLX (MPDG) foram obtidas através da técnica de secagem por pulverização. Os grupos experimentais foram preparados com a adição de 1% de CLX nas suas formas livres ou microencapsuladas em CIV, no grupo controle do experimento não houve incorporação de CLX, constituindo os seguintes grupos: CIV (controle), DA, DG, MPDA e MPDG. Realizaram-se análises qualitativas de estabilidade da CLX livre, espectroscopia no infravermelho por transformada de Fourier (FTIR), liberação cumulativa de CLX (%) e imagens em microscopia eletrônica de varredura (MEV), seguidas de testes de tempo de presa, escoamento e resistência à compressão. Para a análise do efeito antibacteriano, foi utilizado inóculo de *S. mutans* em meio de cultura triptona de soja (TSB) enriquecido com extrato de levedura e 1% de sacarose, submetendo os espécimes à formação de biofilme por até 5 dias. Após 24 h da adesão inicial e com intervalos subseqüentes de 1 dia, foi determinado o número de unidades formadoras de colônia (UFC/ml) do biofilme formado sobre espécimes recém preparados (após 24 h de tempo de presa - Grupo imediato) e espécimes envelhecidos em água em 37 ºC por 15 dias (Grupo envelhecido). A análise estatística para os testes físico-químicos e efeito antibacteriano foi realizada através da análise de variância (ANOVA) e Mann Whitney, ambos seguidos por testes de comparação de médias aos pares, com nível de significância pré-estabelecido em 5%. Resultados: O DG em solução na presença do CIV apresentou maior estabilidade em temperatura ambiente e a 37 ºC quando comparado ao DA. A análise de FTIR não mostrou indicativos de reação química entre o CIV e a CLX nas concentrações testadas. As formulações microencapsuladas aumentaram, enquanto que o DG diminuiu o tempo de presa ($p<0,05$). A inclusão de DG aumentou a resistência à compressão ($p<0,05$) e o escoamento foi diminuído pela inclusão das formas livres de CLX ($p<0,05$). Os grupos microencapsulados mostraram um perfil de liberação mais tardio e gradual quando comparado aos grupos com incorporação de CLX livre. A incorporação de CLX mostrou efeito antibacteriano significativo quando comparado

ao CIV puro, porém sem diferença estatística significativa quando se compara os grupos com as formas livres ou microencapsuladas, bem como imediatos e envelhecidos. Conclusão: A incorporação de CLX resultou em cimentos ionoméricos com efeito antibacteriano e propriedades físico-químicas apropriadas para o uso clínico.

Palavras chave: Cárie dentária, biofilme, polímeros e *Streptococcus mutans*.

ABSTRACT

Chlorhexidine (CHX) is the most investigated antimicrobial agent in dental caries control and its incorporation in glass ionomer cement (GIC) has been proposed to reduce the microorganism number in patients with high caries activity. Thus, the aim of this study was to evaluate the effect of adding CHX salts in their free forms (diacetate - DA or digluconate - DG) and incorporated in polymeric microparticles of poly (lactic-co-glycolic acid) (PLGA), in the physicochemical properties and antibacterial action of a chemically activated GIC. Materials and Methods: PLGA microparticles containing CHX diacetate (MPDA) or digluconate (MPDG) were obtained by the spray drying technique. The experimental groups were prepared with the addition of 1% CHX in their free or microencapsulated forms into GIC, and the control group had no CHX incorporation, constituting the following groups: GIC (control), DA, DG, MPDA and MPDG. Stability of CHX, Fourier transform infrared spectroscopy, CHX cumulative release (%) and scanning electron microscopy (SEM) analysis were performed, followed by physic testing of setting time, flowability and compressive strength. For antibacterial effect determination, *S. mutans* was inoculated in culture medium tryptone soy broth supplemented with yeast extract and 1% sucrose by forming biofilms over the specimens up to 5 days. After 24 h of initial adhesion and subsequent 1-day intervals, the number of colony forming units (CFU/ml) of the biofilm formed on freshly prepared samples (after 24 h of setting time- immediate group) and samples aged in water at 37 °C for 15 days (aged group) was determined. Statistical analysis for physicochemical tests and antibacterial effect were performed by analysis of variance (ANOVA) and Mann Whitney test, both followed by post-hoc tests at a pre-set 5% significant level. Results: DG in solution in GIC presence was more stable at room temperature and at 37 °C when compared to DA. FTIR analysis didn't indicate chemical reaction between GIC and CHX in tested concentrations. Microencapsulated formulations have increased setting time, while DG decreased it ($p<0.05$). DG inclusion increased compressive strength ($p<0.05$) and flowability was reduced by CHX free forms inclusion ($p<0.05$). MPDA and MPDG showed a later and gradual releasing profile when compared to DA and DG groups. Incorporating CHX showed significant antibacterial effect when compared to pure GIC, but without statistically significant differences when comparing groups with free or microencapsulated forms, as well as immediate and aged GICs. Conclusion: The incorporation of CHX resulted in glass ionomer cements with antibacterial effect and appropriate physical properties for clinical use.

Key words: Dental caries, biofilm, polymers and *Streptococcus mutans*.

SUMÁRIO

1 INTRODUÇÃO GERAL

A cárie dentária ainda é uma doença altamente prevalente em várias regiões do mundo (PETERSEN *et al*., 2005; MARINHO *et al*., 2013), sendo o seu controle um grande desafio para a atuação clínica na Odontologia e para o desenvolvimento de materiais odontológicos. A busca de novos produtos que controlem a instalação e/ou progressão das lesões cariosas impulsiona cada vez mais o número de pesquisas científicas nesta área.

Lesões de cárie ativas representam um importante sítio para bactérias como *Streptococcus mutans* e estes têm sido apontados como os principais responsáveis pelo início das lesões de cárie (KRZYŚCIAK *et al*.,2014). Isto se deve ao fato deste micro-organismo estar presente em altos níveis imediatamente antes do surgimento das lesões e sua habilidade em degradar carboidratos fermentáveis, promovendo a formação de ácidos, além da sua capacidade de viver em ambientes com baixo pH (SVENSATER *et al*., 2001). Adicionalmente, sua patogenicidade está relacionada à capacidade de formar biofilmes em superfícies sólidas mediada pela presença de adesinas e polissacarídeos extracelulares (SENADHEERA *et al*., 2005).

Na tentativa de melhorar a saúde bucal dos pacientes, busca-se realizar a adequação do meio bucal, que se caracteriza por uma abordagem clínica que diminui o risco/atividade de cárie pela redução do número de micro-organismos cariogênicos. Alguns dos procedimentos clínicos realizados são o selamento provisório de cavidades abertas, eliminação de fatores retentivos de placa bacteriana e uso de antimicrobianos resultando em ambiente favorável à paralisação do processo carioso, demonstrando-se ser um procedimento eficaz na redução do número de *Streptococos mutans* na saliva (VOLPATO *et al*., 2011). No entanto, tais patógenos podem permanecer viáveis por longos períodos na cavidade dentária, o que pode favorecer a progressão das lesões de cárie ao redor de restaurações e consequente falha do tratamento restaurador (LULA *et al*., 2009; FARRUGIA; CAMILLERI, 2015).

Em abordagens relacionadas à prática da Odontologia minimamente invasiva, uma remoção mais conservadora do tecido cariado é sugerida na literatura (PETERS; Mc LEAN, 2001). Tem sido relatado que a remoção completa da cárie em cavidades dentárias prévias à restauração é difícil e muito pouco provável. Além disso, defende-se que após a remoção conservadora do tecido dentinário, ainda haja bactérias residuais no tecido afetado, como ocorre na técnica do tratamento restaurador atraumático (ART), onde parte dos tecidos dentários desmineralizados é removida apenas com instrumentos manuais (FRENCKEN *et*

al., 1996). Sendo assim, o uso de materiais restauradores antimicrobianos seria o ideal para evitar a propagação da cárie e/ou cáries recorrentes. No entando, o uso de antimicrobianos deve ser controlado, pois a sua administração por tempo prolongado pode afetar o equilíbrio biológico da cavidade oral e levar à resistência microbiana (KOUIDHI; AL QURASHI; CHAIEB, 2015).

O uso de cimentos de ionômero de vidro (CIV) é bastante amplo na odontologia restauradora. Eles são derivados de ácidos orgânicos, geralmente um ácido polimérico aquoso e um componente de vidro, como o fluor-aluminio-silicato, embora possam ser encontrados outros componentes em produtos comerciais diferentes (VAN NOORT, 2002; MOSHAVERINIA *et al*., 2011). O CIV é considerado um material restaurador de alta relevância clínica, especialmente por sua capacidade de adesão química à estrutura dentária, sem a necessidade de um agente de união adicional (LIN; McINTYRE; DAVIDSON, 1992; YAP *et al*., 2003; YLI-URPO *et al*., 2005; SIDHU, 2011). Além disso, apresenta estética aceitável, adequado coeficiente de expansão térmica (NAASAN; WATSON, 1998), boa compatibilidade biológica e ação anticárie através da liberação prolongada de fluoretos (HATTON; HURRELL-GILLINGHAM; BROOK, 2006; NICHOLSON; CZARNECKA, 2008). Estas propriedades juntas promovem longevidade às restaurações, justificando a indicação do CIV para uma variedade de situações clínicas, tais como forramento de cavidades, cimentação de próteses, selamento de cicatrículas e fissuras, reparo de perfuração em raizes e restaurações dentárias (FORSTEN, 1998; GLASSPOOLE; ERICKSON; DAVIDSON, 2001; SIDHU, 2011).

Na técnica do tratamento restaurador atraumático (ART), o CIV é utilizado como material restaurador no procedimento de adequação do meio bucal, devido a sua capacidade de alterar o crescimento e o metabolismo microbiano dos *Streptoccocos mutans* e pela ação do flúor, diminuindo a velocidade dos processos de desmineralização e facilitando os processos de remineralização dentária (HAMILTON, 1990; WEERHEIJM *et al*., 1999; FEATHERSTONE, 2006; WIEGAND; BUCHALLA; ATTIN, 2007). Acreditava-se que o CIV apresentava efeito antibacteriano proporcional à quantidade de flúor liberada, que ocorre em grande quantidade durante a reação de presa inicial, decaindo após esse período, tornando-se insuficiente para eliminar a microbiota cariogênica remanescente após 6 semanas de sua inserção, estando sua ação limitada ao efeito anticárie (SEPPÄ; KORHONEN; NUUTINEN, 1995; VERMEERSCH; LELOUP; VREVEN, 2001; MARTINS *et al*., 2006; WIEGAND; BUCHALLA; ATTIN, 2007).

No intuito de melhorar o efeito inibitório contra patógenos cariogênicos, pesquisadores incorporaram agentes antimicrobianos, como a clorexidina, ao CIV convencional e obtiveram considerável ação antimicrobiana (SANDERS *et al*., 2002; TAKAHASHI *et al*., 2006; TÜRKÜN *et al*., 2008; DEEPALAKSHMI *et al*., 2010; TÜZÜNER *et al*., 2011). A clorexidina é um fármaco bastante investigado no controle da atividade cariogênica (EMILSON, 1994; VAN RIJKOM; TRUIN; VANT'T HOF, 1996; ZHANG *et al*., 2006) e apresenta ação antimicrobiana imediata e amplo espectro de ação contra bactérias gram-positivas, gram-negativas, anaeróbias, aeróbias, leveduras e fungos. Entre os diferentes agentes antimicrobianos usados para controlar micro-organismos dentários, a clorexidina tem sido considerada uma das substâncias mais eficazes (HENNESSEY, 1973; KOO *et al*., 2003). Seu efeito antimicrobiano ocorre através da interação das suas moléculas catiônicas com a superfície aniônica dos micro-organismos, provocando alterações na permeabilidade da membrana celular e, consequentemente, no desequilíbrio osmótico da célula (DELANY *et al*., 1982; OLIVEIRA *et al*., 2009).

Para se obter uma adequada atuação dos agentes anticárie, é necessário que haja uma liberação em níveis terapêuticos ao longo do tempo (FEATHERSTONE, 2006; MARSH; HEAD; DEVINE, 2015), dependendo, frequentemente, da colaboração do paciente. Neste contexto, a libertação controlada representa várias vantagens na administração de fármacos, uma vez que este sistema pode se adequar às condições terapêuticas necessárias, por exemplo, garantindo a liberação inicial de fármaco, seguido pela manutenção de doses eficazes (BRUCK, 1983). Este sistema aumenta a atividade terapêutica local da droga por um período prolongado, reduzindo o número de administrações e, consequentemente, sua toxicidade (WEISER; SALTZMAN, 2014). Dentre os sistemas de liberação controlada, estão as micropartículas constituídas por polímeros biodegradáveis, como o Poli (ácido láctico-co-glicólico) (PLGA) (WU; WANG, 2001; MAKADIA; SIEGEL, 2011), dispositivo de liberação que tem atraído a atenção de pesquisadores. O PLGA é formado por copolímeros para aplicação terapêutica com propriedades favoráveis, tais como biocompatibilidade, biodegradação, bioreabsorção, resistência mecânica e facilidade de fabricação por diversas técnicas, sendo considerado um material padrão-ouro no desenvolvimento destes sistemas para uso clínico em humanos (ANDERSON; SHIVE, 1997; JAIN, 2000; SCHNIEDERS *et al*., 2006; JI *et al*., 2010; CORREIA *et al*., 2015).

Um dos principais problemas clínicos do uso de soluções de clorexidina é a dificuldade em eliminar ou suprimir *S. mutans* por um período de tempo prolongado (GUPTA *et al*., 2015). Além disso, esta solução confere um sabor amargo forte e desagradável em

enxaguatórios bucais, possuindo, adicionalmente, outros efeitos adversos relacionados ao seu uso prolongado, como pigmentação do esmalte dentário, alteração da sensibilidade do paladar e lesões na mucosa oral (AUTIO-GOLD, 2008; GUPTA *et al*., 2015). Sendo assim, o uso de micropartículas de PLGA carregadas com CHX poderia, além de manter a biodisponibilidade do fármaco através da liberação controlada, diminuir ou inibir os efeitos colaterais anteriormente citados, pela possibilidade de ser administrado em doses menores e locais, contribuindo para o uso mais seguro da droga.

A associação entre o flúor liberado pelo cimento e a incorporação de micropartículas poliméricas de CHX poderia somar o efeito antimicrobiano ao efeito anticárie do CIV, possibilitando, assim, a ampliação da sua aplicação clínica. No entanto, o uso do CIV, para obter efeito antibacteriano, requer uma abordagem cuidadosa, devendo-se inserir uma dose apropriada de agentes antibacterianos, sem comprometer as propriedades físico-químicas do material original. Estudos mostram que a incorporação do digluconato e diacetato de clorexidina pode aumentar a atividade bactericida sem comprometer seriamente as propriedades físicas e mecânicas do cimento (TAKAHASHI *et al*., 2006; TÜRKÜN *et al*., 2008; TÜZÜNER *et al*., 2011), porém, a efetividade da ação antimicrobiana do CIV restaurador ativado quimicamente contendo micropartículas poliméricas de clorexidina ainda não foi esclarecida.

Diante do exposto, a busca por um material restaurador biologicamente aceitável e com propriedades antibacterianas enfatiza a significância clínica deste estudo, que teve como objetivo avaliar o efeito da incorporação de sais CHX nas formas livre e microencapsulada sobre as propriedades físico-químicas e biológicas de um CIV restaurador ativado quimicamente, através de um estudo *in vitro*.

2 PROPOSIÇÃO

Esta dissertação será apresentada em um capítulo, tendo como objetivos:

Capítulo 1:

-Avaliar o efeito da adição de sais de clorexidina (diacetato ou digluconato), em suas formas livre ou incorporados em micropartículas poliméricas de PLGA, nas propriedades físico-químicas (tempo de presa, resistência à compressão, escoamento e liberação de clorexidina em água) de um cimento de ionômero de vidro restaurador ativado quimicamente.

-Adicionalmente, avaliar o efeito desta adição na inibição da formação de biofilme sobre espécimes de cimento de ionômero de vidro, 24 h e 15 dias após a confecção dos espécimes.

3 CAPÍTULO

Esta dissertação está baseada no artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado, e permite a inserção de artigos científicos de autoria e coautoria do candidato. Desta forma, esta dissertação é composta por um capítulo, contendo um artigo a ser submetido para publicação em revista científica, conforme descrito abaixo:

Capítulo 1

"Incorporation of chlorhexidine loaded-PLGA microparticles and chlorhexidine salts into a glass-ionomer cement - physicochemical and antibacterial properties." Weslanny de Andrade Morais, Jacqueline Santiago Nojosa, Cícero Leonardo do Nascimento Braga, Ramille Araújo Lima, Francisco Fábio Oliveira de Sousa, Monica Yamauti, Lidiany Karla Azevedo Rodrigues. Este artigo será submetido à publicação no periódico "Journal of Biomedical Materials Research- Part A" (ANEXO).

3.1 Capítulo 1

Title: Incorporation of chlorhexidine loaded-PLGA microparticles and chlorhexidine salts into a glass-ionomer cement- Physicochemical and antibacterial properties.

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Abstract

This *in vitro* study aimed to evaluate the effects of chlorhexidine (CHX) salts (diacetate - DA or digluconate - DG) free base or encapsulated into poly(lactic-co-glycolic acid) (PLGA) microparticles - MP on physicochemical and antibacterial properties of a chemically activated glass ionomer cement (GIC). CHX was not incorporated into control specimens and experimental materials were prepared by adding 1% (w/w) of CHX loaded-PLGA microparticles or pure CHX salts into the GIC, constituting the groups: GIC (Control), DA, DG, MPDA and MPDG. Specimens were evaluated for determining CHX stability test, FTIR spectroscopy, setting time (ST), compressive strength (CS), flowability (F), CHX cumulative release (CR) and anti-biofilm activity (AA). Microencapsulated formulations enlarged, while DG reduced ST ($p<0.05$). DG increased resistance to CS ($p<0.05$); and F was decreased by DA and DG ($p<0.05$). Microencapsulated groups presented a later and gradual profile of CHX-release when compared to CHX free base groups. Both CHX incorporating forms for DA and DG showed significant AA increase when compared to control. However, no statistically significant differences were found when comparing CHX free base or microencapsulated forms, as well as fresh and aged material. Experimental GICs have presented antibacterial activity, without detrimentally affect other material properties.

Keywords: Anti-Bacterial Agents, Biofilms, Polymers, Poly lactic–co-glycolic acid copolymer, *Streptococcus mutans*

INTRODUCTION

The use of glass-ionomer cements (GICs) is quite wide in restorative dentistry since its invention. They are derived from organic acids, generally an aqueous polymeric acid and a glass component, usually a fluoroaluminosilicate.[1] It is a relevant clinical restorative material especially due to its ability of adhering chemically to tooth structure without necessity of any additional bonding agent.[1-4] Furthermore, GICs have acceptable aesthetics and anticariogenic action[5,6] by providing prolonged period of F^- releasing because its ability to act as a reservoir of fluoride.[2,3,5] These properties, together with its biocompatibility, promote longevity and make this material useful for a variety of clinical situations, such as pulp protection, bonding, cementing and restoring. In sealing cavity caries lesions procedures and in atraumatic restorative treatment technique (ART), [2,3] where demineralized dental substrates are partially removed by the use of hand instruments, cavities are usually restored with auto-setting GIC,[7,8] and recurrent caries inhibition has been achieved.

In an attempt to improve antimicrobial characteristics to anti-caries effect of conventional GIC, chlorhexidine (CHX) incorporation into this cement has been investigated.[7,9-12] Since immediate antimicrobial action against Gram positive bacteria, Gram negative, aerobic and facultative anaerobic bacteria, yeasts and fungi has been found, and chlorhexidine has been considered as one of the most effective and used substances.[13,14] For obtained prolonged antibacterial effect of CHX it is necessary being released slowly to the oral environment. In this context, controlled release systems present several advantages in administrating medicines. These systems can fit required therapeutic conditions, ensuring initial release of drugs, followed by maintenance of effective doses,[15] enhancing therapeutic activity and reducing drug amounts. One important and usable system is the Poly (lactide-co-glycolide acid) (PLGA), which is a biodegradable polymer that has great potential as a drug delivery device.[16] PLGA is a copolymer microsphere controlled release system with favorable

properties such as biocompatibility, biodegradability, mechanical strength and facility to be fabricated by different techniques.[17-19] Thus, the use of PLGA microparticles loaded with CHX could, in addition to maintaining the bioavailability of the drug through the controlled release, decrease or inhibit the side effects associated with prolonged use of CHX solutions, as tooth enamel pigmentation and change in taste,[20,21] for possibility of being administered in smaller and local doses, contributing to safer use of drug.

The association between fluoride and microencapsulated chlorhexidine can improve GIC antibacterial properties and contribute for increasing glass ionomer cement applications. However, GICs for use in antibacterial approach requires an appropriate dose of antibacterial agents without compromising basic physicochemical properties of the material. To the best of our knowledge, the association of this material and chlorhexidine loaded-PLGA microparticles (diacetate or digluconate) was not previously studied. Therefore, the aim of this study was to compare and to evaluate the effect of CHX salts incorporation in their free and microencapsulated forms on the physicochemical and antibacterial properties of a chemically activated restorative GIC.

MATERIALS AND METHODS

The experimental design of study is described in Figure 1.

Preparation of chlorhexidine microparticles

The materials and chemicals used in this study are described in Table 1.

Microparticles were prepared with Poly (lactide-co-glycolide acid) (PLGA 50:50, Sigma-aldrich, Germany) by means of spray-drying technique[22] since is the most used for obtaining dry microparticles.[23] Additionally, is very rapid, convenient and has very few processing parameters, making it suitable for industrial scalable processing.[22,24] Chlorhexidine digluconate (DG) (Panreac, Barcelona, Spain) and chlorhexidine diacetate (DA) (Evonik®,

Barcelona, Spain) were used to prepare two formulations of microparticles, resulting in drug loading of 4.07% (w/w) for DA and 2.44% (w/w) for DG (unpublished data).

Chlorhexidine stability test

The stability study was conducted based on procedures defined by RE Nº 01/2005 and RDC 45/2012 with modifications.[25, 26]

CHX-stability experiment was performed to evaluate interaction between CHX salts (DA or DG) in different concentrations, after storing these solutions together with GIC specimens in aqueous medium. GIC samples were prepared with a polystyrene matrice (6.0 mm diameter x 2.0-mm thick) and put into each CHX concentration randomly selected. One low (2 µg/mL) and other higher concentration (5 µg/mL) were stored at room temperature 25 ºC (R). Other two concentrations (4 and 20 µg/mL) were stored in an incubator (oven) at 37 ºC (O) and all solutions were measured at pre-set time intervals along 1,600 hours (66 days).

FTIR spectroscopy analysis

Fourier Transform Infrared (FTIR) Spectroscopy (Perkin-Elmer Spectrum 100, Perkin Elmer, Shelton, CT, USA) was used to verify the interaction between glass ionomer cement and chlorhexidine. One sample of each material was produced: 1) DG, 2) DA, 3) GIC, 4) GIC+DG and 5) GIC+DA. Each material sample was dispersed into a small agate mortar and thoroughly mixed with potassium bromide (KBr) using a pestle. Pellets of KBr/CHX solution and KBr/GIC+CHX solution were prepared with a hand press (Hand Press Kit 161-1100, PIKE Technology, Madison, WI, USA). The glass ionomer was handled according to manufacturer's instructions, and after setting reaction mixture was brought to the ATR. The explored frequency ranged from 500 to 4,000 cm^{-1} at 4 cm^{-1} resolution in transmission mode, looking for the presence of bands related to vibrations modes: 1) Water ν HO in (3500 cm^{-1}) and δ HOH in (1600 cm^{-1}); [27,28] 2) Si-O bands in the GIC: ν_{as} SiOSi (1050 cm^{-1}) and ν_{s} SiOSi

(730 cm^{-1}), which form the vitreous part of the material[27,28] and 3) CHX: band characteristic to vibration modes of amine (cationic) in (1,650 cm^{-1}).[29]

Experimental materials formulation

The conventional restorative glass ionomer cement (GIC) chosen for the current study was Maxxion R (FGM, Joinville, SC, Brazil). The materials formulations tested in this study were prepared by incorporating free base forms (DA or DG) and microencapsulated forms (MPDA or MPDG) of chlorhexidine in the powder of GIC to obtain a final formulations containing 1% (w/w) of CHX (Table 2). Previously, each powder portion of GIC and CHX was calculated and weighted in an analytic balance. In the control group, GIC was used without any modification. Concentrations of chlorhexidine were chosen based on a previous pilot test, where the amount of chlorhexidine released was monitored. Cements were manually manipulated according to the manufacturer's instructions (powder/liquid rate) for all tests at a room temperature (23 ± 1 ºC) and relative humidity of 50 ± 10% as recommended by ISO 9917-1:2003 specification.[30]

Surface morphology of GIC samples with and without microparticles was examined by scanning electron microscopy (FEM-SEM ULTRA PLUS, Carl Zeiss, Baden-Württemberg, Germany) (Figure 2).

Chlorhexidine release measurement

Three samples of each group were prepared with an individual cylindrical polystyrene matrix (2.0 mm diameter x 4.0 mm tick). The materials were manipulated on a glass plate during 1 min and the matrices were filled with each GIC formulation. After 24 h of curing material, samples were placed in a polystyrene tubes containing 1 mL of ultrapure water and stored in an incubator (BOD- Biochemical Oxygen Demand- TE-391, Tecnal, Piracicaba, Brazil) at 37 ºC. Aliquots of 1 mL were collected, and the same amount was immediately

replaced with fresh release medium at pre-determined time intervals. Release studies were carried out for 4,800 h, when sex consecutive CHX measurements presented very similar values. CHX cumulative release (%) and drug relesed (µg) was quantified in a spectrophotometer (Amersham Biosciences Ultrospec 1100 Pro, Cambridge, England).

Analysis of physical properties

Physical properties of setting time and compressive strength were evaluated based on the procedures defined in ISO 9917-1:2003 without modifications.[30] Flowability was evaluated based in a previously study.[31]

Setting Time

Three discs (5 mm in diameter, 2 mm thick) were used to determine the setting time for each type and concentration of CHX. Samples were obtained by dispensing GICs in a silicone matrice that was covered with a polyester tape and a 1 mm thick glass slide, on digital pressure was performed during 2 s for accommodation and elimination of material excess. After losing the brightness, a Gilmore needle with (mass=400 g ± 5 g, flat tip diameter = 1.0 ± 0.1 mm) was carefully introduced perpendicularly to the cement surface during 5 s. The penetration was repeated at 30 s intervals until the needle could no mark the surface material when viewed at 2 x magnification. Setting time was measured in an incubator at 37 ºC ± 1 ºC with 90% of humidity and was defined as the period of time from the end of mixing the material and the moment when the indenter is not able to make marks on cement surface anymore.

Compressive strength

Five samples (6 mm in diameter, 4 mm thick) for each experimental and control groups were prepared using a cylindrical acrylic mould. The material was mixed and dispensed into the mould with a Centrix syringe and covered with a polyester tape and a 1

mm thick glass slide, and digital pressure was carried out for accommodation and elimination of material excess. After 20 min of the initial material cure, samples were ejected from the matrice and stored in deionized water at 37 °C for 24 h under static conditions until testing. Each specimen was manually polished without irrigation using grit silicon carbide paper (Carbimet® 2 - Buehler®, USA) to eliminate irregularities, and the compressive strength was performed with a universal testing machine (3455, Instron Co., Canton, Mass, USA) at a crosshead 1.0-mm/min speed and 2 kN load cell until failure occurred. Compressive strength values (kgf/cm²) were calculated by dividing the load (F) by the cross-sectional area and converted into MPa.

Flowability Test

In order to determine GIC flowability, a sufficient amount of GIC required to fill a 3 mm internal diameter ring, was dispensed over a glass plate with standard dimensions (5 cm x 5 cm x 5 mm). Then, another plate presenting the same size was placed over the first one so that a wire material was formed between the plates. A weight of 2.5 kg was applied on the two plates during 10 min. After this time, the 2.5-kg weight was removed and the biggest and smallest diameters of the material disks were measured using a digital caliper. To validate the test, each material was required to produce a disc with a diameter bigger than 20 mm and the difference between the measured diameters should not be more than 1 mm.[31]

Antimicrobial properties

Inoculum and biofilm model

Streptococcus mutans UA159 (ATTCC) was obtained from single colonies isolated on blood agar plates, inoculated in Tryptone yeast-extract broth containing 1% glucose (w/v) and incubated for 18-24 h at 37 ºC under micro-aerophilic conditions in partial atmosphere of 5% CO_2. Mono-species *S. mutans* biofilms were formed on saliva-coated GIC discs placed in bath cultures at 37 ºC in 5% CO_2 up to 5 days in 24-well polystyrene plates. The biofilms

were grown in tryptone yeast-extract broth containing 1% sucrose (w/v) and were kept undisturbed for 24 h to allow initial biofilm formation. During the biofilm formation period, once daily the discs were dip-washed three times in a plate containing of NaCl 0.89% solution in order to remove the loosely bound biofilm and they were transferred to new 24-well plates with sterile medium.[32]

Biofilm analysis

To analyze the antimicrobial effect, discs (6.0 mm diameter x 1.5 mm thick) of GIC incorporated with 1% CHX in the free (DA or DG) and microencapsulated forms (MPDA or MPDG) were produced (Table 2). Materials were dispensed in a silicone mould, covered with a polyester tape and then submitted to digital pressure during 2 s in order to better accommodate the material. After 24 h of setting time, half of the samples were used for immediate biofilm formation (Immediate group) and the other half was placed in a 24-well plates containing 1 mL of distilled water and stored in an incubator (BOD- Biochemical Oxygen Demand- TE-391, Tecnal, Piracicaba, Brazil) at 37 ºC for aging the samples before started the antimicrobial test (Aged group). Aliquots of 1 mL were collected, and the same amount was immediately replaced with fresh release medium at pre-determined time intervals as in CHX released test. Samples were sterilized by exposure to ultraviolet irradiation in a laminar flow hood during 30 min on each side before starting biofilm formation.

Three discs of each experimental groups (Immediate and Aged) were removed after 1, 2, 3, 4 and 5 days of initial biofilm formation and were transferred to pre-weighed microtubes containing 1 mL of NaCl 0.89% solution. Biofilms were then dispersed with 3 pulses of 15 s with 15 s of interval at a 7-W output (Branson Sonifier 150; Branson Ultrassonics, Danburry, CT). An aliquot (0.05 mL) of the homogenized biofilm was serially diluted (10^{-1}–10^{-6}) and plated in duplicate onto BHI (Brain Heart Infusion) agar, plates were then incubated at 37 ºC, 5% CO_2 during 48 h before enumerating viable microorganisms. Results were expressed as

colony forming units (CFU)/mL and transformed in $\log_{10}$ CFU in order to reduce variance heterogeneity.[32]

Statistical Analysis

Mechanical properties data were submitted to analysis of variance with one factor (One way ANOVA), followed by Tukey test for multiple comparisons. Released chlorhexidine data were analyzed by means a Two-way ANOVA followed by Bonferroni post-test, both results were expressed as Mean±SD. For analyzing antimicrobial effects, Mann Whitney test and Unpaired T test analysis of variance was used to detect differences, followed by an F test for pair wise comparisons. Significance level was set at 5%. The program respectively used to perform the analyses was StatPlus (Microsoft, CA, USA) and Prism 5.0 (GrafPad Software, Inc.; La Jolla, CA, USA)

RESULTS

Chlorhexidine stability test

CHX stability profile is shown in Figure 3 (DA-3A and DG-3B). Chlorhexidine DA presented a decrease in its concentration overtime among all the groups. However, this behavior was more intense in the higher concentrations when stored at 37 °C, as it can be noticed in Figure 3A. For instance, a bigger decline in CHX levels just after 200 h in the group (DA20O) stored in such condition can to be seen. Intermediate DA concentrations were stable up to 1,000 h, when the concentration notoriously decreased. The least concentrated group (DA2R) remained stable during the entire period. DG groups presented a better performance when compared to DA groups, mainly noticeable within the groups at lower drug levels (DG2R, DG4O and DG5R), even if stored at 37 °C, and the group DG20O showed an unstable behavior as observed in Figure 3B.

FTIR spectroscopy

FTIR spectra of GIC in contact with DA and DG (Figure 4A and 4B) showed the presence of bands related to vibration modes of water ν HO in (3500 cm^{-1}) and δ HOH in (1,600 cm^{-1}). However, in the pure GIC spectrum, lower band intensity related to the vibration mode ν OH in water (3500 cm^{-1}) can be observed. In this spectrum (pure GIC) were also observed vibrations modes related to Si-O bands: ν_{as} SiOSi (1050 cm^{-1}) and ν_{s} SiOSi (730 cm^{-1}). In the CHX (DA or DG) spetra, the presence of band characteristic to vibration modes of amine (cationic) in (1650 cm^{-1}) was observed.

Chlorhexidine release measurement

Statistically significant differences were found between groups with free CHX and microencapsulated CHX at same elapsed time ($p<0.0001$). CHX release profile of GIC is shown in Figure 5. Mean CHX release is related to CHX type that was incorporated ($F=697.02$; $p<0.0001$) and time ($F=82.96$; $p<0.0001$). The groups containing free CHX-forms (DA or DG) presented a discrete release in the first 6 h and amount of CHX begins to stabilize at approximately 744 h, without statistically difference between DA and DG groups up to 4,800 h ($p>0.05$). Groups containing free CHX forms presented lower released amounts compared to groups containing microparticles after 6 months (4,320 h).

GICs containing CHX loaded-PLGA microparticles showed differences in cumulative CHX release when compared to control group ($p<0.0001$). GIC containing CHX loaded-PLGA microparticles exhibit a late and gradual release after 96 h (4 days) showing statistic difference in cumulative release between MPDA and MPDG just up to 1,080 hours ($p<0.05$). For microencapsulated groups, the highest release was detected in MPDG group. Generally, the GICs with CHX loaded-PLGA microparticles exhibited higher release rates than GIC CHX free base forms. Point mass (μg) of CHX released by each formulation material is shown in Figure 5C. Mean point mass is related to CHX type that was incorporated ($F=99.40$;

$p<0.0001$) and time ($F=116.85$; $p<0.0001$). The drug released in (μg) was greatest in the MPDG group, followed by the MPDA group.

Mechanical properties

Mean values ± SD of results are described in Table 3. One-way ANOVA for setting time test showed differences between groups ($F=91.37$; $p<0.0001$). DA did not change setting time compared with the control group (GIC). When DG was incorporated, setting time was reduced. Groups where CHX microparticles (MPDA or MPDG) were incorporated significantly increased setting time, being longest time observed for MPDG group.

Differences among tested groups were observed in compressive strength test ($F=5.17804$; $p=0.00024$). In general, addition of free and microparticulated forms of CHX diacetate or digluconate in GIC did not negatively alter compressive strength when compared to control group. DG showed increased resistance. There was no difference in results between microparticulated groups.

Free CHX incorporation (DA or DG) in GIC decreased material flowability when compared to control group ($F=7.64802$; $p<0.0001$). However, no statistically significant difference in flowability of microparticulated groups (MPDA or MPDG) compared with control group was observed. Besides, no difference between microparticulated groups was found.

Bacterial viability

Figure 6 displays median values, as well as minimum and maximum values of bacterial inhibition for each studied group, according to the analyzed conditions (Immediate or Aged). GIC without CHX (control) had no antibacterial activity against *S. mutans*. Antibacterial results showed significant differences between control and experimental groups at all time periods ($p<0.05$). The addition of 1% chlorhexidine diacetate and digluconate in

free or microencapsulated form promoted inhibition on biofilm formation. However, no statistically significant differences between encapsulate and non encapsulated groups were found, regardless the conditions (Immediate or Aged) and times (1, 2, 3, 4, or 5 days) studied ($p>0,05$).

DISCUSSION

Glass ionomer cements have been suggested for restoring carious teeth that have been prepared with dental hand instruments, where secondary caries and restoration failure can occur easier over time, since higher level of cariogenic bacteria may be found in caries active patients.[8,33,34] Teeth restored with chlorhexidine-containing glass ionomers showed lower microorganism counts than those restored with conventional glass ionomer cements, with significantly reduction in mutans streptococci.[10] Since no antibacterial effect has been attributed to fluoride released by restorative materials,[35] benefits may be obtained from combining antibacterial agents with glass ionomer cements to control oral bacteria.

This study showed the addition of microencapsulated CHX could be a great therapeutic promise in view of its minimal impact on physical properties of conventional glass ionomer cement. Chlorhexidine stability test was used to evaluate a possible chlorhexidine adsorption by GIC or drug degradation caused by cement components. A decrease in CHX concentration over time was observed for both CHX salts, being more evident for DA, mainly when stored at 37 ^{o}C (Figure 3A and 3B). A possible reason for this reduction is the CHX attraction to the negative groups (COO^{-} F^{-1} and OH^{-1})[28] present in GIC matrix, which could bring it to solution and form insoluble precipitates. As a consequence, the higher CHX level, the higher the equilibrium displacement bringing to instability. The unstable behavior showed by DG20O might be explained as a constant exchange (loaded-unloaded) of the drug to the GIC specimen (Figure 3B). Conversely, even if the lower

concentration groups (DG2R, DG4O and DG5R) may have showed this behavior, it was not noticeable due to the smaller effect related to drug levels.

In the FTIR spectrum, band related to the vibration mode ν_{as} SiOSi (1050 cm^{-1}) may have been deleted and/or displaced when in contact with DA (Figure 4A) or DG (Figure 4B), indicating changes in type and quantity of modified cations, which act in vitreous matrix depolymerization;[28] or this band might even have been changed and only suppressed by coincidence peaks with the drug. Considering that in both CHX spectra (Figures 4A and 4B), when in contact with GIC, amine peaks (cationic) were not displaced (1634cm^{-1}),[28] this second hypothesis gains further strengthening. Thus, there is greater chance of interactions between GIC and anionic components of DA and DG occur in solution, as previously mentioned.

With regard to handling parameter, groups incorporated with free CHX digluconate presented decreased setting time, in contrast to a previous study previous study, which showed increase in setting time when 1%-DG CHX was incorporated in GIC (Ketak Molar Easymix).[36] On the other hand, DA group showed no statistically difference from the control group, coinciding with the findings reported for CHX incorporation as diacetate at 1% into Fuji Type II.[7] CHX microparticles groups showed an expanded setting time when compared to CHX free forms and control groups, this enlargement was more evident with DG use (MPDG group). A possible reason for microparticulate drug had increased setting time is the difficulty of reaction between polyacrylic acid and ions of glass particles, since CHX have this ability to react chemically with ionomer matrix, affecting the initial polymerization.[31]

Small changes in powder/liquid proportions in addition of CHX salts also can influence the mechanical strength and time of polymerization,[1,37,38] which probably was the case with inclusion of loaded CHX polymeric microparticles. However, changes in setting

time could be a clinically acceptable time, maybe considering the benefits of antibacterial action of restorative GIC.

Compressive strength is one the most commonly test used to characterize dental cements. In this study, for both types of CHX-added materials no significant changes in compressive strength compared to control were observed, except for DG, whose resistance was augmented. Flowability of DA and DG groups decreased with CHX incorporation and groups containing microparticles (MPDA or MPDG) did not significantly change compared to control. These results are in line with previous studies that have showed incorporation of CHX diacetate at 2% or greater significantly decreased compressive strength, while no influence on mechanical strength was determined for CHX diacetate incorporation when a 1% concentration was used.[7,12]

In a previous study, no decrease in physical properties of materials were observed when low concentrations (0.5%) of CHX digluconate were added to GIC. In compressive strength test, high concentrations (1.25% and 2.5%) by addition of CHX diacetate resulted in lower values compared to control. Setting time of all experimental groups were not different of control group, corroborating with results of this study to groups diacetate 1%.[12] Others studies show that increasing concentration of antibacterial agent had increasing adverse effects on physical properties.[7,40,41,43]

The ability of a restorative material to resist masticatory forces is an important aspect for its long-term clinical performance. Incorporation of 1% CHX diacetate showed optimal antimicrobial activity while it did not affect the mechanical properties, quality of connections and setting time in a previously study.[7] These reports corroborate with findings in this study, both for setting time and for compressive strength test, where no significant change in these properties was observed when added concentrations of CHX diacetate 1% in a direct or microencapsulated way, except for MPDA group that increased setting time.

Currently, in order to assess the antibacterial action of CHX-loaded GIC, a biofilm accumulation model was used for better simulating oral environment and showing the antibacterial action for these materials. Most researches used agar diffusion tests to assess this antibacterial effect is important to highlight.[7,12,36,39] Adding 1% free or microencapsulated CHX to GIC promoted *S. mutans* inhibition, a finding corroborating similar previous studies that used CHX free salts incorporation.[7,11,12,33] Recently, a research presented conflicting results since CHX DG incorporated to GIC at 1% concentration was not able to reduce *S. mutans* biofilms when compared to the control group, while CHX DA was effective[40] Different antibacterial activity of glass ionomer cements depend of evaluated cement, bacterial specie and period of evaluation.[41,42,44]

In this study conditions, the pure form of CHX diacetate can be preferable as a material which is more stable and can be easily added to powder of glass ionomer cement. CHX digluconate, when added to glass ionomer inhibits *S. mutans* growth, but there are reports which can also result in a decrease of physical properties of material.[11] This reduction associated with digluconate form is related to fact that this compound is a liquid and therefore released faster than powder form (CHX diacetate salts).[11]

Controlled drug release could be important in minimally invasive treatment approach to caries control. The main advantage of microencapsulation is an effort to protect drugs from the influence of its environment (degradative processes) and also serves to regulate the drug release through controlled release mechanism[15,16] without seriously affecting the physical properties of materials such were demonstrated in this study. However, no different antimicrobial performances could be observed among CHX free or encapsulated groups in this study. Consequently, although CHX microparticles constitute an attractive study field with innumerable opportunities for further research and developmental work, further studies

are needed to examine interactions between microparticles loaded with CHX and the matrix of one reinforced GIC that can be stayed on teeth such as restorative material for more than 30 days. Another point to be highlighted is 1% concentration used in the present study was sufficiently high to exterminate the most of bacterial cells for both free and encapsulate CHX groups. It can be suggested that lower CHX concentrations could make the efficacy differences more evident between these groups.

Based on tested condition of this study, it is suggest that more time of analysis and using other CHX concentration, antibacterial effect of CHX microparticles could be different, according to cumulative release test, since the concentration of drug used in this study was lethal, showing no advantage in the encapsulation technique. However, based on the knowledge that the moment in witch, patients most need for antimicrobial action is when they are learning to control biofilm (first days of adequacy of oral environment). Therefore, association of CHX with a GIC shows a good perspective for controlling dental caries progression and residual caries such as antimicrobial temporary restorative material.

CONCLUSION

- The addition of both CHX salts (diacetate or digluconate) either directly or microencapsulated forms had the same antibacterial effect being different only with pure GIC in the immediate use of the material or before 15 days of aging the samples in biofilm of 1, 2, 3, 4 or 5 days .

-Addition of 1% CHX to chemically activated restorative GIC in free base forms should produce antimicrobial activity and no changed negatively the tested physical properties comparable to original material.

REFERENCES

1. Van Noort R. Glass-ionomer cements and resin-modified glass-ionomer cements. Introduction to dental materials, Edinburgh, UK: Mosby; 2000; 2:129-130.
2. Sidhu SK. Glass-ionomer cement restorative materials: a sticky subject? Aust Dent J. 2011;56 Suppl 1:23-30.
3. Moshaverinia A., Nima Roohpour N, Cheea WWL. and Schricker SR. A review of powder modifications in conventional glass-ionomer dental cements. J Mater Chem, 2011; 21:1319–1328.
4. Lin A, McIntyre NS, Davidson RD. Studies on the adhesion of glass-ionomer cements to dentin. J Dent Res, 1992; 71:1836-1841.
5. Forsten L. Fluoride release and uptake by glass-ionomers and related materials and its clinical effect. Biomater, 1998;19:503-508.
6. Glasspoole EA, Erickson RL, Davidson CL. A fluoride-releasing composite for dental applications. Dent Mater, 2001; 17:127-133.
7. Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. Dent Mater, 2006; 22:647-652.
8. Frencken JE, Pilot T, Sangpaisan Y, Phantumvanit P. Atraumatic Restorative Treatment (ART): rationale, technique and development. J Public Health Dent, 1996; 56:135-140.
9. Hoszek A, Ericson D. In vitro fluoride release and the antibacterial effect of glass ionomers containing chlorhexidine gluconate. Oper Dent, 2008; 33:696-701.

10. Frencken JE, Imazato S, Toi C, Mulder J, Mickenautsch S, Takahashi Y. Antibacterial effect of chlorhexidine containing glass ionomer cement in vivo: a pilot study. Caries Res, 2007;41:102-107.
11. Sanders BJ, Gergory RL, Moore K, Avery DR. Antibacterial and physical properties of resin modified glass-ionomers combined with chlorhexidine. J Oral Rehabil, 2002;29:553-558.
12. Türkün LS, Türkün M, Ertuğrul F, Ates M, Brugger S. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent. 2008; 20:29-44.
13. Hennessey TD. Some antibacterial properties of chlorhexidine. J Period Res, 1973; 8(12):61-67.
14. Emilson CG. Susceptibility of various microorganisms to chlorhexidine. Scand J Dent Res, 1977; 85: 255-265.
15. Bruck SD. Pharmacological basis of controlled drug delivery. In: Controlled drug delivery, vol. I, Bruck, S. D. (ed.): Boca Raton, FL: CRC Press, 1983; p.1-13.
16. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers, 2011, 3:1377-1397.
17. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev, 1997; 28:5-24.
18. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly (lactide-*co*-glycolide) (PLGA) devices. Biomat, 2000, 21:2475-2490.
19. Wu XS. Preparation, characterization, and drug delivery applications of microspheres based on biodegradable lactic/glycolic acid polymers. In: Wise et al., editors. Encyclopedic handbook of biomaterials and bioengineering. New York: Marcel Dekker, 1995; p.1151-200.

20. Autio-Gold J. The Role of Chlorhexidine in Caries Prevention. Oper Dent 2008; 33(6):710-716.
21. Gupta, D.; Nayan, S.; Tippanawar, H. K.; Patil, G. I.; Jain, A.; Momin R. K.; Gupta R. K. Are herbal mouth wash efficacious over chlorhexidine on the dental plaque? Phcog Res 2015; 7(3):277–281.
22. Sousa FF, Luzardo-Alvarez A, Pérez- Estévez A, Seoane- Prado R, Blanco-Méndez J. Development of a novel AMX- loaded PLGA/zein microsphere for root canal desinfection. Biomed Mater 2010; 5(5):055008.
23. Jin Y, Chen, XD. Entropy production during the drying process of milk droplets in an industrial spray dryer. Int J Therm Sci 2011; 50(4):615-625.
24. Makadia, H. K., Siegel, S. J. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers 2011; 3:1377-1397.
25. Brasil. Agência Nacional de Vigilância Sanitária. ANVISA. Resolução RE nº 1/2005 de 29 de Julho de 2005. Guia para a realização de estudos de estabilidade. Diário Oficial da União, Brasília, 2005.
26. Brasil. Agência Nacional de Vigilância Sanitária. ANVISA Resolução RDC 45/2012. Guia para a realização de estudos de estabilidade, 2012.
27. Farrugia C, Cassar G, Valdramidis V, Camilleri J. Effect of sterilization techniques prior to antimicrobial testing on physical properties of dental restorative materials. J Dent 2015; 43:703-714.
28. Bertolline MJ, Zaghete MA, Rossano G. Uso das técnicas de infravermelho e de ressonância magnética nuclear na caracterização da reação ácido-base de um cimento odontológico experimental. Quim Nova 2009;32(5):1231-1234.
29. Kim J, Uchiyama T, Carrilho M , Agee KA, Mazzoni A, Breschi L, Carvalho RM, Tjäderhane L, Looney S, Wimmer C, Tezvergil-Mutluay A, Tay FR, Pashley DH.

Chlorhexidine binding to mineralized versus demineralized dentin powder. Dent Mat 2010; 26:771–778.

30. ISO. ISO 9917-1 dentistry – water based cements – Part 1. Powder liquid acid–base cements. Geneva, Switzerland: International Organization for Standardization; 2003.
31. Al Zraikat H, Palamara JE, Messer HH, Burrow MF, Reynolds EC. The incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass ionomer cement. Dent Mat 2011;27(3):235-243.
32. Duarte S, Gregoire S, Singh AP, Vorsa N, Schaich K, Bowen WH, Koo H. Inhibitory effects of cranberry polyphenols on formation and acidogenicity of Streptococcus mutans biofilms. FEMS Microbiol Lett 2006; 257(1):50-56.
33. Massara ML, Alves JB, Brandao PR. Atraumatic restorative treatment: clinical, ultrastructural and chemical analysis. Caries Res 2002; 36:430-436.
34. Anusavice KJ. Present and Future Approaches for the Control of Caries. J Dent Educ 2000; 69(5):538-554.
35. Sousa RP, Zanin IC, Lima JP, Vasconcelos SM, Melo MA, Beltrão HC, Rodrigues LK. In situ effects of restorative materials on dental biofilm and enamel demineralisation. J Dent 2009; 37(1):44-51.
36. Marti LM, Mata M, Ferraz-Santos B, Azevedo ER, Giro EMA, Zuanon ACC. Addition of chlorhexidine gluconate to a glass ionomer cement: A Study on mechanical, physical and antibacterial properties. Braz Dent J 2014; 25(1):33-37.
37. Billington RW, Williams JA, Pearson GJ. Variation in powder/liquid ratio of a restorative glass-ionomer cement used in dental practice. Br Dent J 1990;169:164-167.
38. Botelho MG. Inhibitory effects on select oral bacteria of antibacterial agents incorporated in a glass ionomer cement. Caries Res 2003; 37:108-114.

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

40. Marti LM, Becci AC, Spolidorio DM, Brighenti FL, Giro EM, Zuanon ACC. Incorporation of chlorhexidine gluconate or diacetate into a glass-ionomer cement: Porosity, surface roughness, and anti-biofilm activity. Am J Dent 2014; 27(6):318-322**.**

41. Botelho MG. Compressive strength of glass ionomer cements with dental antibacterial agents. SADJ 2004; 59(2):51-53.

42. Castilho ARF, Duque C, Negrini TC, Sacono NT, Paula AB, Costa CAS, Spolidório DMP, Puppin-Rontani RM. In vitro and in vivo investigation of the biological and mechanical behaviour of resin-modified glass-ionomer cement containing chlorhexidine . J Dent 2013; 41:155-163.

43. Palmer G, Jones FH, Billington RW, Pearson GJ. Chlorhexidine release from an experimental glass ionomer cement. Biomat 2004; 25(23):5423-5431.

44. Jedrychowski JR, Caputo AA, Kerper S. Antibacterial and mechanical properties of restorative materials combined with chlorhexidines. J Oral Rehabil 1983; 10:373-381.

Table 1 - Materials and chemicals used in the study

Materials (Abbreviation)	Manufacturer	Batch number	Basic Formulation
Maxxion R Glass ionomer cement	FGM, Produtos Odontológicos, Joinville, SC, Brazil.	140612	Liquid: Polycarboxylic acid 45% Tartaric acid < 10% Powder: Fluor-alumino silicate glass >75%
Chlorhexidine diacetate (DA)	Evonik®, Barcelona, Spain	8320017837	Anhydrous salt
Chlorhexidine digluconate (DG)	Panreac, Barcelona, Spain	9418600021	20% solution
Poli (D-Lactide-co-glycolide acid) - (PLGA 50-50)	Sigma-Aldrich, Toufkirchen, Germany	STBC263V	_____

Table 2 - Description of the experimental groups

Groups	Free chlorhexidine and microparticles incorporated
GIC	Control
DA	Containing 1% (w/w) of chlorhexidine diacetate
DG	Containing 1% (v/w) of chlorhexidine digluconate
MPDA	Containing 1% (w/w) chlorhexidine diacetate loaded in microparticles
MPDG	Containing 1% (w/w) chlorhexidine digluconate loaded in microparticles

Table 3 - Values of setting time (min ± SD), compressive strength (MPa ± SD) and flow test (mm ± SD) of glass ionomer cement with free chlorhexidine and microencapsulated forms.

Test	GIC	DA	DG	MPDA	MPDG
Setting Time	7.4 ± 0.6^{a}	$5.7\pm0.5^{a,b}$	4.6 ± 1.2^{b}	13.4 ± 0.7^{c}	15.8 ± 0.3^{d}
Compressive Strength	9.1 ± 2.7^{a}	$14.9\pm2.3^{a,b}$	17.5 ± 1.2^{b}	$12.0\pm4.0^{a,b}$	9.9 ± 2.4^{a}
Flowability	33.4 ± 4.5^{a}	23.2 ± 3.3^{b}	25.1 ± 5.3^{b}	33.1 ± 5.8^{a}	31.4 ± 5.9^{a}

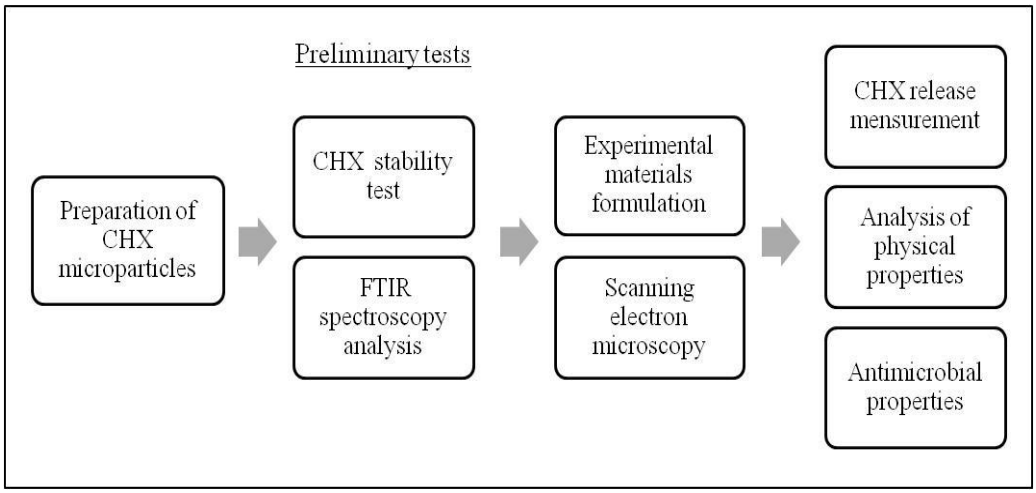

Fig.1- Experimental design of study.

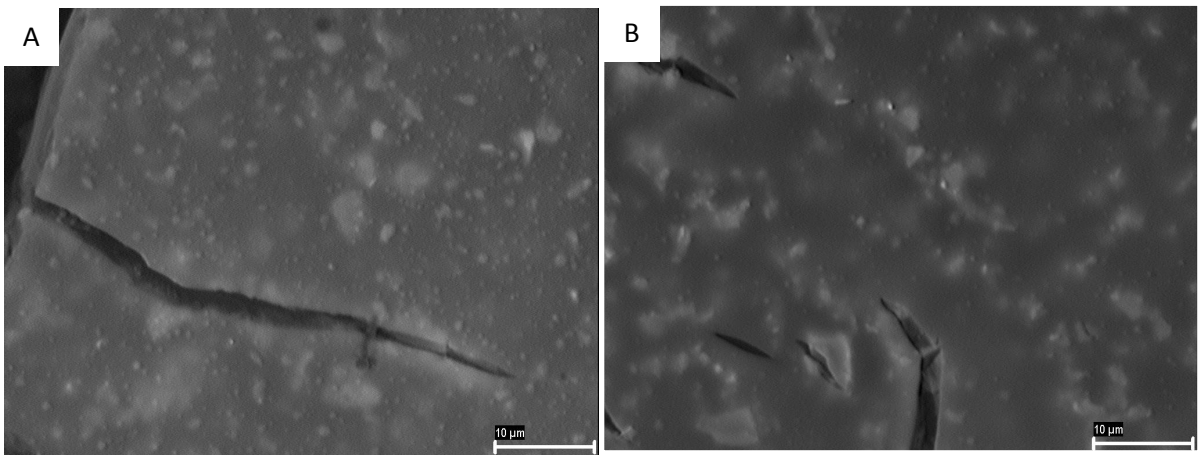

Fig.2- Scanning electron micrographs (SEM). Surface observations of GIC. A) GIC incorporated with chlorhexidine loaded-PLGA microparticles. B) GIC without microparticles.

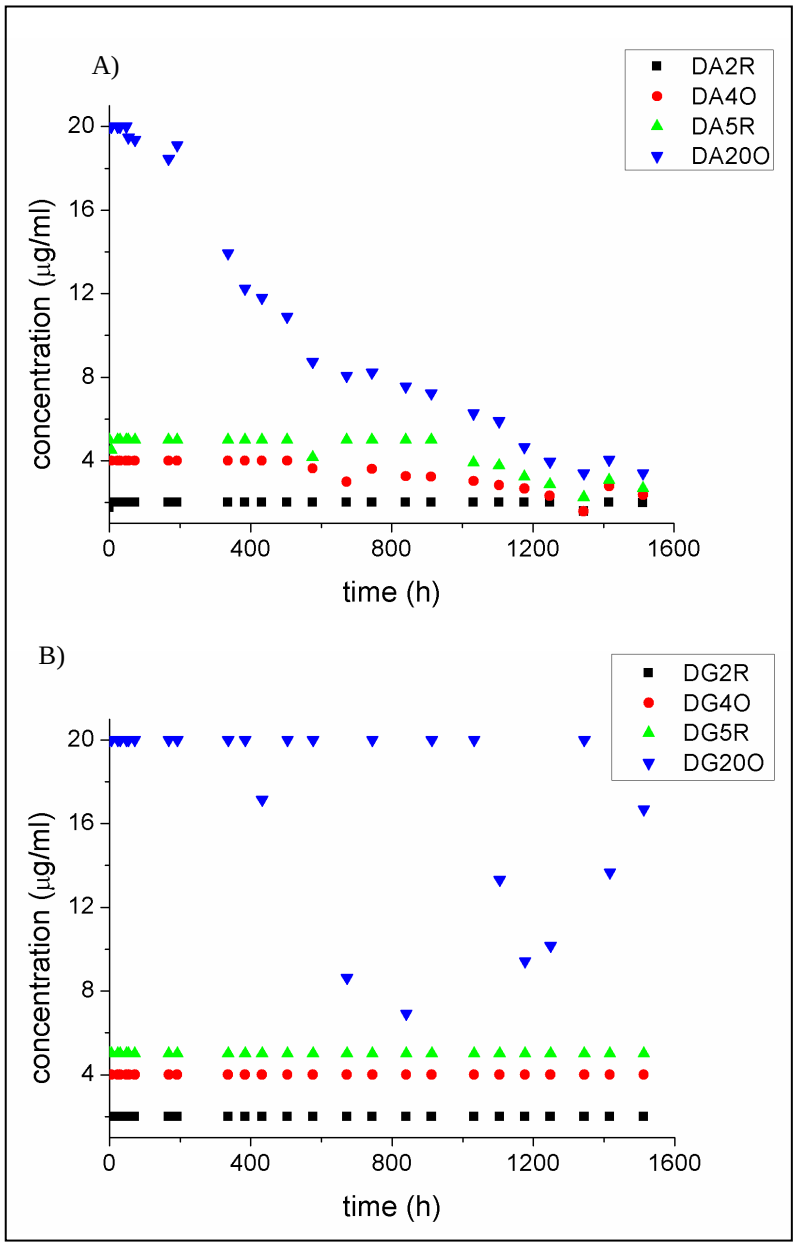

Fig.3- Chlorhexidine solution stability in the presence of glass ionomer cement in aqueous medium. A) Chlorhexidine diacetate B) Chlorhexidine digluconate. * R (Room temperature at 25ºC); O (Oven at 37ºC)

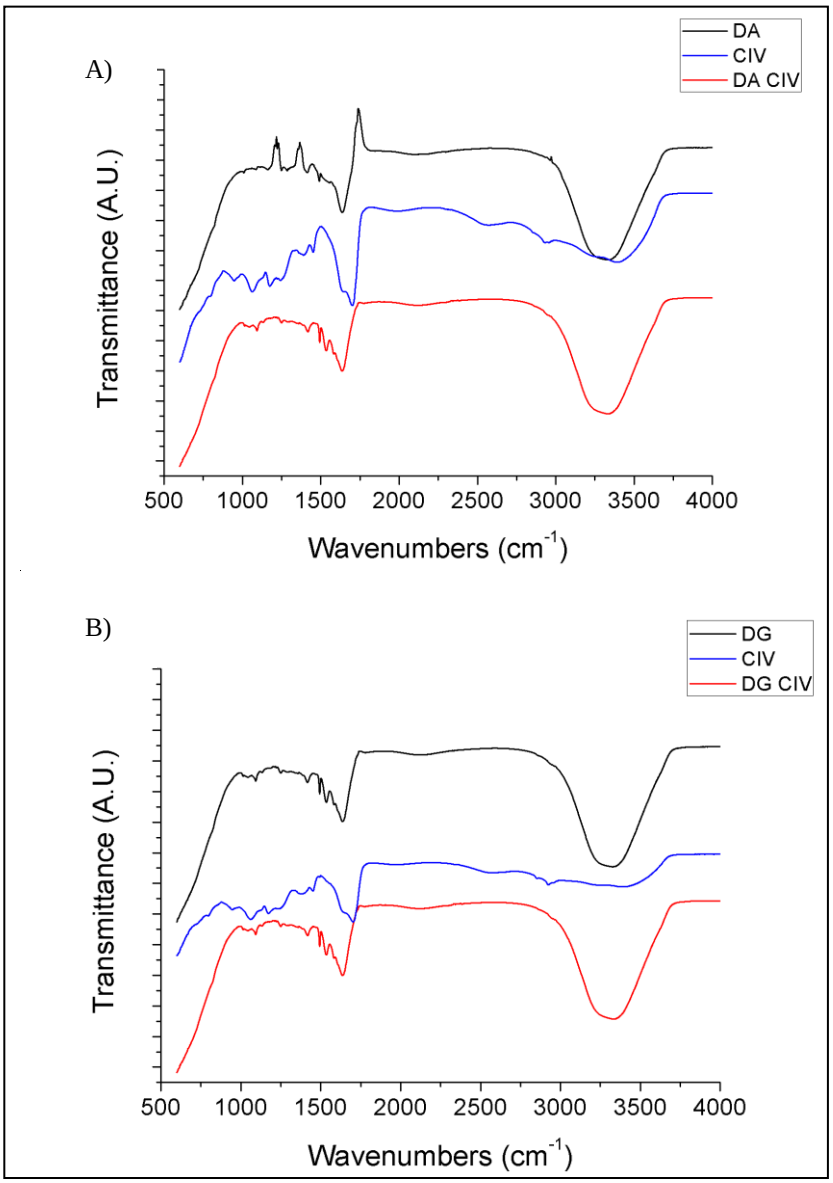

Fig.4- FTIR spectroscopy analysis of interaction between glass ionomer cement and chlorhexidine.

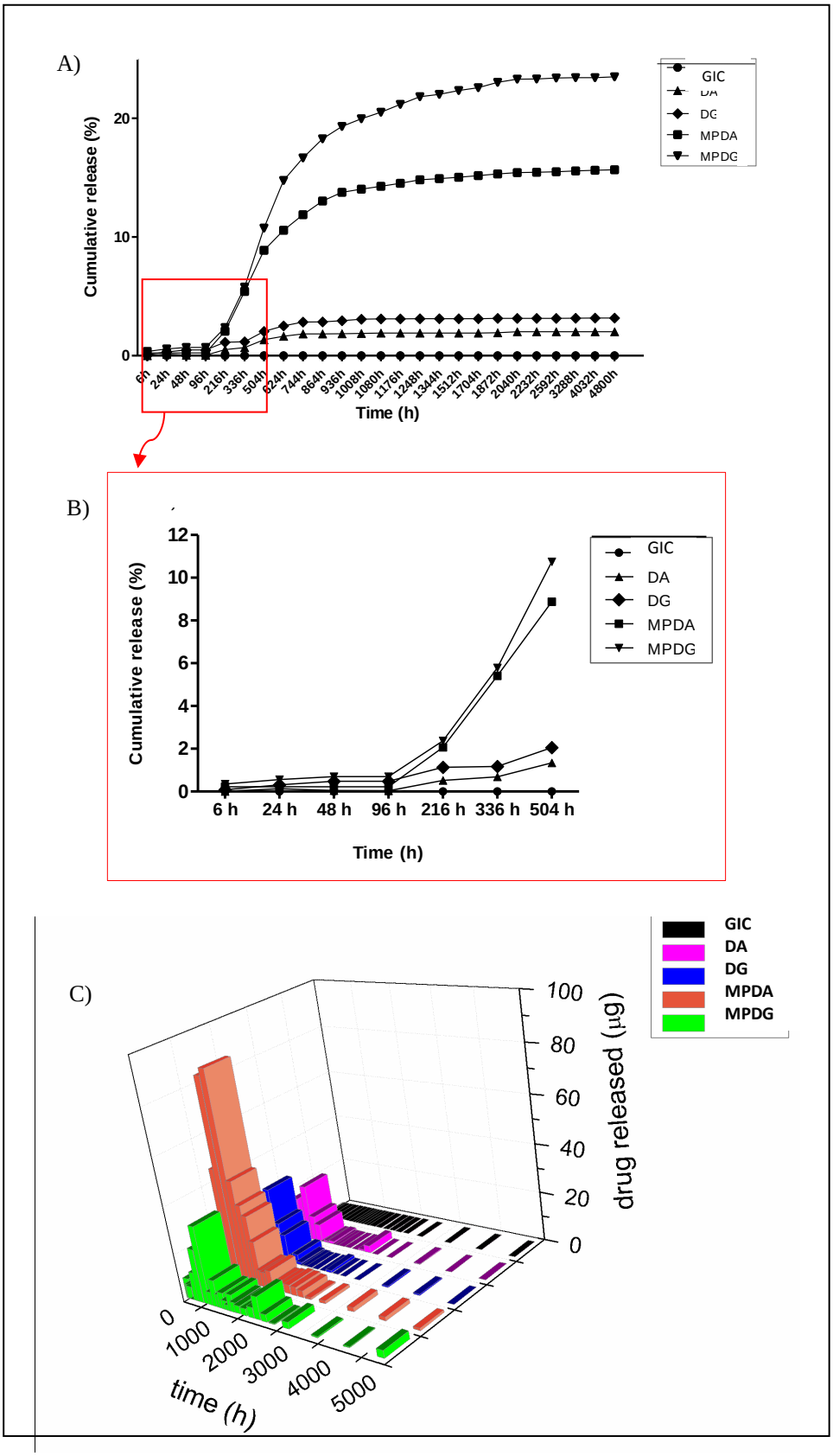

Fig.5 - Cumulative chlorhexidine release from commercial glass ionomer cement in aqueous medium. A) Cumulative release (%) during the entire evaluation period (4800 h). B) Cumulative release (%) at the first 504 h. C) Drug released (µg) of chlorhexidine during the entire evaluation period (4800 h).

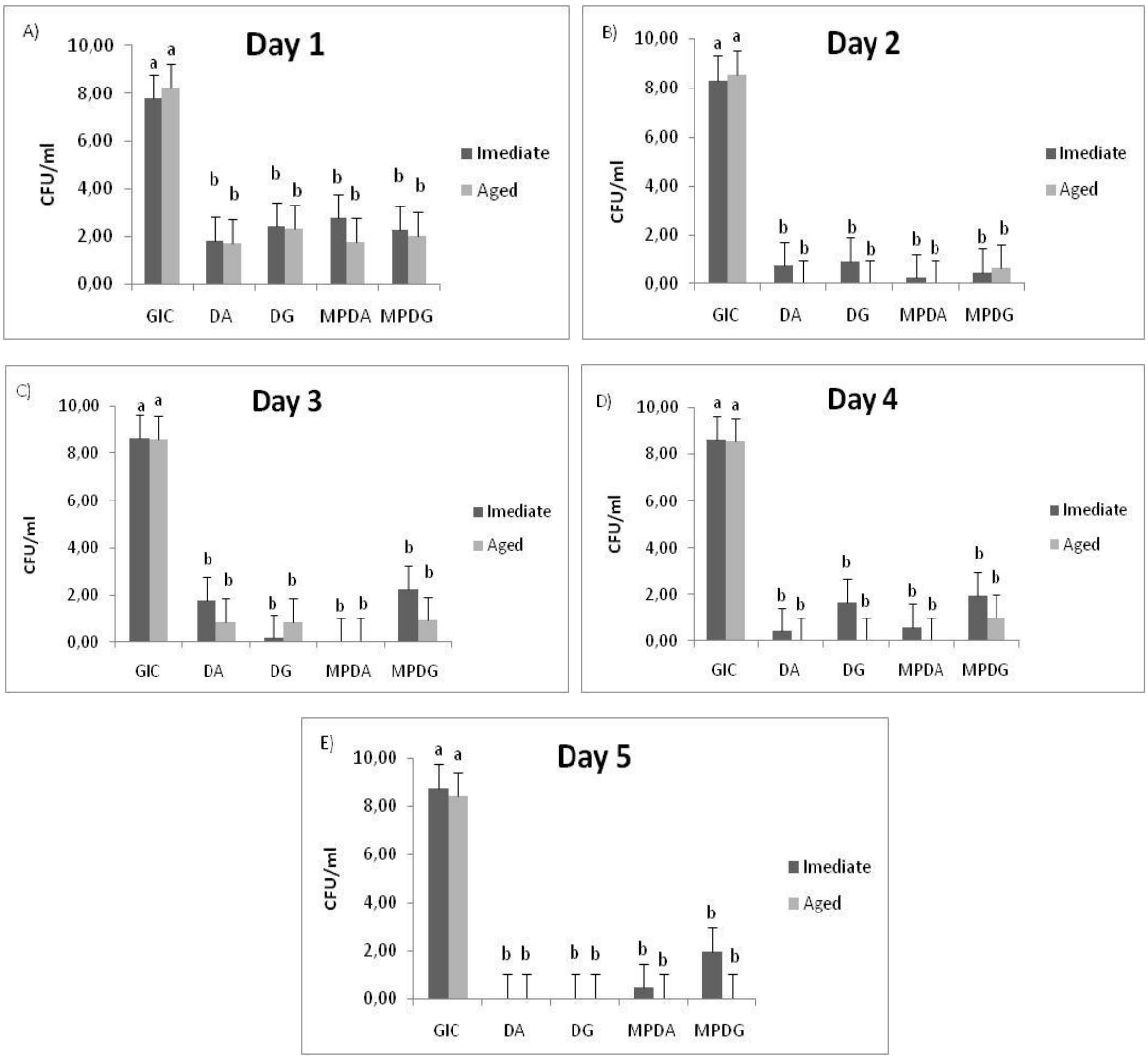

Fig.6 – Antibacterial effect of incorporation of free and microencapsulated CHX 1% diacetate and digluconate in GIC. A, B, C, D and E represent antibacterial effect in biofilm of 1, 2, 3, 4 and 5 days respectively. *Identical letter indicate no significant difference between groups ($p<0,05$).

FIGURE LEGENDS

Fig.1- Experimental design of study

Fig.2- Scanning electron micrographs (SEM) Surface observations of GIC. A) GIC incorporated with chlorhexidine loaded-PLGA microparticles. B) GIC without microparticles.

Fig.3- Chlorhexidine solution stability in the presence of glass ionomer cement in aqueous medium. A) Chlorhexidine diacetate B) Chlorhexidine digluconate. * R (Room temperature); O (Oven at 37ºC).

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Fig.6- Antibacterial effect of incorporation of free and microencapsulated CHX 1% diacetate and digluconate in GIC. A, B, C, D and E represent antibacterial effect in biofilm of 1, 2, 3, 4 and 5 days respectively. *Identical letter indicate no significant difference between groups ($p<0,05$).

4 CONCLUSÃO GERAL

-A incorporação de CLX resultou em cimentos ionoméricos com efeito antibacteriano e propriedades físico-químicas apropriadas para o uso clínico. As micropartículas de PLGA carregadas com CLX apresentaram um perfil de liberação lento e gradual do fármaco.

-A adição de ambos os sais de CLX (diacetato ou digluconato), nas formas livres e microencapsuladas, tiveram o mesmo efeito antibacteriano, tanto no grupo imediato, quanto no grupo envelhecido, com biofilme de 1, 2, 3, 4 e 5 dias, diferindo apenas do grupo crontrole.

-A adição de 1% de CLX nas formas livres (DA ou DG) ao CIV ativado quimicamente, apresentou um bom efeito antibacteriano sem alterações negativas das propriedades físicas testadas, quando comparado ao material original, mostrando-se uma boa opção de material para o tratamento restaurador contra a progressão da cárie.

-A adição 1% CLX carregado em micropartículas ao CIV ativado quimicamente apresentou um bom efeito antibacteriano e boas propriedades físicas, exceto para o aumento do tempo de presa, que podería ser considerado irrelevante frente à importância do efeito antibacteriano apresentado.

-As micropartículas de PLGA carregadas com CLX descritas no presente estudo podem ser úteis para a liberação localizada do mesmo no tratamento da cárie, quando uma liberação prolongada e controlada é desejada. Sendo assim, mostra-se um material com potencial para o tratamento resturador em pacientes odontopediátricos e em pacientes especiais com dificuldades motoras, principalmente na abordagem do tratamento restaurador atraumático.

REFERÊNCIAS GERAIS

ANDERSON, J. M.; SHIVE, M. S. Biodegradation and biocompatibility of PLA and PLGA microspheres. **Advanced Drug Delivery Reviews**, v.28, p.5-24, 1997.

AUTIO-GOLD J. The Role of Chlorhexidine in Caries Prevention. **Operative Dentistry**, v.33, n.6, p.710-716, 2008.

BRUCK, S. D. Pharmacological basis of controlled drug delivery. In: **Controlled drug delivery**, Bruck, S. D. (ed.): Boca Raton, FL: CRC Press, v.1, p.1-13, 1983.

CORREIA, R. C.; JOZALA A. F.; MARTINS, K. F.; PENNA, T. C. V.; DUEK, E. A. R.; RANGEL-YAGUI, C. O.; LOPES, A. M. Poly(lactic-co-glycolic acid) matrix incorporated with nisin as a novel antimicrobial biomaterial. **World Journal of Microbiology and Biotechnology**, v.31, p.649–659, 2015.

DELANY, G.M.; PATTERSON, S.S.; MILLER, C.H.; NEWTON, C. W. The effect of chlorhexidine gluconate irrigation on the root canal flora freshly extracted necrotic teeth. **Oral surgery, Oral medicine, Oral pathology**, v.53, n.5, p.518-523, 1982.

DEEPALAKSHMI M.; POORNI S.; MIGLANI R.; RAJAMANI I.; RAMACHANDRAN S. Evaluation of the antibacterial and physical properties of glass ionomer cements containing chlorhexidine and cetrimide: an in-vitro study. **Indian Journal Dental Research**, v.21, n.4, p.552-556, 2010.

EMILSON, C. G. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. **Journal of Dental Research,** v.3, p.682-691, 1994.

FARRUGIA, C.; CAMILLERI, J. Antimicrobial properties of conventional restorative filling materials and advances in antimicrobial properties of composite resins and glass ionomer cements-A literature review. **Dental Materials**, v.31, p.89-99, 2015.

FEATHERSTONE, J. D. Delivery challenges for fluoride chlorhexidine and xylitol. **BHC Oral Health**, v.6 (Suplemento1): S8, 2006.

FORSTEN, L. Fluoride release and uptake by glass-ionomers and related materials and its clinical effect. **Biomaterials**, v.19, p.503-508, 1998.

FRENCKEN, J. E.; PILOT, T.; SANGPAISAN, Y.;PHANTUMVANIT, P. Atraumatic Restorative Treatment (ART): rationale, technique and development. **Journal of Public Health Dentistry**, v.56, p.135-140, 1996.

GLASSPOOLE, E. A.; ERICKSON, R. L., DAVIDSON, C. L. A fluoride releasing composite for dental applications. **Dental Materials**, v.17, p.127-133, 2001

GUPTA, D.; NAYAN, S.; TIPPANAWAR, H. K.; PATIL, G. I.; JAIN, A.; MOMIN R. K.; GUPTA R. K. Are herbal mouth wash efficacious over chlorhexidine on the dental plaque? **Pharmacognosy Research**, v.7, n.3, p.277–281, 2015.

HAMILTON, I. R. Biochemical effects of fluoride on oral bacteria**. Jornal of Dental Research,** v. 69 (Spec No), p.660-667; discussion p. 682-683, 1990.

HATTON, P. V.; HURRELL-GILLINGHAM, K.; BROOK, I. M. Biocompatibility of glass ionomer bone cements**. Journal of Dentistry**, v.34, p. 598- 601, 2006.

HENNESSEY, T. D. Some antibacterial properties of chlorhexidine. **Journal of Periodontal Research**, v.8, n.12, p.61-67, 1973.

JAIN, A. R. The manufacturing techniques of various drug loaded biodegradable poly (lactide-*co*-glycolide) (PLGA) devices. **Biomaterials**, v.21, p. 2475-2490, 2000.

JI, Y.; XU, G. P.; ZHANG, Z. P.; XIA, J. J.; YAN, J. L.; PAN, S. H. BMP-2/PLGA delayed-release microspheres composite graft, selection of bone particulate diameters, and prevention of aseptic inflammation for bone tissue engineering. **Annals of Biomedical Engineering**, v. 38, n.3, p.632-639, 2010.

KOO, H.; HAYACIBARA, M. F.; SCHOBEL, B. D.; CURY, J. A.; ROSALEN, P. L.; PARK, Y. K.; VACCA-SMITH, A. M.; BOWEN, W. H. Inhibition of Streptococcus mutans biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. **Journal of Antimicrobial Chemotherapy**, v.52, n.5, p.782-789, 2003.

KOUIDHI, B., AL QURASHI, Y. M. A., CHAIEB, K. Drug resistance of bacterial dental biofilm and the potential use of natural compounds as alternative for prevention and treatment. **Microbial Pathogenesis**, v.80, p.39-49, 2015.

KRZYŚCIAK, W.; JURCZAK, A.; KOŚCIELNIAK, D.; BYSTROWSKA, B.; SKALNIAK, A. The virulence of Streptococcus mutans and the ability to form biofilms. **European Journal of Clinical Microbiology Infectious Diseases,** v.33, p.499–515, 2014.

LIN, A.; McINTYRE, N. S.; DAVIDSON, R. D. Studies on the adhesion of glass-ionomer cements to dentin. **Journal of Dental Research**, v.71, p.1836-1841, 1992.

LULA, E. C.; MONTEIRO-NETO, V.; ALVES, C. M.; RIBEIRO C. C. Microbiological analysis after complete or partial removal of carious dentin in primary teeth: a randomized clinical trial. **Caries Research,** v.43, n.5, p.354-358, 2009.

MAKADIA, H. K., SIEGEL, S. J. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. **Polymers**, v.3, p.1377-1397, 2011.

MARINHO, V.C.C.; WORTHINGTON, H. V.; WALSH, T.; CLARKSON, J. E. Fluoride varnishes for preventing dental caries in children and adolescents (Review). **Cochrane Database of Systematic Reviews**, 2013.

MARSH, P. D.; HEAD, D. A.; DEVINE, D. A. Ecological Approaches to Oral Biofilms: Control without killing. **Caries Research**, v.49, n.1, p.46-54, 2015.

MARTINS, L. R. M.; SILVA, A. L. F.; CURY, J. A.; FRANCISCHONE, C. E. Liberação de flúor de restaurações de ionômero de vidro e a sua incorporação ao esmalte dental após ciclos de desmineralização/remineralização. **Revista Odonto Ciência**, v.21, p.30-36, 2006.

MOSHAVERINIA, A.; ROOHPOUR, N.; CHEEA, W. W. L.; SCHRICKER, S. R. A review of powder modifications in conventional glass-ionomer dental cements. **Journal of Materials Chemistry**, v. 21, p.1319-1328, 2011.

NAASAN, M. A.; WATSON, T. F. Conventional glass ionomers as posterior restorations. A status report for the American Journal of Dentistry. **American Journal of Dentistry**, v. 11, p.36-45, 1998.

NICHOLSON, J. W.; CZARNECKA, B. The biocompatibility of resin modified glass ionomer cements for dentistry. **Dental Materials**, v. 24, p.1702-1708, 2008.

OLIVEIRA, T. M.; SAKAI, V. T.; SILVA, T. C.; MORETTI, A. B. S.; PASSOS, V. A. B.; TESSAROLLI, V.; MACHADO, M. A. A. M. Efeito do gel de clorexidina em diferentes concentrações no controle do biofilme bacteriano e da inflamação gengival em crianças. **Revista da Faculdade de Odontologia de Porto Alegre**, v.50, n.1, p.29-33, 2009.

PETERS, M. C.; McLEAN, M. E. Minimally invasive operative care. Part I: minimum intervention and concepts for minimally invasive cavities. **Journal of Adhesive Dentistry**, v.3, p.7-16, 2001.

PETERSEN, P. E.; BOURGEOIS, D.; OGAWA, H.; ESTUPINAN-DAY, S.; NDIAYE, C. The global burden of oral diseases and risks to oral health. **Bulletin of the World Health Organization**, v.83, p. 661-669, 2005.

SANDERS, B. J.; GERGORY, R. L.; MOORE, K.; AVERY, D. R. Antibacterial and physical properties of resin modified glass-ionomers combined with chlorhexidine. **Journal Oral Rehabilitation**, v.29, p.553-558, 2002.

SCHNIEDERS, J.; GBURECK, U.; THULL, R., KISSEL, T. Controlled release of gentamicin from calcium phosphate-poly (lactic acid-co-glycolic acid) composite bone cement. **Biomaterials**, v.27, n.23, p. 4239-4249, 2006.

SEPPÄ, L.; KORHONEN, A.; NUUTINEN, A. Inhibitory effect on S. mutans by fluoride-treated conventional and resin-reinforced glass ionomer cements**. European Journal of Oral Sciences,** v.103, p. 182-185, 1995.

SENADHEERA, M. D.; GUGGENHEIM, B.; SPATAFORA, G. A.; HUANG, Y. C.; CHOI, J.; HUNG, D. C. I.; TREGLOWN, J. D.; GOODMAN, S. D.; ELLEN, R. P.; CVITKOVICH, D. G. A. VicRK Signal Transduction system in Streptococcus mutans affects gtfBCD, gbpG, and ftf expression, biofilm formation and genetic competence development. **Journal of Bacteriology**, v.187, n.12, p. 4064-4076, 2005.

SIDHU, S. K. Glass-ionomer cement restorative materials: a sticky subject? **Australian Dental Journal**, v.56, n.1, p. 23-30, 2011.

SVENSATER, G.; WELIN, J.; WILKINS, J.; BEIGHTON, D.; HAMILTON, I.R. Protein expression by planktonic and biofilm cells of *Sreptococcus mutans*. **FEMS Microbiology Letters,** v.205, p.139-146, 2001.

TAKAHASHI, Y.; IMAZATO, S.; KANESHIRO, A. V.; EBISU, S.; FRENCKEN, J. E; TAY, F. R. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. **Dental Materials,** v. 22, n.7, p.647-652, 2006.

TÜRKÜN L. S; TÜRKÜN M.; ERTUGRUL F.; ATES M.; BRUGGER S. Long-term antibacterial effects and physical properties of a chlorexidine-containing glass ionomer cement. **Journal of Esthetic and Restorative Dentistry**, v.20, n.1, p.29-44, 2008.

TÜZÜNER, T.; KUSGÖZ, A.; KÜRSAT, E. R.; TASDEMIR, T.; BURUK, K.; KEMER, B. Antibacterial activity and physical properties of conventional glass-ionomer cements containing diacetate/cetrimide mixtures. **Journal of Esthetic and Restorative Dentistry**, v.23, p.46–56, 2011.

VAN NOORT R. Glass-ionomer cements and resin-modified glass-ionomer cements, Introduction to dental materials, Edinburgh, UK: Mosby; v.2, p.129-130, 2002.

VAN RIJKOM, H. M.; TRUIN, C. J.; VANT'T HOF, M. A. A MTA-analysis of clinical studies on the caries-inhibiting effect of chlorhexidine treatment. **Journal of Dental Research,** v.75, p.790-795, 1996.

VERMEERSCH, G.; LELOUP, G.; VREVEN, J. Fluoride release from glass-ionomer cements, compomers and resin composites. **Jornal of Oral Rehabilitation**, v.28, p.26-32, 2001.

VOLPATO, F. C.; JEREMIAS, F.; SPOLIDÓRIO, D. M. P.; CORRÊA DA SILVA, S. R, VALSECKI JUNIOR, A.; ROSELL, F. L. Effects of oral environment stabilization procedures on *Streptococcus mutans* counts in pregnant women. **Brazilian Dental Journal,** v.22, n.4, p.280-284, 2011.

WEERHEIJM, K. L.; KREULEN, C. M.; DE SOET, J. J.; GROEN, H. J.; VAN AMERONGEN, W. E. Bacterial counts in carious dentine under restorations: 2-year in vivo effects. **Caries Research**, v.33, p.130-134, 1999.

WEISER, J. R.; SALTZMAN, W. M. Controlled release for local delivery of drugs: barriers and models. **Journal of Controlled Release**, v.190, p. 664-673, 2014.

WIEGAND, A.; BUCHALLA, W.; ATTIN, T. Review on fluoride-releasing restorative materials – Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. **Dental Materials**, v.23, p.343-362, 2007.

WU, X.S.; WANG, N. Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers. Part II: Biodegradation. **Journal of Biomaterials Science, Polymer Edition**, v.12, p.21-34, 2001.

YAP, A. U.; TAN, A. C.; GOH, A. T.; GOH, D. C.; CHIN, K. C. Effect of surface treatment and cement maturation on the bond strength of resin-modified glass ionomers to dentin. **Operative Dentistry**, v.28, p.728-733, 2003.

YLI-URPO, H.; LASSILA, L. V. J.; NARHI, T.; VALLITTU, P. K. Compressive strength and suface caracterization of glass ionomer cements modified by particles of bioactive glass. **Dental Materials, v.**21, p.201-209, 2005.

ZHANG, Q.; VAN PALENSTEIN HELDERMAN, W. H.; VAN'T HOF, M. A.; TRUIN, G. J.Chlorhexidine varnish for preventing dental caries in children, adolescents and young adults: a systematic review. **European Journal of Oral Sciences**, v.114, n. 6, p.449–455, 2006.

ANEXO

NORMAS DO PERIÓDICO JOURNAL OF BIOMEDICAL MATERIALS RESEARCH. PART A

Aims and Scope

The Journal of Biomedical Materials Research Part A is an international, interdisciplinary, English-language publication of original contributions concerning studies of the preparation, performance, and evaluation of biomaterials; the chemical, physical, toxicological, and mechanical behavior of materials in physiological environments; and the response of blood and tissues to biomaterials. The Journal publishes peer-reviewed articles on all relevant biomaterial topics including the science and technology of alloys,polymers, ceramics, and reprocessed animal and human tissues in surgery,dentistry, artificial organs, and other medical devices. The Journal also publishes articles in interdisciplinary areas such as tissue engineering and controlled release technology where biomaterials play a significant role in the performance of the medical device.

The Journal of Biomedical Materials Research is the official journal of the Society for Biomaterials (USA), the Japanese Society for Biomaterials, the Australasian Society for Biomaterials, and the Korean Society for Biomaterials.

Articles are welcomed from all scientists. Membership in the Society for Biomaterials is not a prerequisite for submission.

Instructions for Manuscript Preparation

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2. Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons; 1990. 1223 p.
3. Gilmor ML, Rouse ST, Heilman CJ, Nash NR, Levey AI. Receptor fusion proteins and analysis. In: Ariano MA, editor. Receptor localization. New York: Wiley-Liss; 1998. p 75-90. Please note that journal title abbreviations should conform to the practices of Chemical Abstracts.

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