UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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ANÁLISE DAS PROPRIEDADES DE MATERIAIS INFILTRANTES EM FUNÇÃO DA COMPOSIÇÃO: MONÔMEROS BASE E ANTIMICROBIANO

Dissertação de Mestrado apresentada a Faculdade de Odontologia de Piracicaba, da UNICAMP, para obtenção do título de Mestre em Odontologia, na área de Odontopediatria

Orientadora: Profa. Dra. Regina Maria Puppin-Rontani

Este exemplar corresponde à versão final da Dissertação defendida pelo aluno, e orientada pela Profa. Dra. Regina Maria Puppin-Rontani.

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"O valor das coisas não está no tempo em que elas duram,
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Por isso existem momentos inesquecíveis,
coisas inexplicáveis e pessoas incomparáveis."
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RESUMO

Esta dissertação foi dividida em dois capítulos: **Capítulo 1** avaliou a atividade antimicrobiana de misturas não polimerizadas e polimerizadas por meio da mensuração da zona de inibição após difusão em ágar (Pour Plate); e determinou a Concentração Inibitória Mínima (CIM) e Concentração Mínima Bactericida (CMB) de misturas resinosas experimentais. Os ensaios microbiológicos foram realizados em triplicata, sendo o digluconato de clorexidina 0,12% utilizado como referência (antibacteriano padrão) e o infiltrante Icon® (DMG) utilizado como controle comercial. Cepas de *Streptococus mutans* UA159 e *Lactobacillus acidophilus* LYO50DCU-S foram utilizadas em todos os testes. **Capítulo 2** determinou o grau de conversão e a dureza Knoop das misturas experimentais tendo como controle comercial o infiltrante Icon®. As misturas utilizadas nos ensaios experimentais foram: TEGDMA, TEGDMA/CHX 0,1%, TEGDMA/CHX 0,2%, TEGDMA/UDMA, TEGDMA/UDMA/CHX 0,1%, TEGDMA/UDMA/CHX 0,2%, TEGDMA/BisEMA, TEGDMA/BisEMA/CHX 0,1% e TEGDMA/BisEMA/CHX 0,2%. Os testes estatísticos utilizados nos ensaios de ambos os estudos foram ANOVA um critério seguido por teste de Tukey, e teste-t para comparação entre os grupos ($p<0,05$). O CIM e CMB mostraram que as misturas demonstraram ter atividade antibacteriana com baixas concentrações de CHX para as duas cepas bacterianas testadas. Antes da polimerização, a atividade antibacteriana da maioria das misturas foi maior que o Icon®. Para *S. mutans,* as misturas TEGDMA/BisEMA/CHX 0,1% e TEGDMA/BisEMA/CHX 0,2%, a concentração de CHX foi fator relevante para aumentar a zona de inibição. Após a polimerização, a mistura TEGDMA/UDMA/CHX 0,1% mostrou maior zona de inibição para *S. mutans*. Para o *L. acidophilus*, a atividade antibacteriana antes da polimerização foi maior para TEGDMA/CHX 0,2% e TEGDMA/UDMA/CHX 0,1%, independente da concentração de CHX. Após a polimerização, todas as misturas experimentais e Icon® não apresentaram atividade antibacteriana. Quando as zonas de inibição das misturas polimerizadas e não polimerizadas foram comparadas, a maioria das

misturas não polimerizadas apresentaram maior efeito antibacteriano para as duas cepas. A adição de CHX não reduziu o DC das misturas; contudo, para as misturas a base de TEGDMA, o CHX influenciou positivamente e causou o aumento do DC. As misturas TEGDMA/UDMA, TEGDMA/UDMA/CHX 0,1% e TEGDMA/UDMA/CHX 0,2% apresentaram os maiores valores de DC e as misturas TEGDMA/BisEMA, TEGDMA/BisEMA/CHX 0,1% e TEGDMA/BisEMA/CHX 0,2% os menores valores de DC. Todas as misturas apresentaram valores de DC menores que o Icon®. Em relação à microdureza, TEGDMA/UDMA e TEGDMA/UDMA/CHX 0,2% apresentaram os maiores valores de dureza Knoop. A adição de CHX não afetou a dureza de superfície das misturas experimentais. Quando comparadas ao Icon®, todas as misturas apresentaram maior dureza Knoop. Assim, dentre as misturas avaliadas, a mistura TEGDMA/UDMA/CHX 0,1% apresentou os melhores resultados para o DC e para a atividade antibacteriana após polimerização.

PALAVRAS-CHAVE: Cárie dentária, Clorexidina, Dureza, Espectroscopia Infravermelho Transformada de Fourier

ABSTRACT

This dissertation was divided into two chapters: **Chapter 1** aimed to evaluate the antimicrobial activity of cured and uncured resin blends though measurement of inhibition zone using agar diffusion (Pour Plate), Minimal Inhibiting Concentration (MIC) and Minimum Bactericidal Concentration (MBC). For microbiological assays, that were performed in triplicate, the 0.12% chlorhexidine digluconate solution was used as reference standard antimicrobial and infiltrant Icon® (DMG) was used as commercial control group. *Streptococcus mutans* UA159 and *Lactobacillus acidophilus* LYO50DCU-S strains were selected for all assays. **Chapter 2** aimed to determine DC and Knoop hardness of mixtures having Icon® as commercial control group. Mixtures were set as follow: TEGDMA, TEGDMA/0.1% CHX, TEGDMA/0.2% CHX, TEGDMA/UDMA, TEGDMA/UDMA/0.1% CHX, TEGDMA/UDMA/0.2% CHX, TEGDMA/BisEMA, TEGDMA/BisEMA/0.1% CHX and TEGDMA/BisEMA/0.2% CHX. Data obtained from all mixtures and Icon® were submitted to one-way ANOVA, followed by Tukey test, and in order to compare the groups t-test was used ($p<0.05$). The MIC and MBC tests showed that the mixtures demonstrated antibacterial activity in low concentrations of CHX against both of strains. Analyzing antibacterial activity against *S. mutans* before light curing process, the most of blends provided larger inhibition zones than Icon®, and for TEGDMA/BisEMA/0.1% CHX and TEGDMA/BisEMA/0.2% CHX the CHX concentration was significant factor to increase the inhibition zones. After light curing, the mixture TEGDMA/UDMA/0.1% CHX showed the highest inhibition zone against *S. mutans*. Analyzing antibacterial activity against *L. acidophilus* before light curing, the addition of CHX to the blends, regardless concentration, provided inhibition zones and TEGDMA/0.2% CHX and TEGDMA/UDMA/0.1% CHX showed the highest antibacterial effects. After light curing, no significant difference between all experimental blends, including Icon®, was observed. When the inhibition zones of uncured and cured blends were compared, the major of uncured blends

demonstrate grater antimicrobial activity to both strains. The addition of CHX didn't reduce the DC of experimental infiltrants blends, but CHX had positive influence for TEGDMA neat monomer, increasing DC. TEGDMA/UDMA, TEGDMA/UDMA/0.1% CHX and TEGDMA/UDMA/0.2% CHX showed the highest DC than other mixtures, while TEGDMA/BisEMA, TEGDMA/BisEMA/0.1% CHX and TEGDMA/BisEMA/0.2% CHX the lowest DC. All mixtures showed significant lower values of DC than commercial infiltrant. Concerning hardness, TEGDMA/UDMA and TEGDMA/UDMA/0.2% CHX showed the highest Knoop hardness values. The addition of CHX didn't change surface hardness of mixtures. When Knoop hardness values of nine mixtures were compared with Icon®, all mixtures showed significant higher values than commercial infiltrant. Thus, among the experimental resin mixtures evaluated, TEGDMA/UDMA/0.1% CHX showed the best results to DC and to antimicrobial effect after polymerization.

KEY WORDS: Chlorhexidine, Dental caries, Hardness, Spectroscopy Fourier Transform Infrared

SUMÁRIO

INTRODUÇÃO

A lesão de cárie se inicia a partir de um desequilíbrio iônico, observado entre o esmalte e a saliva, produzido pelo biofilme cariogênico aderido à superfície do esmalte. Os estágios iniciais da dissolução do esmalte envolvem uma distinta desintegração da superfície, com espaços intercristalinos mais ampliados levando a formação de microcavidades (Fejerskov *et al*., 2005). As lesões de cárie se desenvolvem nos locais adequados para os depósitos bacterianos como regiões interproximais, margem gengival e superfícies oclusais de dentes posteriores (Fejerskov *et al*., 2005). Durante o desenvolvimento da lesão cariosa o mineral é removido da estrutura do esmalte, deixando-o com porosidades, visualmente reconhecidas como manchas brancas opacas (Robinson *et al*., 2001; Fejerskov *et al*., 2005).

Na lesão de mancha branca a zona superficial do esmalte aparece relativamente intacta, com menor perda de minerais (por volta de 8%), enquanto nas camadas subjacentes a perda pode variar de 20 a 90% e o volume dos poros se encontra muito aumentado (Bergman & Lind, 1966). Essa hipermineralização da camada superficial da lesão de mancha branca torna esse esmalte mais resistente ao condicionamento ácido (Lee *et al*., 1995). Com o intuito de evitar a remoção de tecido lesionado e sadio, métodos menos invasivos como a aplicação tópica de fluoretos e instrução de higiene bucal ao paciente são frequentemente utilizados na tentativa de promover a remineralização dessas lesões iniciais de cárie (Mejàre *et al*., 1998; Paris *et al*., 2006). Contudo, são práticas que necessitam da colaboração do paciente para que haja sucesso no tratamento (Robinson et al., 2001).

O uso de selantes nas fóssulas e fissuras, principalmente em molares e pré-molares recém-irrompidos, tem sido uma medida muito eficaz na prevenção da cárie dentária (Mejàre *et al*., 2003; Martignon *et al*., 2006). A partir do princípio

do selamento oclusal, pensou-se numa alternativa para o tratamento das lesões iniciais não cavitadas em esmalte, inicialmente para as superfícies proximais, na tentativa de paralisá-las (Garcia-Godoy *et al*., 1997 e Gray & Shellis, 2002). Os selantes, quando aplicados diretamente sobre lesões de cárie de fissuras, diminuem a quantidade de microrganismos no local somente se mantiverem intactos (Feigal, 2002; Simonsen, 2002). Isso dificilmente ocorre porque os prolongamentos resinosos produzidos a partir do condicionamento ácido da superfície do esmalte não proporcionam um eficiente vedamento dos poros da lesão (Feigal, 2002; Simonsen, 2002; Paris *et al*., 2006; Hevinga *et al*., 2007). A camada de resina inserida à zona superficial da cárie, promovida pelos selantes, não impede a progressão da lesão; além do fato da presença de excessos marginais servirem como meios de retenção de placa bacteriana e propiciar o desenvolvimento de novas lesões cariosas (Feigal, 2002; Simonsen, 2002; Paris *et al*., 2006; Hevinga *et al*., 2007; Paris *et al*., 2007a).

Os poros do esmalte cariado são como um caminho para a difusão de ácidos e minerais dissolvidos. No entanto, a obstrução desses poros pela infiltração de um material resinoso pode cessar a progressão da lesão cariosa e estabilizar mecanicamente a estrutura frágil do esmalte comprometido. Paris *et al*. (2006) e Paris *et al*. (2007a) afirmaram que a penetração de um material resinoso altamente fluido nos poros do corpo da lesão é fator determinante para que a inibição da progressão da lesão seja efetiva, além de fornecer a esse esmalte mais poroso um reforço mecânico estrutural. O termo “infiltrante” foi estabelecido por Paris *et al*., (2007c) para diferenciar esses materiais de selantes de fóssulas e fissuras e de sistemas adesivos.

A paralização da progressão da cárie incipiente por meio de materiais infiltrantes, como monômeros resinosos fotoativados, é uma alternativa para a Odontologia menos invasiva. Muitos trabalhos científicos comprovaram a capacidade de infiltração em lesões naturais e artificiais de esmalte por meio de adesivos comercialmente utilizados (Davila *et al*., 1975; Robinson *et al*., 1976;

Robinson *et al*., 2001; Mueller *et al*., 2006; Meyer-Lueckel *et al*. 2006; Paris *et al*., 2007b; Paris *et al.,* 2009).

A utilização de monômeros resinosos é um passo importante para a interceptação da lesão de cárie em seus estágios iniciais; e para essa função há a necessidade de se utilizar um material com alta capacidade de penetração nos poros da lesão cariosa que, ao se polimerizar, produza o fortalecimento da área mais porosa do esmalte (Kantovitz *et al*., 2010) . De acordo com os mesmos autores, selantes de fóssulas e fissuras e sistemas adesivos comerciais foram utilizados para essa finalidade, entretanto, não tiveram a capacidade de penetrar adequadamente na lesão, pois a fluidez do material está diretamente relacionada com a capacidade de penetração, isto é, quanto mais fluido o material maior o coeficiente de penetração.

Para a melhor penetração dos infiltrantes nas lesões cariosas naturais não cavitadas há a necessidade de condicionamento prévio para a erosão da camada superficial que é contaminada por água e substâncias orgânicas (Meyer-Lueckel *et al*., 2007; Meyer-Lueckel & Paris, 2008; Paris & Meyer-Lueckel, 2010). Num estudo realizado por Paris *et al*. (2007b) os autores avaliaram a penetração de adesivo no esmalte dental após 120 segundos de condicionamento ácido com ácido fosfórico a 37% e ácido clorídrico a 15%, tendo este último apresentado os melhores resultados.

A influência do coeficiente de penetração e da adição de solventes na penetrabilidade das resinas de baixa viscosidade foi verificada por Meyer-Lueckel & Paris (2010) num estudo *in vitro* que mostrou que resinas de baixa viscosidade como o TEGDMA (Trietilenoglicol Dimetacrilato), com maior coeficiente de penetração, são capazes de penetrar em até 100 µm nas lesões naturais de cárie. Entretanto, a adição de etanol ao TEGDMA resultou em ligeira diminuição em relação à profundidade de penetração quando comparada à lesão cariosa artificial. Este efeito, de acordo com os autores, pode ter sido causado pela polimerização

incompleta do material nas partes mais profundas da lesão, onde a intensidade de polimerização da luz foi baixa. Deste estudo pode-se concluir que os materiais com alto coeficiente de penetração (infiltrantes), baseados principalmente em TEGDMA, são capazes de penetrar profundamente no corpo das lesões naturais de cárie.

Araujo (2010), Araújo (2011) e Sfalcin (2011) também mostraram em seus estudos que o etanol e o HEMA (Metacrilato de Hidróxi Etila) incorporados nas misturas monoméricas à base de TEGDMA, UDMA (Uretano Dimetacrilato) e BisEMA (Bisfenol A Glicidil Dimetacrilato Etoxilado) afetaram negativamente as características dos infiltrantes. Para Araujo (2010), o etanol e o HEMA, apesar de diminuirem a viscosidade desses materiais, reduziram o grau de conversão monomérico, a dureza e o modo de elasticidade dos mesmos. Araujo (2010), Araújo (2011) e Sfalcin (2011) afirmaram que as misturas sem adição de solventes apresentaram os melhores resultados quanto ao grau de conversão, ao grau de penetração, a densidade de ligações cruzadas, ao módulo de elasticidade e à resistência de união.

A adição de agentes antibacterianos aos materiais restauradores resinosos pode diminuir ou impedir a adesão de biofilme na superfície do material polimerizado e, dessa forma, evitar que áreas adjacentes ao esmalte infiltrado sejam acometidas por novos processos de desmineralização (Yoshida *et al.*, 1999; Bürgers *et al.*, 2009; de Fúcio *et al.*, 2009; Aydin Sevinç & Hanley, 2010).

A clorexidina é uma molécula catiônica simétrica que consiste de dois anéis 4-clorofenis e dois grupos biguanidas ligados à cadeia central de hexametileno e, sendo uma base forte, é mais estável na forma de sal. Devido às suas propriedades catiônicas, a clorexidina se liga à hidroxiapatitia do esmalte dentário, à película adquirida na superfície dentária, às proteínas salivares, às bactérias e às proteínas extracelulares de origem bacteriana; por isso possui amplo espectro de ação contra cepas gram-positivas e gram-negativas, além de

fungos, anaeróbios facultativos e aeróbios (Fardal & Turnbull, 1986). Entre as bactérias gram-positivas, os *Streptococcus mutans* são particularmente mais sensíveis à clorexidina que as espécies *Lactobacillus sp* (Emilson, 1994).

O mecanismo de ação da clorexidina reside na capacidade de se adsorver na parede celular do microrganismo que provoca a liberação de componentes intracelulares. Em baixas concentrações, a clorexidina provoca a liberação de substâncias com baixo peso molecular, como potássio e fósforo, exercendo um efeito bacteriostático. Por outro lado, em altas concentrações, a clorexidina possui efeito bactericida devido à precipitação e coagulação do citoplasma, provavelmente causado por ligações cruzadas protéicas (Fardal & Turnbull, 1986). A inibição da formação do biofilme dentário pela clorexidina pode ser explicada, de acordo com Ribeiro *et al*. (2008), pelo fato da clorexidina desativar a enzima glicosiltransferase secretada pelo *Streptococcus mutans*, importante na aderência bacteriana à superfície dentária; e também por deslocar cálcio dos grupos sulfatos, desintegrando o biofilme já estabelecido. Assim, devido ao seu amplo espectro de ação, a clorexidina tem sido usada no tratamento e prevenção de doenças periodontais e cárie dentária (Emilson, 1994; van Rijkom *et al*., 1996; Autio-Gold, 2008). A clorexidina, para a prevenção da cárie dentária, tem sido utilizada em várias formulações como enxaguatórios bucais, géis e vernizes, sendo estes últimos considerados os mais eficazes (Autio-Gold, 2008).

A adição de clorexidina aos materiais restauradores como sistemas adesivos e cimentos ionoméricos tem apresentado inibição do crescimento de colônias bacterianas na interface dente/restauração, além disso, quando incorporada aos sistemas adesivos utilizados na colagem de braquetes, mostrou ter bons resultados quanto à resistência ao cisalhamento (Damon *et al.,* 1997; Bishara *et al*., 1998; Ribeiro *et al*., 2008). Estudos têm sido realizados com sais de clorexidina (digluconato de clorexidina e diacetato de clorexidina) adicionados aos cimentos de ionômero de vidro convencionais, aos cimentos de ionômero de vidro modificados e aos materiais resinosos com o intuito de aumentar a efetividade

clínica por meio da atividade antibacteriana (Riggs *et al*., 2000; Leung *et al*., 2005; Cacciafesta *et al*., 2006; Hiraishi *et al*., 2008; Mehdawi *et al*., 2009; Castilho, 2010; Hiraishi *et al*., 2010; Tüzüner *et al*., 2011).

Assim, diante dos trabalhos científicos consultados, a incorporação de antimicrobianos, como a clorexidina, na composição dos infiltrantes poderia aumentar a efetividade desses materiais quanto à atividade antimicrobiana, principalmente em relação aos microrganismos cariogênicos residuais presentes nas lesões cariosas incipientes, e ainda diminuir a colonização bacteriana do biofilme sobre a área infiltrada.

Com base nos pressupostos descritos, este estudo teve como objetivos[1]:

1 – Avaliar o efeito antibacteriano *in vitro* de nove misturas resinosas experimentais contendo como base os monômeros TEGDMA, UDMA e BisEMA com adição de duas concentrações diferentes de diacetato de clorexidina;

2 – Avaliar o grau de conversão e dureza Knoop de nove misturas resinosas experimentais contendo como base os monômeros TEGDMA, UDMA e BisEMA, comparando-as com um infiltrante disponível comercialmente.

[1] Esta dissertação de mestrado foi realizada no formato alternativo, com base na resolução da CCPG/002/06, a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP.

CAPÍTULO 1[2]

Antibacterial properties of experimental resin materials with infiltrant characteristics

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ABSTRACT

The aim of this study was to evaluate the antibacterial properties of experimental infiltrants with addition of chlorhexidine diacetate salt (CHX). Nine blends were prepared: TEGDMA, TEGDMA/0.1% CHX, TEGDMA/0.2% CHX, TEGDMA/UDMA, TEGDMA/UDMA/0.1% CHX, TEGDMA/UDMA/0.2% CHX, TEGDMA/BisEMA, TEGDMA/BisEMA/0.1% CHX, TEGDMA/BisEMA/0.2% CHX. The 0.12% chlorhexidine digluconate solution was used as reference standard antimicrobial

[2] Trabalho submetido ao periódico **Journal Biomedical Materials Research Part A** (Anexo)

and Icon® (DMG) was used as commercial control group. In order to measure the antibacterial activity of the blends, the Minimal Inhibiting Concentration (MIC) and Minimal Bactericidal Concentration (MBC) assays were conducted. Inhibition zone was measured using agar diffusion assay (Pour Plate) for cured and uncured blends. The strains of *Streptococcus mutans* UA159 *a*nd *Lactobacillus acidophilus* LYO50DCU-S were selected for all assays which were performed in triplicate. Data were submitted to ANOVA and t-test ($p < 0.05$). In the MIC and MBC assays, mixtures containing CHX showed no bacterial growth in lower concentrations after dilutions. In the Pour Plate assay, uncured blends showed larger inhibition zone than cured blends for both strains. TEGDMA/UDMA/0.1% CHX showed the highest antibacterial effect after cured against *S. mutans.* Addition of CHX increases the bactericidal properties of resin blends. Polymerization can affect the antimicrobial activity of some blends.

Key words: Dental caries, Infiltrant, Chlorhexidine, Antibacterial, Dimethacrylate monomers

INTRODUCTION

Non invasive approaches using adhesive materials are currently used to arrest incipient caries such light curing infiltrant resin monomers. It can be an alternative to less invasive dentistry. Many scientific studies confirmed the infiltration capacity of natural and artificial lesions in enamel by adhesive systems used commercially, but the porous surface of enamel caries lesion haven´t been filled completely.[1-3] To obtain deep penetration of the porous layer, the surface have been etched with 15% of hydrochloric acid gel for 2 min and filled with low viscosity light curing resins solvent-free having triethylene glycol dimethacrylate (TEGDMA) as the main constituent. [3-5]

The TEGDMA, urethane dimethacrylate (UDMA) and ethoxylated bisphenol A glycol dimethacrylate (BisEMA) show a significantly lower viscosity when compared with 2,2 - bis [4 - (3 – methacryloyloxy – 2 – hydroxypropoxy) phenyl] propane (Bis-GMA).[6,7] BisEMA is a monomer with a structure almost identical to Bis-GMA, except for the fact that hydroxyl groups are not present, providing decreased viscosity values.[7] UDMA and TEGDMA are the most frequently used cross-linkers in adhesive systems because these di-methacrylates exhibit flexibility properties which compensate the rigidity of Bis-GMA.[8] Due to these characteristics, TEGDMA, UDMA and BisEMA could be used in experimental infiltrants composition, providing a low viscosity and high level of crosslink that assure penetration and mechanical properties.

The infiltration of caries lesions with low-viscosity light-curing resins (infiltrants) has been shown to slowdown further demineralization *in vitro*[5] but not hamper that. The mineral plots average shows that the mineral dissolution was slightly higher beneath the surface, but affected the whole lesion body. This observation might be attributed to a partial dissolution of remaining mineral in lesion body that isn't completely embedded within the resin matrix.[9] Moreover, cleft-like structures that have previously been described, probably caused by material shrinkage during light curing, might cause leakage and thus reduce the acid resistance.[9] Actually, it could be shown that a repeated application of resin can reduce this leakage[1] but not forbids it.

In development of caries lesions *S. mutans* and *Lactobacillus sp* are considerable the major dental pathogens.[10] The meaning for adding chlorhexidine diacetate salt (CHX) in low viscosity experimental monomer blends with infiltrant characteristics was based in studies that incorporated antimicrobials into materials like glass-ionomer cements, resin-modified glass-ionomer cements and methacrylates to improve and/or extend the antimicrobial properties of these materials against cariogenic bacteria.[11-13]

The addition of soluble antimicrobials into resin matrix is a way to release the agent from the materials in a wet environment as oral one, and chlorhexidine is the most frequently used.[10,14] The addition of an antibacterial agent would mean an improving in ability of arresting incipient caries lesions and to inhibit plaque accumulation on surface of materials and tooth around the restoration.[15] A little inhibition of caries-associated bacteria was also observed in few brands of restorative resins when uncured specimens were tested, but antibacterial activities haven't been demonstrated after being cured.[16] However, the addition of CHX in light cured glass-ionomer showed increased antibacterial properties for a period of three weeks.[17]

The chlorhexidine have been described as the gold standard for antibacterial application because it's wide spectrum of action.[10] At low chlorhexidine concentrations, small molecular weight substances, such as potassium and phosphorus, will leach out, exerting a bacteriostatic effect; and in higher concentrations, chlorhexidine have bactericidal action because precipitation or coagulation of cytoplasm, probably caused by protein cross-linking.[18] Chlorhexidine can be released in relatively high percentages from various methacrylate polymers and bone cements.[11] The rate of CHX release is faster when it has been incorporated in hydrophilic resins that have greater sorption of water than hydrophobic resins.[12] The mechanism for CHX releasing from methacrylates hasn't already elucidated. However, it is known that it is dependent of monomer blend hidrophilicity.[12]

Like this, the incorporation of antimicrobial agents such as chlorhexidine in infiltrants composition would increase the effectiveness of these materials as antimicrobial activity, especially in relation to cariogenic microorganisms present in incipient carious lesions, and also could decrease bacterial colonization on infiltrated area. The purpose of this study was to evaluate the antimicrobial activity of experimental resin mixtures with chlorhexidine addition. The hypotheses tested in this study are that CHX added to low viscosity monomer blends enhances its

antimicrobial activity compared with commercial infiltrant and the polymerization affects the antimicrobial activity of the mixtures.

MATERIALS AND METHODS

Mixtures preparation

Nine low viscosity monomer blends with infiltrant characteristics were prepared using the monomers TEGDMA (Sigma-Aldrich, St. Louis, USA), UDMA (Sigma-Aldrich, St. Louis, USA), and BisEMA (Sigma-Aldrich, St. Louis, USA). The curing photoinitiators used in the mixtures were DMAEMA (2-dimethylaminoethyl methacrylate, Sigma-Aldrich, St. Louis, USA), CQ (canphoroquinone, Sigma-Aldrich, St. Louis, USA) and the inhibitor BHT (butylated hydroxytoluene, Sigma-Aldrich, St. Louis, USA), in concentration of 1.0 wt.%, 0.5 wt.% and 0.1 wt.% respectively. Two different concentrations of CHX (Chlorhexidine Diacetate Salt Hydrate, Sigma-Aldrich, St. Louis, USA) were added in each monomer blend. Providing blends formulation as demonstrated in Figure 1. The chxd (0.12% chlorhexidine digluconate solution – Proderma, Piracicaba, Brazil) was used as standard antimicrobial reference and the infiltrant Icon® (DMG – Hamburg, Germany) was used as commercial control group. In order to avoid premature polymerization, the resins were stored at 4 ◦C until use.

Mixture	Composition
M1	TEGDMA (100 wt.%)
M2	TEGDMA (100 wt.%), CHX 0.1 wt.%
M3	TEGDMA (100 wt.%), CHX 0.2 wt.%
M4	TEGDMA (75 wt.%), UDMA (25 wt.%)
M5	TEGDMA (75 wt.%), UDMA (25 wt.%), CHX 0.1 wt.%
M6	TEGDMA (75 wt.%), UDMA (25 wt.%), CHX 0.2 wt.%
M7	TEGDMA (75 wt.%), BisEMA (25 wt.%)
M8	TEGDMA (75 wt.%), BisEMA (25 wt.%), CHX 0.1 wt.%
M9	TEGDMA (75 wt.%), BisEMA (25 wt.%), CHX 0.2 wt.%

Figure 1 – Composition of nine different resin mixtures with infiltrant characteristics.

Microorganisms and microbial susceptibility testing

The test organisms used were *Streptococcus mutans* UA159 and *Lactobacillus acidophilus* LYO50DCU-S from Microbiology and Immunology Laboratory of Piracicaba Dental School – University of Campinas, Piracicaba, São Paulo, Brazil. The preparation of *S. mutans* and *L. acidophilus* strains was performed using a microdilution method following the recommendations of the protocol M7-A6[19] with modifications. Cultures of both strains were prepared for 24 hours in 0.9% saline (5 mL), comparing the turbidity with Mc Farland scale (0.5) and adjusting the absorbance in a spectrophotometer (Genesys 10uv, Thermo Electron Corporation, USA) to obtain an inoculum concentration equivalent to 1.5×10^8 cells/mL. Then, serial dilution was made reaching a concentration of 1.0×10^6 cells/mL in BHI broth culture (Difco Laboratories, USA). In order to measure the antibacterial activity of the mixtures, measurement of inhibition zone was evaluated using Pour Plate assay. In order to evaluate the inhibitory and bactericidal activities of the antimicrobial agent, Minimal

Inhibiting Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays were conducted, respectively.

Pour Plate Assay

This technique was used to observe the inhibition zone formed from low viscosity monomer blends and control group in two different situations: uncured and cured. Each inoculum was adjusted at absorbance of 0.6 to 0.7 A and 550 nm in spectrophotometer (Genesys 10uv, Thermo Electron Corporation, USA), and 1 mL was transferred to a vial containing 50 mL of fused agar – BHI (Difco Laboratories, USA) at 45°C, both were mixed and dispensed into a Petri dish (140 x 15 mm). After solidification of agar, wells (5 mm diameter x 1.5 mm height) were made of equidistant points using sterile metal molds (5 mm diameter). Immediately after preparation of drilling, the wells were completely filled with mixtures of resins, Icon® and chxd. To the assay with cured materials the same process described above was conducted, however, soon after the nine mixtures were put in the wells, they are cured for 60 seconds with light curing Elipar Free Light 2 (3M ESPE, St. Paul, USA). Like this, Petri dishes were kept at room temperature for 2 hours to occur pre-diffusion of substances and subsequently incubated at 37°C in an anaerobic chamber for 24 hours. After incubation, the diameter of the inhibition zones of microbial growth formed around the wells was measured in millimeters with a digital caliper (Mitutoyo, Tokyo, Japan) under reflected light.

MIC and MBC

To determine the MIC of the groups (M1, M3, M4, M6, M7, M9, chxd and Icon®) the strains of both microorganisms were adjusted to the absorbance from 0.08 to 0.10 A and 625 nm in a spectrophotometer (Genesys 10uv, Thermo

Electron Corporation, USA). 100 µL of culture broth (Difco Laboratories, USA) and 100 µL of inoculums were pippeted in each well of 96 well plates. Twenty different dilutions were evaluated and to start the microdilutions 100 µL of each mixture were pippeted in the first well of each plate. Therefore, the dilution started with the highest concentration of CHX (0.2 wt.%) and stopped in zero percent (0.0 wt.%) of the antimicrobial substance in the mixtures. Cultures were performed in an anaerobic chamber at 37°C. The growth of microorganisms was examined after 24 and 48 hours.

Minimum Bactericidal Concentration (MBC) has been done after the last reading of the MIC. To determine the MBC, 100µL of each well were pippeted and spread with a swab in a solid culture medium (Difco Laboratories, USA). Then, the cultures were performed in anaerobic chamber at 37°C to observer the growth of microorganisms after 24 and 48 hours.

Statistical analysis

Data from Pour Plate, concerning antimicrobial effect of monomer mixtures were compared using one-way ANOVA and Tukey tests and from antimicrobial effect of light curing were compared using paired t-test. The significance level set was 0.05 for normal distribution. It was considered each bacteria strain individually for all statistical analysis.

RESULTS

Pour Plate Technique

The mean and standard deviations values of the inhibition zones for the blends tested (M1-9 and control group) for cured and uncured materials are shown in Tables 1 and 2.

The antibacterial activity against *S. mutans* of the major of blends containing or not CHX, regardless concentration, provided larger inhibition zones than Icon® before light curing process, except for TEGDMA/UDMA(M4) that didn't show significant difference on inhibition zone with Icon®. However, for blends containing TEGDMA/BisEMA (M8 and M9), the CHX concentration was significant factor to increase the inhibition zones, the higher CHX concentration, the higher inhibition zone ($p<0.05$). The uncured experimental blends without CHX (M1, M4 and M7) showed similar inhibition zones and the same occurred with TEGDMA 0.1% and 0.2% CHX (M2 and M3), and TEGDMA/UDMA 0.1% and 0.2% CHX (M5 and M6). The most of uncured neat monomer blends presented greater antibacterial effect than cured ones. For *S. mutans* strain there wasn't significant difference between cured neat monomer blends (M1, M4 and M7) and Icon® that didn't show antibacterial effect. Considering individual cured blends, addition of CHX in the TEGDMA monomers didn't add any antibacterial effect to the blend, regardless CHX concentration. However, for TEGDMA/UDMA cured blends, regardless CHX concentration, addition of CHX provided increase on antibacterial effect. This performance was also observed in TEGDMA/BisEMA cured blend when CHX was added, but in this case, the concentration of CHX was a relevant factor since the higher CHX concentration caused the higher antimicrobial effect. Thus, addition of CHX to the blends showed different results according to monomer type. CHX 0.1% added to TEGDMA/UDMA blend showed the higher

inhibition zone after cured compared with other blends following by TEGDMA/UDMA/0.2% CHX and TEGDMA/BisEMA/0.2% CHX.

Analyzing antibacterial activity against *L. acidophilus* before and after light curing, the blends without CHX and Icon® didn't show antibacterial activity. The addition of CHX to the blends, regardless concentration, provided inhibition zones and M3 (TEGDMA/0.2% CHX) and M5 (TEGDMA/UDMA/0.1% CHX) showed the highest antibacterial effects. After light curing, no significant difference between all experimental blends and Icon® was observed. To *L. acidophilus* strain the polymerization reduced substantially the antibacterial capacity.

When the inhibition zones of uncured and cured blends were compared, the major of uncured blends showed greater inhibitory effects to both strains.

Table 1 – Diameter of inhibition zones in millimeters produced by cured and uncured blends and Icon® against *S. mutans* (mean ± SD).

	Mixture	uncured		cured	
Neat monomer blends	**M1 - TEGDMA**	3.4 ± 0.5	Acd	0.0 ± 0.0	Bc
	M4 - TEGDMA/UDMA	2.1 ± 0.4	Ade	0.0 ± 0.0	Bc
	M7 - TEGDMA/BISEMA	2.3 ± 0.5	Ad	0.0 ± 0.0	Bc
CHX monomer blends	**M2 - TEGDMA/0.1% CHX**	10.7 ± 0.8	Ab	1.3 ± 1.3	Bbc
	M3 - TEGDMA/0.2% CHX	10.1 ± 1.0	Ab	2.5 ± 1.5	Bbc
	M5 - TEGDMA/UDMA/0.1% CHX	9.8 ± 0.6	Ab	6.9 ± 1.6	Aa
	M6 - TEGDMA/UDMA/0.2% CHX	10.4 ± 0.7	Ab	4.4 ± 0.6	Bab
	M8 - TEGDMA/BisEMA/0.1% CHX	4.9 ± 1.4	Ac	2.1 ± 0.3	Abc
	M9 - TEGDMA/BisEMA/0.2%CHX	13.0 ± 1.3	Aa	4.4 ± 2.9	Aab
Commercial control	**Icon®**	0.0 ± 0.0	Ae	0.0 ± 0.0	Ac

Similar small letters following average mean no significant statistically difference on column. Similar capital letters mean no significant statistically difference observed in row.

Table 2 – Diameter of inhibition zones in millimeters produced by experimental infiltrants against the *L. acidophilus* (mean ± SD).

	Mixture	uncured		cured	
Neat monomer blends	**M1 - TEGDMA**	0.0 ± 0.0	Ad	0.0 ± 0.0	Aa
	M4 - TEGDMA/UDMA	0.0 ± 0.0	Ad	0.0 ± 0.0	Aa
	M7 - TEGDMA/BISEMA	0.0 ± 0.0	Ad	0.0 ± 0.0	Aa
CHX monomer blends	**M2 - TEGDMA/0.1% CHX**	12.6 ± 1.7	Aac	5.4 ± 5.9	Aa
	M3 - TEGDMA/0.2% CHX	15.0 ± 1.9	Aa	2.9 ± 1.8	Ba
	M5 - TEGDMA/UDMA/0.1% CHX	15.1 ± 0.5	Aa	3.8 ± 1.2	Ba
	M6 - TEGDMA/UDMA/0.2% CHX	14.2 ± 0.9	Aab	5.3 ± 2.6	Ba
	M8 - TEGDMA/BisEMA/0.1% CHX	11.7 ± 0.6	Abc	1.8 ± 3.2	Ba
	M9 - TEGDMA/BisEMA/0.2%CHX	10.9 ± 2.0	Ac	0.0 ± 0.0	Ba
Commercial control	**Icon®**	0.0 ± 0.0	Ad	0.0 ± 0.0	Aa

Similar small letters following average mean no significant statistically difference on column. Similar capital letters mean no significant statistically difference observed in row.

MIC and MBC

The TEGDMA, TEGDMA/UDMA, TEGDMA/BisEMA blends and Icon® didn´t demonstrate any antibacterial activity against *S. mutans* and *L. acidophilus* strains in all dilutions tested. However, 0.2% CHX-added blends demonstrated inhibitory activity on microorganism growth very similar or better than chxd. Thus, the lowest concentrations of CHX (in percentage) that were effective against both strains were 9.76×10^{-5} for TEGDMA/UDMA/0.2% CHX, 2.44×10^{-5} for TEGDMA/BisEMA/0.2% CHX, 6.10×10^{-6} for TEGDMA/BisEMA/0.2% CHX and 3.91×10^{-4} for chxd (Figure 2).

Dilutions		Mixtures						Commercial Control	Positive Control
	[CHX]	M1	M3	M4	M6	M7	M9	Icon®	chxd
D1	5 x 10^{-2}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D2	2.5 x 10^{-2}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D3	1.2 x 10^{-2}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D4	6.2 x 10^{-3}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D5	3.1 x 10^{-3}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D6	1.5 x 10^{-3}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D7	7.8 x 10^{-4}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D8	3.9 x 10^{-4}	+ +	- -	+ +	- -	+ +	- -	+ +	- -
D9	1.9 x 10^{-4}	+ +	- -	+ +	- -	+ +	- -	+ +	+ -
D10	9.7 x 10^{-5}	+ +	- -	+ +	- -	+ +	- -	+ +	+ +
D11	4.8 x 10^{-5}	+ +	- +	+ +	- -	+ +	- -	+ +	- -
D12	2.4 x 10^{-5}	+ +	+ -	+ +	- -	+ +	- -	+ +	+ -
D13	1.2 x 10^{-5}	+ +	- -	+ +	+ -	+ +	- -	+ +	+ +
D14	6.1 x 10^{-6}	+ +	- -	+ +	- -	+ +	- -	+ +	+ +
D15	3 x 10^{-6}	+ +	+ +	+ +	- -	+ +	+ +	+ +	+ +
D16	1.5 x 10^{-6}	+ +	+ -	+ +	+ -	+ +	- +	+ +	+ +
D17	7 x 10^{-7}	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
D18	3 x 10^{-7}	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
D19	1 x 10^{-7}	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
D20	0	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

D1 to D20 represents the dilutions of Minimum Inhibiting Concentration (MIC) of material mixings and controls groups. [CHX] represents chlorhexidine diacetate salt hidrate concentrations in each dilution. Sing (+) means microorganism resistant, sing (-) means microorganism not resistant.

Figure 2 – MBC of *S. mutans* (left signs) and *L. acidophilus* strains (right sign).

DISCUSSION

Minimally invasive dentistry is a concept that involves dental tissue preservation, preferably by preventing disease from occurring and intercepting its progress, but also removing and replacing with as little tissue loss as possible. In this concept, apart for arresting active caries lesions, remineralization of cavitated lesions and the placement of restorations using minimal cavity designs, a reduction in cariogenic bacteria to eliminate the risk of further demineralization and cavitation is one of the important approaches.[15,20] For such treatment or management of caries, there would be advantages if the restorative materials possessed the antibacterial abilities, allied to adhesion to dental structures, because these effects are mainly relevant to inhibition of plaque accumulation on the surface of the materials and tooth around the restoration.[15] In this study, the hypothesis tested were proved. The Pour Plate technique test used in this study is an accepted method to initially discriminating antibacterial activity among materials, but some limitations should be considered because it cannot determine if the materials are bactericidal or bacteriostatic.[21] Data showed that uncured neat monomer blends had minimum and similar antibacterial activity against *S. mutans* and *L. acidophilus* (Table 1 and 2). These results are in according to the fact that Bis-GMA, TEGDMA or UDMA, frequently used in composites, have no antibacterial activity against *Streptococcus species*.[15,22] In spite of there are no papers showing specifically the antibacterial activity of resin monomers using pour plate assay, especially those used in this study.

CHX added to uncured monomers increased the inhibition zone, regardless concentration, for the most of monomer blends, except by TEGDMA/BisEMA to *S. mutans* and TEGDMA to *L. acidophilus* that were CHX concentration-dependent (Table 1 and 2). CHX has been used due to its antimicrobial activity and the results of MIC and MBC tests showed that chxd and CHX (incorporated in resin blends) showed bactericidal activity against *S. mutans*

and *L. acidophilus* even in low concentrations (Figure 2). But the composition of resin blends and uncured and cured situations might have influenced in the inhibition zone formation.

After curing, the most of blends studied showed a significant decrease on inhibition zone against both strains tested, even when CHX was added. However, clearly it can be seen that antibacterial activity was material and CHX concentration-dependent. Against *S.mutans* all neat monomer blends provided a significant decrease on inhibition zone; however, when CHX was added, similar results were found by M2 and M3 (TEGDMA/0.1% and 0.2% CHX) and for M6 (TEGDMA/UDMA/0.2% CHX). M5 (TEGDMA/UDMA/0.1% CHX) exhibited the highest inhibition activity after light curing process and could be considered the best mixture against *S. mutans* strain. Concerning the *L. acidophilus* strain, the inhibition zone only decrease after light curing to M3 (TEGDMA/0.2% CHX), M5 (TEGDMA/UDMA/0.1% CHX), M6 (TEGDMA/UDMA/0.1% CHX), M8 (TEGDMA/BisEMA/0.1% CHX) and M9 (TEGDMA/BisEMA/0.2% CHX). The other blends didn't show significant difference between uncured and cured blends against *L. acidophilus*. However, for this strain, after curing neither blend nor Icon® had statistically significant antibacterial activity and this result showed that CHX added in cured blends lost the effective action against this strain. Similar results were found in the review which showed that cured composite didn't release any antibacterial components like CHX and fluoride.[15] It can be speculate that CHX was entrapped in the polymerized matrix become difficult to be leachable from the bulk of the resin.

An important factor that could be considerable is the viscosity of the monomers. It seems to be related with degree of conversion and consequently, with the polymerization of the resin and the elution of some unpolymerized components as CHX. Viscosity is inversely related to the degree of conversion.[23] TEGDMA, UDMA and Bis-GMA are hydrophobic dimethacrylates that are frequently used in adhesive systems and these monomers have low viscosity.[8,11]

Nevertheless, a problem for the incorporation of antimicrobials into the monomer phase is a supposed adverse influence on mechanical properties. A study reported that addition of 1% chlorhexidine gluconate resulted in the reduction of tensile and compressive strengths.[24] The reason for these phenomena may be the disturbance of curing of monomers or the interference of binding of the filler and matrix phases by the incorporated agent. Moreover, it is clear that the release of the agent produces porous structure in the material, and mechanical properties of materials containing soluble antimicrobials can decrease over time.

Therefore, addition of CHX in low viscosity monomer blend using TEGDMA, UDMA or BisEMA would be a pathway to control the biofilm on material/enamel surface and consequently development of new caries lesions around the infiltrated area. However, further researches have to be conducted to elucidate the effect of those blends in biofilm, since the antibacterial effect is a quite different due to the architecture of and interrelation among the bacteria colonies. This study showed the CHX antibacterial activity in a pour plate assay; this is the first step on this kind of analysis and longitudinal studies should be conducted to assess the permanence of CHX in these experimental infiltrants. However, before clinical trials, it is demand to evaluate the structure of the polymer formed, since CHX can be release from the resin bulk and leave some voids inside. Also, it can affect the physical and mechanical properties of the final material and the longevity of the procedure.

CONCLUSION

Neat resin blends showed no antibacterial activity against both strains and the addition of CHX (0.1% or 0.2%) to resin blends promotes antibacterial effects depending on the type of monomer and the light curing. Nevertheless, light

curing decrease the antibacterial activity of majority experimental resin blends. TEGDMA/UDMA resin blend with 0.1% of CHX (M5) showed the higher antibacterial activity after light curing against *S. mutans*. No resin blends with CHX demonstrated antibacterial activity against *L. acidophilus*.

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CAPÍTULO 2

Effect of chlorhexidine addition on the degree of conversion and hardness of experimental infiltrants

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ABSTRACT

The pores of enamel caries act as diffusion pathways for acids and dissolved minerals. Light curing infiltrants with low viscosity can arrest the lesion progression and stabilize mechanically the fragile lesion structure. The incorporation of chlorhexidine diacetate salt (CHX) in infiltrants composition can increase the performance of these materials as antimicrobial activity. The aim of this study was to evaluate the effect of CHX addition on the polymerization characteristics (hardness and degree of conversion) of experimental infiltrants. Nine blends were prepared using different monomers: TEGDMA, TEGDMA/0.1% CHX, TEGDMA/0.2% CHX, TEGDMA/UDMA, TEGDMA/UDMA/0.1% CHX, TEGDMA/UDMA/0.2% CHX, TEGDMA/BisEMA, TEGDMA/BisEMA/0.1% CHX, TEGDMA/BisEMA/0.2% CHX. The infiltrant Icon® (DMG) was used as commercial control group. Nine specimens of each blends and control group were inserted in silicon molds and light cured for 60 seconds. Degree of conversion was determined by Fourier Transformed Infrared Spectroscopy (FTIR). Knoop hardness measures were obtained with micro hardness tester indenter Future Tech FM-100 (FUTURE-TECH), with a load of 10g for 6s. Data were submitted to one-way ANOVA and

Tukey test for comparisons between groups; and each group were compared to control group using unpaired t-test. Significance level of all statistical analysis was set at 5%. The addition of CHX influenced just TEGDMA and TEGDMA/UDMA blends in conversion degree and hardness, respectively. All experimental monomer blends were different of control group. The characteristics of each monomer in the formulation of resin blends were determinant factors which influenced in conversion degree and hardness.

KEY WORDS: Chlorhexidine, Degree of conversion, Infiltrant, Microhardness

INTRODUCTION

In the beginning of the development of enamel caries lesions, recognized as opaque white spots lesions, the mineral is removed from enamel structure leaving porosities (subsurface lesion), whilst the surface of the lesion visually remains relatively intact (Robinson *et al*., 2001; Belli *et al*., 2011). The pores of enamel caries act as diffusion pathways for acids and dissolved minerals and an occlusion of these pores by infiltrant materials can arrest the lesion progression and mechanically stabilize this fragile lesion structure (Paris *et al*., 2007a). Many scientific studies have confirmed that resin sealants and adhesive system can infiltrate natural and artificial enamel caries lesions, but these materials cannot completely fill them (Davila *et al*., 1975; Robinson *et al*., 1976; Robinson *et al*., 2001; Mueller *et al*., 2006; Meyer-Lueckel *et al*. 2006; Paris *et al*., 2007b; Paris *et al.,* 2009). In order to obtain deep penetration of the subsurface porous layer, it was proposed the enamel etching with 15% of hydrochloric acid gel for 2 min and filling with low viscosity light curing resins (Paris *et al*., 2007b; Meyer-Lueckel & Paris, 2010; Paris *et al*., 2010). Additionally, the time of application of the infiltrants is important and Meyer-Lueckel *et al.* (2011) observed that 3 minutes application of

an infiltrant seems to be ideal to obtain relatively homogenous resin layers in natural caries lesions.

The term “infiltrant” was introduced by Paris *et al.* (2007c) to describe a new class of resin materials used for infiltration of caries lesions. These low-viscosity light-curing resins have been shown to slowdown further demineralization *in vitro* (Paris *et al*., 2010) and *in situ (*Paris & Meyer-Lueckel, 2010*)*. However, they have showed that the infiltration procedure can slowdown the mineral loss and caries lesion progression, not hamper that. The average mineral plots show that the mineral dissolution was slightly higher beneath the surface, but affected the whole lesion body. This observation might be attributed to a partial dissolution of remaining mineral in the lesion body that isn’t completely embedded within the resin matrix. Moreover, cleft-like structures that have previously been described, probably caused by material shrinkage during light curing, might cause leakage and thus reduce the acid resistance (Paris *et al*., 2007a). It was shown that a repeated application of infiltrants can reduce this leakage (Robinson *et al*., 2001), but not prevents it.

The di-methacrylates 2,2 – bis [4 – (3 – methacryloyloxy – 2 – hydroxypropoxy) phenyl] propane (Bis-GMA), urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA) are the most frequently used cross-linkers in adhesive systems because they provide mechanical strength by forming densely cross-linked polymers (Van Landuyt *et al*., 2007). The high molecular weight of Bis-GMA (512 g/mol^{-1}) due to it high viscosity (600 – 1,000 Pa.s) arising of its – OH groups and the two voluminous aromatic rings in the spacer also make this monomer quite rigid (Gonçalves *et al*., 2009; Van Landuyt *et al*., 2007; Vasudeva, 2009). This property has shown to have a negative effect on conversion rate because the polymerizable methacrylate groups will have difficulty finding a mating methacrylate group (Ferracane & Greener, 1986; Van Landuyt *et al*., 2007). The monomers TEGDMA, UDMA and BisEMA have molecular weight 286 g/mol, 470 g/mol and 540 g/mol, respectively, and also a significantly lower

viscosity (0.05 Pa·s, 8-10 Pa·s and 3 Pa·s, respectively) when compared with BisGMA (Moszner *et al*., 2008; Gonçalves *et al*., 2009). BisEMA is a monomer with a structure is almost identical to Bis-GMA, except by the hydroxyl groups are not present, and this characteristic decreased viscosity values and allow a less hidrophilicity blend, adding evidence of the importance of hydrogen bonding in determining viscosity (Gonçalves *et al*., 2009). UDMA and TEGDMA are most frequently used cross-linkers in adhesive systems because these di-methacrylates exhibit flexibility properties which compensate the rigidity of Bis-GMA and admixture will result in resins with higher conversion rate (Van Landuyt *et al*., 2007). Due to these characteristics, TEGDMA, UDMA and BisEMA could be used in the composition of the experimental infiltrants.

An antimicrobial that could be added in composition of infiltrants is the chlorhexidine. This substance is the most intensely researched antimicrobial agent in dentistry. This fact is justified for the reason that chorhexidine have wide spectrum of action and can suppress the growth of *Streptococcus mutans*, and consequently, prevent dental caries (Atac *et al*., 2001; Autio-Gold, 2008). Chlorhexidine is a symmetrical cationic molecule consisting of two 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain, which is considered a strong base and it is stable in the form of salts (Fardal & Turnbull, 1986). At low chlorhexidine concentrations, small molecular weight substances, such as potassium and phosphorus, will leach out, exerting a bacteriostatic effect (Fardal & Turnbull, 1986). Nevertheless, in higher concentrations, chlorhexidine have bactericidal action because of precipitation or coagulation of the cytoplasm, probably caused by protein cross-linking (Fardal & Turnbull, 1986). So, the chlorhexidine could be capable to increase the effectiveness of dental materials as antimicrobial activity, especially in relation to cariogenic microorganisms present in carious lesions, and also decrease bacterial colonization of biofilm on infiltrated area.

The purpose of this study was to evaluate the polymerization characteristics of nine experimental resin blends and to determine the better mixture with infiltrant characteristics. It's prudent to investigate whether the incorporation of CHX into experimental infiltrants can modify their polymerization, thereby affecting their degree of conversion and mechanical properties. The hypothesis tested in this study is that the addition of CHX interfere in degree of conversion and hardness of the experimental materials TEGDMA/UDMA/BisEMA-based.

MATERIALS AND METHODS

Formulation of the experimental mixtures

In this study, nine low viscosity monomer blends with infiltrant characteristic were prepared using the TEGDMA (Sigma-Aldrich, St. Louis, USA), UDMA (Sigma-Aldrich, St. Louis, USA), and BisEMA (Sigma-Aldrich, St. Louis, USA) as demonstrate in Figure 1. The photoinitiator system used in the mixtures was 1.0 wt % DMAEMA (2-Dimethylaminoethyl Methacrylate, Sigma-Aldrich, St. Louis, USA) and 0.5 wt% CQ (canphoroquinone, Sigma-Aldrich, St. Louis, USA). The inhibitor BHT (butylated hydroxytoluene, Sigma-Aldrich, St. Louis, USA) was been additioned in the blends in a concentration of 0.1 wt % to prevent spontaneous initiation and propagation of the free-radical polymerization reaction (Van Landuyt *et al.*, 2007). Two different concentrations (0.1 wt % and 0.2 wt %) of CHX (Chlorhexidine Diacetate Salt Hydrate, Sigma-Aldrich, St. Louis, USA) were added in each mixture as demonstrate in Figure 1. In order to avoid premature polymerization, the resins were stored at 4 ◦C until use.

Mixture	Composition
M1	TEGDMA (100 wt.%)
M2	TEGDMA (100 wt.%), CHX 0.1 wt.%
M3	TEGDMA (100 wt.%), CHX 0.2 wt.%
M4	TEGDMA (75 wt.%), UDMA (25 wt.%)
M5	TEGDMA (75 wt.%), UDMA (25 wt.%), CHX 0.1 wt.%
M6	TEGDMA (75 wt.%), UDMA (25 wt.%), CHX 0.2 wt.%
M7	TEGDMA (75 wt.%), BisEMA (25 wt.%)
M8	TEGDMA (75 wt.%), BisEMA (25 wt.%), CHX 0.1 wt.%
M9	TEGDMA (75 wt.%), BisEMA (25 wt.%), CHX 0.2 wt.%

Figure 1 – Composition of nine different resin mixtures with infiltrant characteristics.

The infiltrant Icon® (DMG – Hamburg, Germany) was used as a commercial control group.

Specimen preparation

Cylindrical specimens with 7 mm in diameter and 1 mm thick of each material (experimental infiltrants and the control Icon®) were prepared (n=9) into polyvinilsiloxane matrix (Express, 3M, St. Paul, USA). The matrix was filled completely with the mixture and a polyester strip was placed over and covered with a glass slide until light curing, in order to obtain a smooth and flat surface. Each specimen was cured for 60 seconds with light curing Elipar Free Light 2 (3M ESPE, USA) with power density of 1000 mW/cm^2 approximately. After light curing, the specimens were stored in 100% humidity at room temperature for 24 hours before evaluations of degree of conversion and hardness.

Degree of conversion

The degree of conversion (DC) of experimental infiltrants was evaluated using Fourier Transformed Infrared Spectroscopy (DS20/XAD, Analect Instruments, Irvine, CA, USA). To analyze the mixtures containing TEGDMA and BisEMA in the composition, the interval of 1590,65 cm^{-1} to 1658,31 cm^{-1} was considered and the peaks heights between 1608 cm^{-1} (aromatic ring of BisEMA) and 1638 cm^{-1} (carbon double bonds) were attributed as references (Borges *et al.*, 2012). To TEGDMA and UDMA mixtures the interval of 1502,05 cm^{-1} to 1658,31 cm^{-1} was considered and the peaks heights between 1537 cm^{-1} (urethanes links of UDMA) 1638 cm^{-1} (carbon double bonds) were attributed (Borges *et al.*, 2012). To TEGDMA mixtures the interval of 1590,65 cm^{-1} to 1786,16 cm^{-1} was considered and the peaks heights between 1716 cm^{-1} (carbonyl group of TEGDMA) and 1638 cm^{-1} (carbon double bonds) were attributed (Borges *et al.*, 2012). The degree of conversion was calculated according to the following formulas:

$$\textit{Residual double bonds}\ (\%) = \left(\frac{\textbf{polymerized specimens}}{\textbf{unpolymerized specimens}}\right) \times \mathbf{100}$$

Rate of residual double bonds

$$\textit{DC (\%) = 100 – Residual double bonds (\%)}$$

Rate of degree of conversion

Knoop hardness

The Knoop hardness test was performed using the microhardness Future Tech FM-100 indenter (FUTURE-TECH CORP., Kawasaki-City, Japan) at automatic procedure with a load of 10gF applied for 6s. Three readings were

performed for each specimen. The values obtained in micrometers were converted to Knoop Hardness Number (KHN), by indenter software. The average of the three indentations was considered for statistical analysis.

Statistical analysis

Data were analyzed using one-way ANOVA and Tukey test for comparisons between groups. Data from each group were compared to control group using unpaired t-test. Significance level of all statistical analysis was set at 5% for normal distribution.

RESULTS

The results of DC and Knoop hardness are demonstrated in Table 1 and 2, respectively. The addition of CHX in resin blends didn't reduce the DC of the experimental infiltrants blends, so that CHX had positive influence for the TEGDMA neat monomer, increasing DC. UDMA blends containing or not CHX (M4, M5 and M6) showed the highest DC than other mixtures, while BisEMA blends containing or not CHX (M7, M8 and M9) the lowest, regardless CHX concentration. TEGDMA neat monomer containing or not CHX (M1, M2 and M3) showed intermediate values of DC. When the DC of the nine experimental mixtures was compared with Icon®, all the mixtures (M1-9) showed significant lower values than the commercial infiltrant. Concerning microhardness, when M1-9 were compared with each other, M4 (TEGDMA/UDMA) and M6 (TEGDMA/UDMA/CHX 0.2%) showed the highest Knoop hardness values. The others resin blends had statistically significant similar values of hardness. The addition of CHX didn't change the surface hardness of the mixtures. When Knoop hardness of the nine mixtures was compared with Icon®, all the mixtures (M1-9) showed significant higher values than commercial infiltrant.

Table 1 – Degree of conversion of experimental infiltrants (M1-9) and commercial infiltrant Icon® (mean ± standard deviation).

	Mixture	Degree of conversion (%)	
No CHX blends	**M1 - TEGDMA**	68.1 ± 3.6	Ac
	M4 - TEGDMA/UDMA	81.0 ± 1.8	Aa
	M7 - TEGDMA/BisEMA	59.5 ± 3.3	Ad
CHX blends	**M2 - TEGDMA/0.1 % CHX**	73.8 ± 3.1	Ab
	M3 - TEGDMA/0.2 % CHX	75.6 ± 0.8	Ab
	M5 - TEGDMA/UDMA/0.1 % CHX	82.7 ± 1.4	Aa
	M6 - TEGDMA/UDMA/0.2 % CHX	82.7 ± 1.7	Aa
	M8 - TEGDMA/BisEMA/0.1 % CHX	60.1 ± 5.4	Ad
	M9 - TEGDMA/BisEMA/0.2 % CHX	61.3 ± 2.0	Ad
Commercial infiltrant	**Icon®**	98.4 ± 2.2	B

Similar small letters following average mean no significant statistically difference when the mixtures (M1-9) was compared with each other. Similar capitals letters mean no significant statistically difference each mixtures (M1-9) and Icon®.

Table 2 – Knoop hardness of experimental infiltrants (M1-9) and commercial infiltrant Icon® (mean ± standard deviation).

	Mixtures	Knoop hardness	
No CHX blends	**M1 - TEGDMA**	8.6 ± 1.1	Ab
	M4 - TEGDMA/UDMA	11.1 ± 1.1	Aa
	M7 - TEGDMA/BisEMA	7.8 ± 0.7	Ab
CHX blends	**M2 - TEGDMA/0.1 % CHX**	10.3 ± 1.0	Aab
	M3 - TEGDMA/0.2 % CHX	8.7 ± 1.2	Ab
	M5 - TEGDMA/UDMA/0.1 % CHX	10.5 ± 1.6	Aab
	M6 - TEGDMA/UDMA/0.2 % CHX	10.9 ± 1.5	Aa
	M8 - TEGDMA/BisEMA/0.1 % CHX	8.6 ± 2.1	Ab
	M9 - TEGDMA/BisEMA/0.2 % CHX	8.6 ± 1.6	Ab
Commercial infiltrant	**Icon®**	6.4 ± 0.5	B

Similar small letters following average mean no significant statistically difference when the mixtures (M1-9) was compared with each other. Similar capitals letters mean no significant statistically difference each mixtures (M1-9) and Icon®.

DISCUSSION

The hypothesis tested in this study was partially proved, since different monomers and CHX addition lead to different DC and surface hardness in some mixtures. Although no study in the literature has evaluated the monomer blends tested in this study, the results agree with Ferracane (1985) and Gonçalves *et al.* (2009). They showed that different monomers and co-monomers can provide different DC due to the properties of their different chain size, viscosity and reactivity. Since a polymeric matrix is a large molecule built up by the repetitive bonding together of many smaller units called monomer; and the extent to which monomer is changed into polymer depends on the chemical structure of the dimethacrylate monomer and the polymerization conditions (atmosphere, temperature, light intensity and photoinitiator concentration). It was observed the higher the conversion of double bonds, the greater the mechanical strength (Sideridou *et al*., 2002).

Sideridou *et al*. (2002) show that DC increase in the followed order: Bis-GMA < BisEMA < UDMA < TEGDMA. It was a little bit different from our results that showed as opposite results from TEGDMA and UDMA. It could be because all blends used in their study were Bis-GMA based. Bis-GMA would change the DC of the blend providing different results due to its high viscosity when compared with the monomers used in this study. In the case of UDMA the higher DC occurred most probably because the chain transfer reactions caused by the – NH – groups with increase the mobility of radical sites on the polymer (Sideridou *et al.* 2002). Gonçalves *et al.* (2009) showed that BisEMA mixtures had a lower DC compared with the TEGDMA mixtures. Partial or total replacement of TEGDMA by BisEMA increased viscosity, which was associated with the observed decreases in DC. Also, it can be attributed to the limitations on the mobility of reactive species imposed by rapid formation of a cross-linked in polymeric matrix. Gradual replacement of TEGDMA with UDMA or/and BisEMA in copolymerization with Bis-

GMA resulted in more flexible resins with lower water sorption and higher solubility values, depending on the TEGDMA content. In this study DC of neat monomers ranged from 59 to 83%, as follow: TEGDMA/BisEMA blend (59.5%) <TEGDMA (68%) <TEGDMA/UDMA blend (81%).

Dickens *et al.* (2003) showed that the structures of the individual monomers and, consequently, the resin viscosities of the co-monomer mixtures strongly influence both the rate and the extent of conversion of the polymerization process. The aromatic group of the central part of the BisEMA molecule causes much larger to matrix rotation and certain more difficult to maintain the reaction of double carbon bonds (Vasudeva, 2009). In addition, the degree of conversion of dimethacrylates may be very high if the distance between the methacrylate groups is long (Vasudeva, 2009). This fact can explain the results of this study where UDMA have methacrylates groups more distant than TEGDMA and showed the highest DC.

It has to be evidenced in this study that CHX addition at different monomer blends did not changed the profile found in the DC. TEGDMA/UDMA/ 0.1% or 0.2% CHX (M5 and M6) showed the highest DC, followed by TEGDMA/0.1% or 0.2% CHX (M2 and M3) and TEGDMA/BisEMA/0.1% or 0.2% CHX (M8 and M9). However, when observed DC of TEGDMA neat monomer (M2 and M3), addition of CHX, regardless concentration, increases DC. These results are according to Cadenaro *et al.*, (2009) who showed that addition of CHX in monomer blends didn't change DC, and this property is material-dependent. The authors also claim that for the most hydrophilic resins blends, the addition of 1% or 5% of CHX significantly increased the DC, regardless of CHX concentration.

In this study the blends used were focused on the low viscosity monomers in order to penetrate into the enamel porosities provided by the subsurface caries lesions. The main target of the blends besides the penetration was to reach a high DC providing a high dense polymer and adding CHX to reach

antibacterial properties against biofilm formation. It was observed that TEGDMA neat monomers provided an increased DC when CHX was added regardless CHX concentration (0.1% and 0.2%). However, for the studied monomer and co-monomer blends there wasn't observed alteration on DC when CHX was added to the mixture, but it was dependent of the kind of monomer. The results obtained in this study concerning monomer blends is according to Cadenaro *et al*., (2009) that verified that 1% CHX added to monomer blends in experimental adhesive had no effect on the DC.

In addition, it is notorious that cure extension can exert an effect near to the properties of the final material as such mechanical properties, solubility, dimensional stability, color change and biocompatibility. Thus, using specific combinations of monomers it is possible to reach mixtures with properties for specific applications. In this study it can be seen an association between DC and surface hardness, even in the presence of CHX. It was observed, similar to DC, that TEGDMA/UDMA added or not with CHX showed the highest hardness values followed by TEGDMA and TEGDMA/BisEMA with or without CHX. Indirect methods, as surface hardness, for determining DC provide relative data and can be correlated positively to the results obtained by direct testing modes, such as infrared analysis (Rueggeberg & Craig, 1988). In addition, Ferracane (1985) observed that for only a specific resin, increase in hardness correlates well with increases in DC during setting, and is possible that two resins with different degrees of conversion may have identical properties when tested at room temperature. Ferracane & Greener (1986) suggested that some mechanical properties could be affected by DC, but an analysis of the amount of crosslinking monomers could provide a closer correlation to properties. On the other hand, each resin monomer has an intrinsic polymerization characteristic, and the composition of monomers has an influence on curing of resin-based materials (Asmussen, 1982; Ferracane and Greener, 1986).

The idea of using infiltrants as vehicles for the delivery of therapeutic agents designed to improve the caries arresting in white spot lesions is appealing. CHX has been incorporated into glass-ionomer cements, resin-modified glass-ionomer cements and methacrylates to improve and/or extend the antimicrobial properties of these materials and its is according to Ribeiro & Ericson, 1991; Riggs *et al*., 2000; Sanders *et al*., 2002; Palmer *et al*., 2004; Hiraishi *et al*., 2010. However, the rate of release CHX is much faster if incorporated into hydrophilic resins that absorb more water than hydrophobic ones (Hiraishi *et al*., 2008). Leung *et al*. (2005) showed that 50% of CHX incorporated into a HEMA-based resin composite was released within 1 week. If CHX-doped resins release CHX before it is depleted from primer-delivered CHX, there will be no residual CHX available to "recharge" caries lesion surface. It is very important to measure the modulus of elasticity of polymers that have been doped with therapeutic agents such as CHX. The results of this study show that TEGDMA/UDMA is an appropriate co-monomer blend for CHX since it demonstrated higher DC and surface hardness. In addition, it is important to consider evaluate the CHX releasing from these mixtures. Other properties as water sorption and solubility, abrasion resistance, color stability, biocompatibility and hygroscopic expansion and of resin materials have to be tested.

CONCLUSION

- The characteristics of each monomer in the formulation of resin blends were considerable factors which more influenced in conversion degree and hardness than the addition of CHX.
- M4 (TEGDMA/UDMA), M5 (TEGDMA/UDMA/0.1% CHX) and M6 (TEGDMA/UDMA/0.2% CHX) were the mixtures that showed the best results in the tests that evaluated degree of conversion and hardness properties.

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CONCLUSÃO

Por meio dos resultados obtidos neste estudo, pode-se concluir que:

1. A adição de 0,1% e 0,2% de CHX às misturas monoméricas experimentais contendo como base os monômeros TEGDMA, UDMA e BisEMA foi efetiva quanto à ação antimicrobiana das misturas antes da polimerização para as duas cepas bacterianas. Quanto aos microrganismos utilizados nos experimentos, *L. acidophilus* foi mais resistente que *S. mutans* após a polimerização das misturas. A polimerização das misturas reduziu consideravelmente a capacidade antibacteriana das mesmas e a mistura M5 (TEGDMA/UDMA/CHX 0,1%) apresentou a maior capacidade antibacteriana para *S. mutans*.

2. O tipo de monômero influenciou as propriedades físico-química e mecânica avaliadas, e a concentração do CHX não foi fator determinante para alterar o DC e dureza Knoop das misturas. A adição de CHX aumentou a dureza e o DC das misturas com apenas TEGDMA. As misturas M4 (TEGDMA/UDMA) e M6 (TEGDMA/UDMA/CHX 0,2%) tiveram os maiores valores de dureza Knoop, e as misturas M4 (TEGDMA/UDMA), M5 (TEGDMA/UDMA/CHX 0,1%) e M6 (TEGDMA/UDMA/CHX 0,2%) tiveram os maiores valores de DC. Quando todas as misturas foram comparadas ao Icon®, todas tiveram menor DC e maior dureza Knoop

3. A mistura M5 (TEGDMA/UDMA/CHX 0,1%) foi considerada a melhor mistura resinosa experimental por ter apresentado a maior capacidade antibacteriana após polimerização e maior grau de conversão.

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[3] De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Commitee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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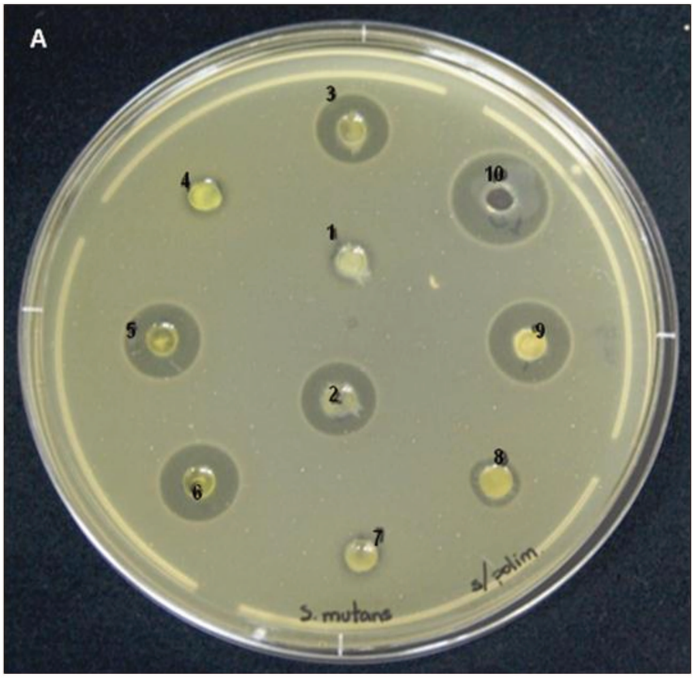
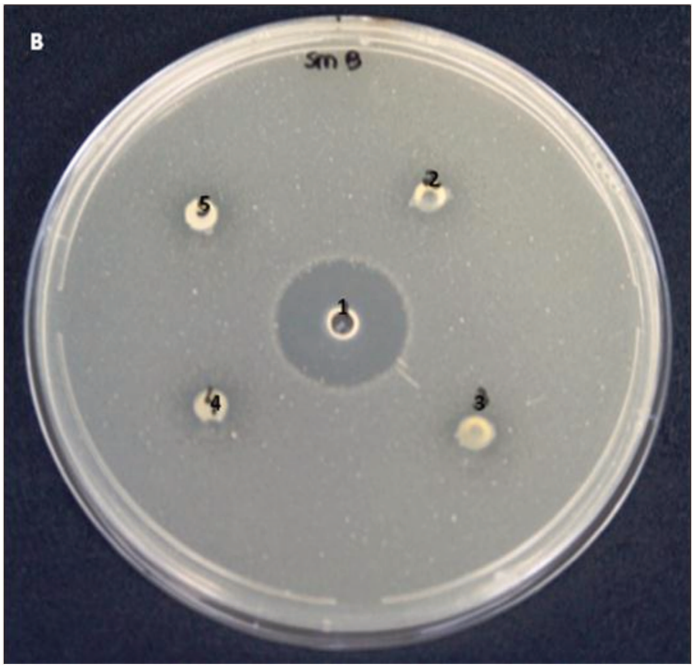
APÊNDICE

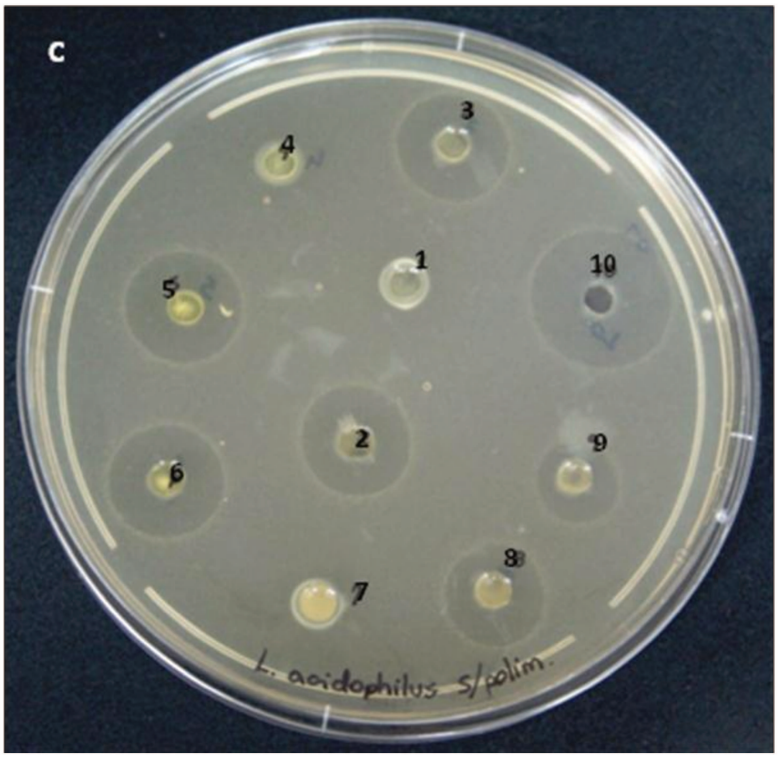
Ensaios Microbiológicos (Capítulo 1)



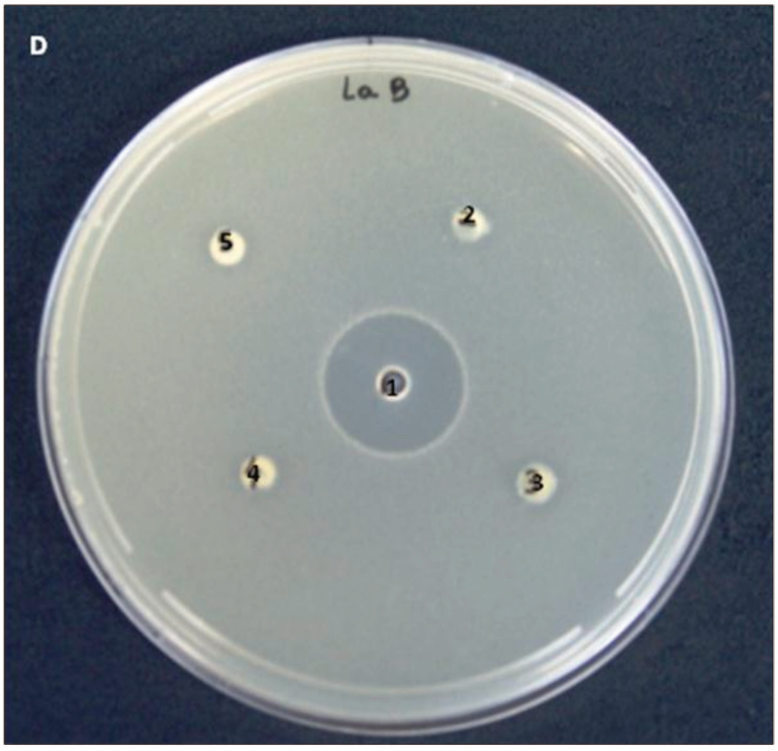
(A) Halo de inibição de misturas não polimerizadas com cepas de *S. mutans*; os números correspondem às seguintes misturas: 1- TEGDMA, 2-TEGDMA/CHX 0,1%, 3-TEGDMA/CHX 0,2%, 4-TEGDMA/UDMA, 5-TEGDMA/UDMA/CHX 0,1%, 6-TEGDMA/UDMA/CHX 0,2%, 7-TEGDMA/BisEMA, 8-TEGDMA/BisEMA/CHX 0,1%, 9-TEGDMA/BisEMA/CHX 0,2% e 10- controle positivo (solução de digluconato de clorexidina).



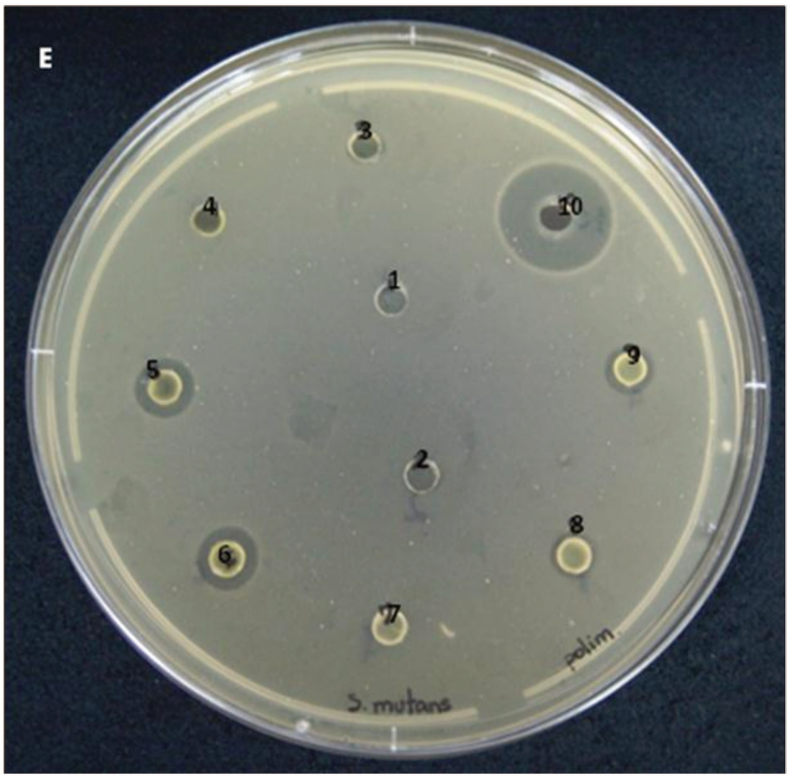
(B) Halo de inibição de misturas não polimerizadas com cepas de *S. mutans*; os números correspondem às seguintes misturas: 1-Controle positivo (solução de digluconato de clorexidina), 2-Controle negativo (Icon®), 3-TEGDMA, 4-TEGDMA/UDMA, 5-TEGDMA/BisEMA.



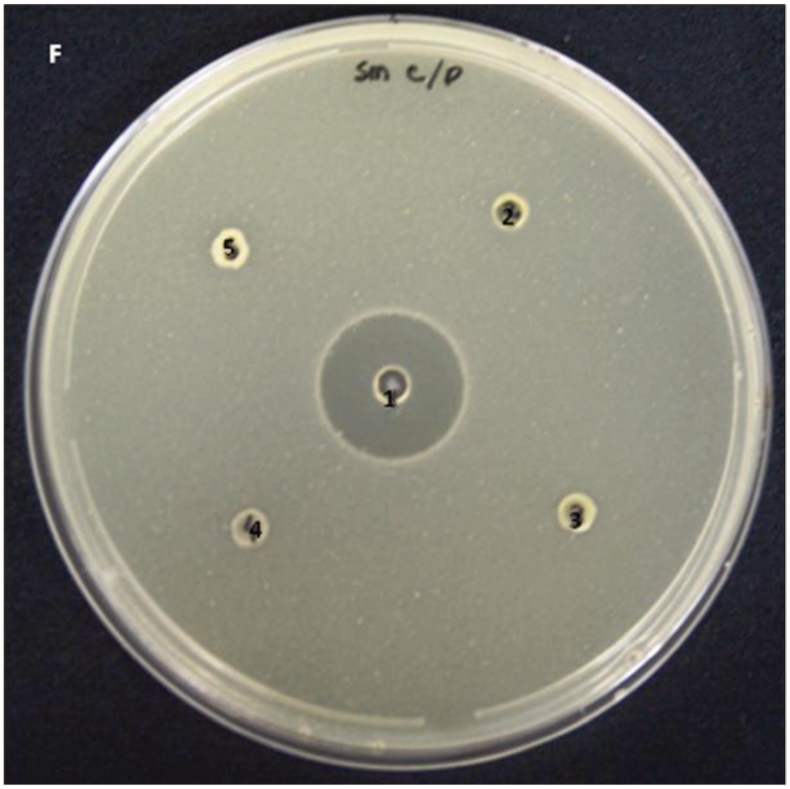
(C) Halo de inibição de misturas não polimerizadas com cepas de *L. acidophilus*; os números correspondem às seguintes misturas: 1- TEGDMA, 2- TEGDMA/CHX 0,1%, 3- TEGDMA/CHX 0,2%, 4- TEGDMA/UDMA, 5- TEGDMA/UDMA/CHX 0,1%, 6- TEGDMA/UDMA/CHX 0,2%, 7- TEGDMA/BisEMA, 8- TEGDMA/BisEMA/CHX 0,1%, 9- TEGDMA/BisEMA/CHX 0,2% e 10- controle positivo (solução de digluconato de clorexidina).



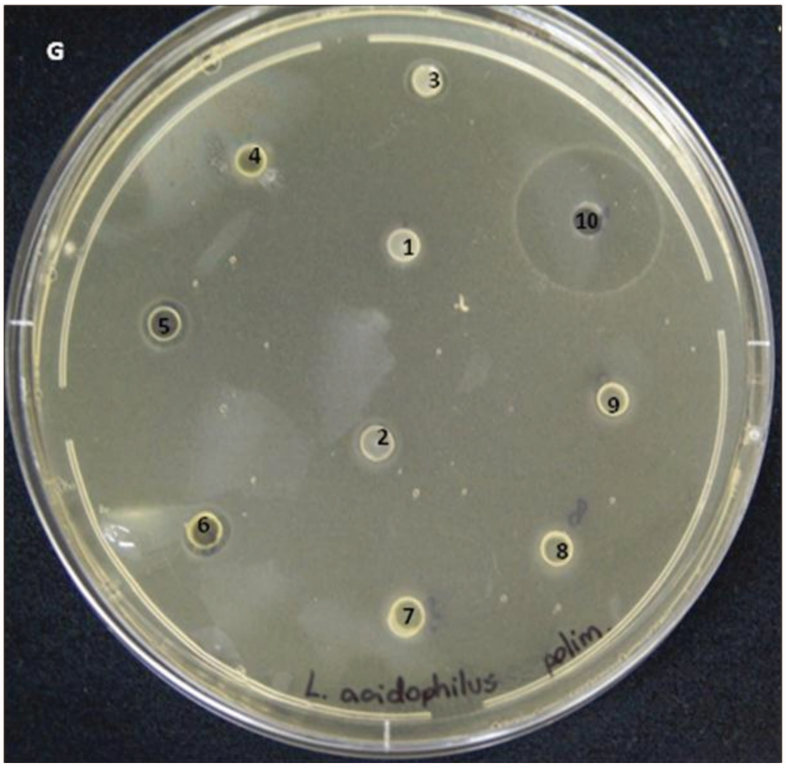
(D) Halo de inibição de misturas não polimerizadas com cepas de *L. acidophilus*; os números correspondem às seguintes misturas: 1- Controle positivo (solução de digluconato de clorexidina), 2- Controle negativo (Icon®), 3- TEGDMA, 4- TEGDMA/UDMA, 5- TEGDMA/BisEMA.



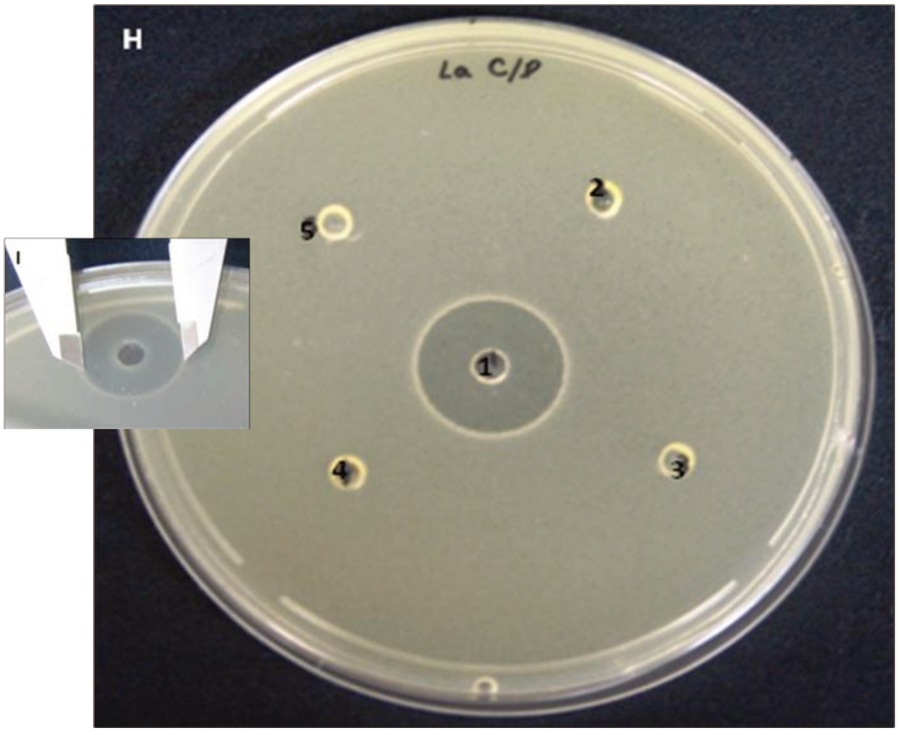
(E) Halo de inibição de misturas polimerizadas com cepas de *S.mutans*; os números correspondem às seguintes misturas: 1- TEGDMA, 2-TEGDMA/CHX 0,1%, 3-TEGDMA/CHX 0,2%, 4-TEGDMA/UDMA, 5-TEGDMA/UDMA/CHX 0,1%, 6-TEGDMA/UDMA/CHX 0,2%, 7-TEGDMA/BisEMA, 8-TEGDMA/BisEMA/CHX 0,1%, 9-TEGDMA/BisEMA/CHX 0,2% e 10-controle positivo (solução de digluconato de clorexidina).



(F) Halo de inibição de misturas polimerizadas com cepas de *S. mutans*; os números correspondem às seguintes misturas: 1-Controle positivo (solução de digluconato de clorexidina), 2-Controle negativo (Icon®), 3-TEGDMA, 4-TEGDMA/UDMA, 5-TEGDMA/BisEMA.



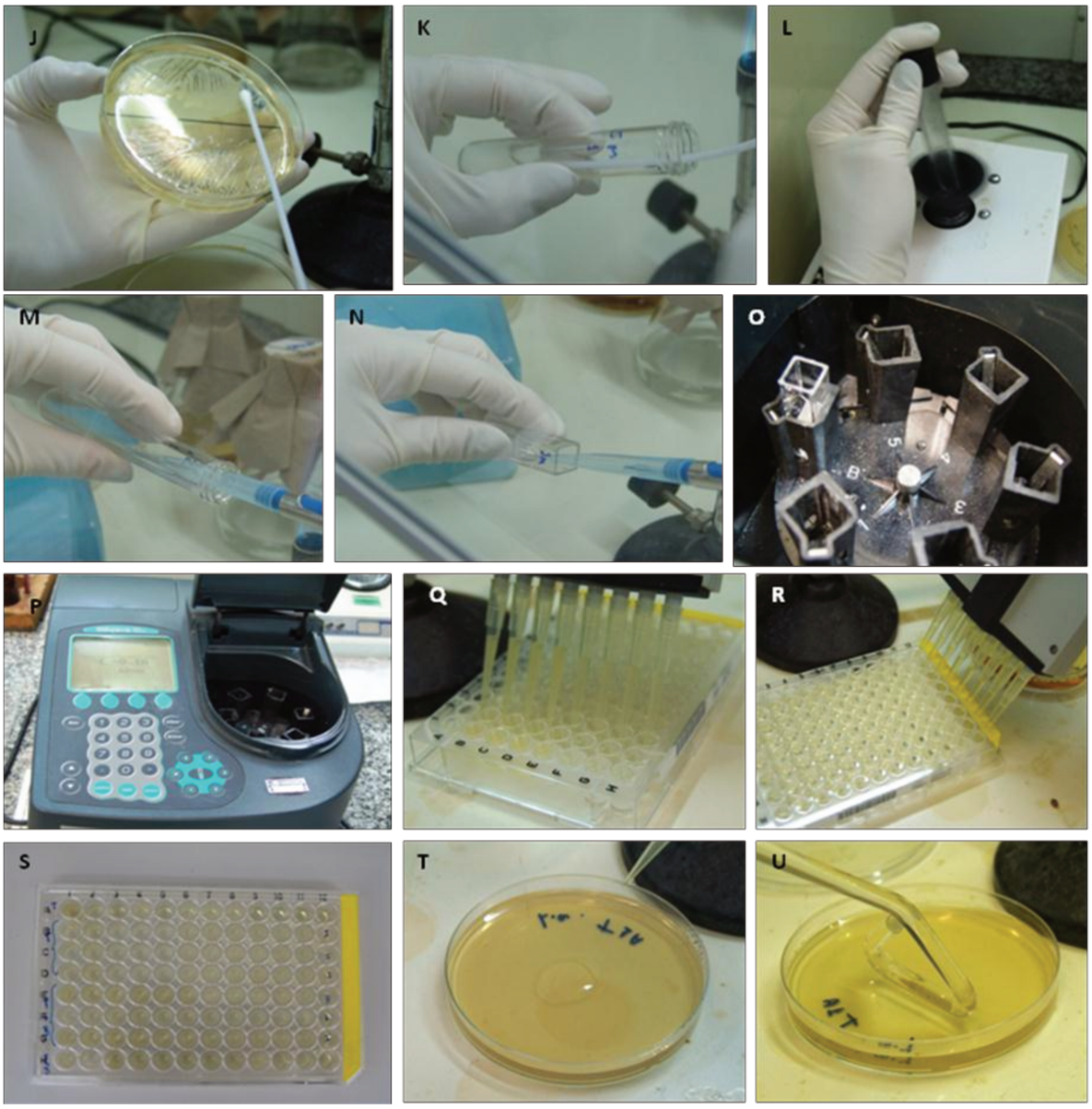
(G) Halo de inibição de misturas polimerizadas com cepas de *L. acidophilus*; os números correspondem às seguintes misturas: 1- TEGDMA, 2-TEGDMA/CHX 0,1%, 3-TEGDMA/CHX 0,2%, 4-TEGDMA/UDMA, 5-TEGDMA/UDMA/CHX 0,1%, 6-TEGDMA/UDMA/CHX 0,2%, 7-TEGDMA/BisEMA, 8-TEGDMA/BisEMA/CHX 0,1%, 9-TEGDMA/BisEMA/CHX 0,2% e 10-controle positivo (solução de digluconato de clorexidina).



(H) Halo de inibição de misturas polimerizadas com cepas de *L. acidophilus*; os números correspondem às seguintes misturas: 1-Controle positivo (solução de digluconato de clorexidina), 2-Controle negativo (Icon®), 3-TEGDMA, 4-TEGDMA/UDMA, 5-TEGDMA/BisEMA.

(I) Mensuração do halo de inibição com paquímetro digital.

Microdiluição do inóculo para determinação do CIM e CMB (Capítulo 1)



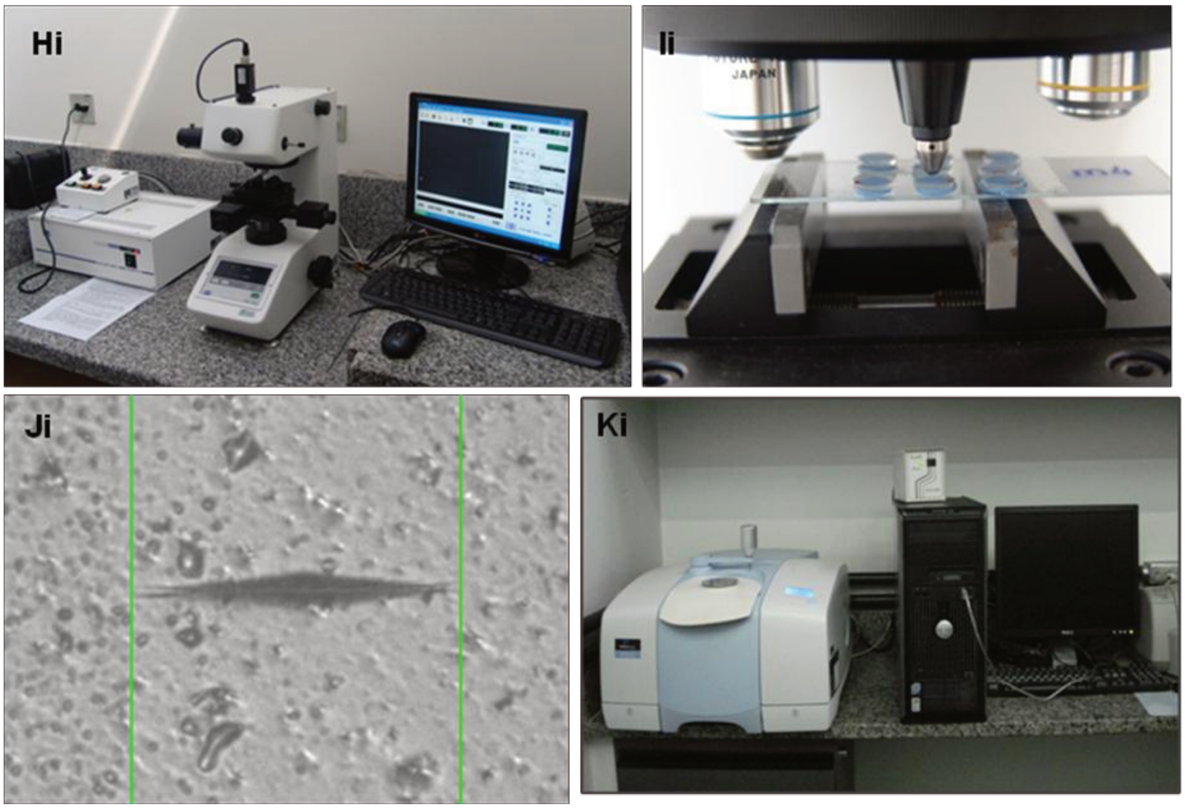
(J) Coleta de inóculo com uma *Swab para* microdiluição.
(K) Diluindo inóculo em salina.
(L) Homogeneização da salina com inóculo.
(M, N, O, P) Ajuste do inóculo em Espectrofotômetro Genesys 10uv, Thermo Electron Corporation, USA.
(Q) Diluição das misturas para determinação do CIM.
(R) Inoculando os poços para determinação do CIM.
(S) Placa de 96 poços do CIM após 48h em estufa de CO^2.
(T, U) Plaqueamento dos poços do CIM para determinação do MCB.

Preparo dos corpos-de-prova para a determinação de dureza Knoop e grau de conversão (Capítulo 2)

(V) Inserção do material experimental na matriz de silicona.
(X) Inserção de tira matriz de poliéster sobre o molde de silicone preenchido com a mistura.
(Y) Conjunto molde/tira matriz de poliéster/lâmina de vidro.
(W) Fotoativação do conjunto
(Z, Ai, Bi) Remoção do corpo-de-prova polimerizado

(Ci) Corpo-de-prova logo após a polimerização
(Di) Armazenamento por 24h em água deionizada
(Ei) Corpos-de-prova fixados em massa de modelar e planificados
(Fi) Misturas resinosas experimentais
(Gi) Aparelho LED Free Light Elipar 2 (3M ESPE, St. Paul, EUA) para fotoativação das misturas

Aparelhos utilizados na determinação da Dureza Knoop e Grau de Conversão (capítulo 2)



(Hi, Ii) Microdurômetro Future Tech FM-100 (FUTURE-TECH CORP., Kawasaki-City, Japan) – FOP/UNICAMP
(Ji) Indentação em superfície de corpo-de-prova
(Ki) Espectroscopia Transformada de Fourier – FTIR (DS20/XAD, Analect Instruments, Irvine, CA, USA)

ANEXO

15-Feb-2012

Manuscript number: JBMR-A-12-0143

Dear Dr. Puppin-Rontani:

We are pleased to receive your manuscript entitled Antibacterial properties
of experimental resin materials with infiltrant characteristics by Inagaki,
Luciana; Alonso, Roberta; Anibal, Paula; Araujo, Giovana; Höfling, José; Puppin-Rontani, Regina. We will be sending it out for review shortly.

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Sincerely,
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Assistant Managing Editor
Journal of Biomedical Materials Research