UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

VANESSA BENETELLO DAINEZI

INFLUÊNCIA DA INCORPORAÇÃO DE CLOREXIDINA NAS PROPRIEDADES FÍSICAS, QUÍMICAS E BIOLÓGICAS DE UM INFILTRANTE COMERCIAL

EFFECT OF CHLORHEXIDINE-ADDING COMMERCIAL INFILTRANT ON BIOLOGICAL, PHYSICAL AND CHEMICAL PROPERTIES*

PIRACICABA
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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Doutora em Odontologia, na área de Odontopediatria.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor, in the area of Pediatric Dentistry.

Orientadora: Profa. Dra. Regina Maria Puppin-Rontani

Coorientadora: Profa. Dra. Fernanda Miori Pascon

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"Combati o bom combate, terminei a corrida, mantive a fé..."

2 Timóteo 4:7

RESUMO

O uso do infiltrante Icon® tem mostrado eficácia ao redor de 60% quando aplicado em lesões iniciais de cárie, em relação à progressão das mesmas. Entretanto, foi observada alta rugosidade de superfície após a aplicação sobre lesões de mancha branca, podendo aumentar o acúmulo de biofilme dental sobre a superfície e assim comprometer a longevidade do procedimento. A adição de um agente antimicrobiano como o diacetato de clorexidina (CHX) ao material infiltrante (Icon®) poderia auxiliar no controle da adesão do biofilme na superfície do material, evitando a reincidência de lesões cariosas ou a progressão das mesmas, e também aumentar a eficácia da terapêutica. Deste modo, o objetivo desta Tese foi avaliar o efeito da adição de CHX no infiltrante Icon® em relação: a atividade antimicrobiana, por meio de teste de difusão em ágar (n=3); a rugosidade de superfície dos infiltrantes antes e após o processo de biodegradação (n=10); o grau de conversão (n=10); a dureza Knoop e amolecimento do polímero (n=10); o módulo de elasticidade (n=12); o coeficiente de penetração por meio do ângulo de contato (n=10); e a sorção e a solubilidade em água (n=5). A densidade de ligações cruzadas foi avaliada indiretamente através da taxa de redução de dureza após imersão em etanol. Neste estudo foram utilizados três grupos: infiltrante Icon®, Icon® + 0,1% CHX e Icon® + 0,2% CHX. Os dados de biodegradação foram submetidos à ANOVA para medidas repetidas dois critérios e teste de Tukey (α=5%) fatores de estudo foram o material e o tempo; os dados de ângulo de contato foram submetidos à ANOVA dois critérios e teste de Tukey (α=5%) fatores de estudo foram material e superfície; e os demais dados a ANOVA um critério, e teste de Tukey (α=5%). O Icon® não apresentou atividade antimicrobiana no teste de difusão em ágar para *Streptococcus mutans* e *Lactobacillus acidophillus*; a adição de CHX a 0,1 e 0,2% apresentou efeito antimicrobiano e não diferiram significativamente entre si, para ambas as cepas ($p>0,05$). Após a biodegradação a superfície do grupo Icon® apresentou maior rugosidade quando comparada aos grupos com adição de CHX ($p<0,05$). As análises de grau de conversão, amolecimento do polímero e ângulo de contato não diferiram significativamente entre os grupos em estudo ($p>0,05$). As análises de módulo de elasticidade, dureza Knoop e sorção de água apresentaram maiores valores para os grupos com adição de CHX ($p<0,05$). A adição de CHX diminuiu a solubilidade ($p<0,05$). Em conclusão, os grupos com adição de CHX independente da

concentração obtiveram satisfatória atividade antimicrobiana, e diminuíram a biodegradação superficial. Além disso, mantiveram ou melhoraram as propriedades físico/químicas do material infiltrante, exceto o aumento da sorção de água.

Palavras chave: resina infiltrante, antimicrobiano, clorexidina.

ABSTRACT

Considering the 60% efficacy of Icon®-infiltrant. However, the surface roughness higher after the used, and can increased accumulation of biofilm, and decreased material lifetime. The antimicrobial as chlorhexidine diacetate (CHX) added to infiltrant can increase the effectiveness of this material concerning the biofilm adhesion on its surface, decreasing of caries lesion progression. This study aims to assess the effect of addition of CHX to Icon® infiltrant on antimicrobial activity and physical/chemical properties. Groups were set up as follows: Icon®, Icon® + 0.1% CHX, Icon® + 0.2% CHX. Test were performed: agar diffusion test (n=3), biodegradation (n=10), degree of conversion (n=10), elastic modulus (n=12), Knoop hardness (n=10), polymer softening (n=10), water sorption and solubility (n=5), contact angle (n=10). Data biodegradation were subjected to two-way ANOVA repeated measure and a Tukey's test (α=5%), study factors were material and time; and contact angle were subjected to two-way ANOVA and a Tukey's test (α=5%), study factors were material and surface. Data the agar diffusion test, degree of conversion, elastic modulus, Knoop hardness, polymer softening, water sorption and solubility were subjected to one-way ANOVA and a Tukey's test (α=5%). The Icon® group not showed antimicrobial activity by agar diffusion test, the 0.1 and 0.2% CHX showed similar effect antimicrobial. The biodegradation test showed higher roughness for the Icon® groups to compare the groups added CHX ($p<0.05$). The degree of conversion, polymer softening and contact angle showed similar results among the groups. The elastic modulus, Knoop hardness and water sorption showed higher values for the groups added of CHX ($p<0.05$). However, the solubility showed the lowest values for the groups added of CHX ($p<0.05$). In conclusion, the groups added of CHX showed be promising, for the activity antimicrobial showed growth inhibition bacterial and biodegradation analyses showed low degradation of resin infiltrant. The physics and chemical properties are maintained or improved, except for the water sorption.

Keywords: resin infiltrant, antimicrobial, chlorhexidine.

SUMÁRIO

1 INTRODUÇÃO

Relata-se na literatura que a prevalência de lesões cariosas no Brasil é entre 32,4% a 66,3%, levando em consideração crianças de 3 a 12 anos (Fernándes et al., 2015; Pinto-Sarmento et al., 2016) sendo que 88% apresentam lesões ativas e 24% destas encontram-se não cavitadas (ICDAS escore 1 e 2) (Pinto-Sarmento et al., 2016).

Lesões não cavitadas são passíveis de remineralização e aproximadamente 36% destas remineralizam-se sem que qualquer intervenção profissional seja utilizada, levando em consideração que os indivíduos fazem uso de água e dentifrício fluoretados (Parisotto et al., 2012). Porém, tem se observado que 10% destas lesões de mancha branca progridem e para esta parcela várias estratégias vêm sendo empregadas como métodos de orientação e instrução de higiene bucal e também o emprego da fluorterapia tópica tem mostrado redução das lesões de cárie ativa em 46% quando do uso de verniz fluoretado (Marinho et al., 2013); e para a utilização de flúor tipo gel a fração de prevenção é de aproximadamente 20% (Van Rijkom et al.,1998; Marinho et al., 2003; Truin e van't Hof, 2005). Dessa forma, observa-se que o efeito do flúor no controle/remineralização das lesões de cárie ativa é parcial.

Sendo assim, alternativas foram pesquisadas como o uso de adesivos comercialmente disponíveis, que mostraram-se efetivos na penetração nas lesões de cárie ativas (Davila et al., 1975; Robinson et al., 1976; Robinson et al., 2001; Mueller et al., 2006; Meyer-Lueckel et al., 2006; Paris et al., 2007a). Entretanto, devido à presença de solventes na composição apresentam alta solubilidade no meio bucal O mecanismo de ação destes materiais se dá pela obstrução dos poros do esmalte cariado pelo material resinoso que pode paralisar a progressão da lesão cariosa e estabilizar mecanicamente a estrutura frágil do esmalte comprometido, bloqueando o caminho para a difusão de ácidos e minerais dissolvidos. Este mesmo mecanismo de ação é preconizado para o uso dos materiais infiltrantes e para que este objetivo seja alcançado o material deve apresentar baixa viscosidade, de modo que se espalhe rapidamente sobre a superfície do dente, e penetre nas áreas do corpo da lesão de esmalte, como nos poros das lesões (Robinson et al., 1976; Paris et al., 2006; Paris et al., 2007b), uma vez inacessíveis para outros tratamentos como o uso de fluoretos.

A utilização de monômeros resinosos é um passo importante para a interceptação da lesão de cárie em estágios iniciais (Kantovitz et al., 2010). 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 viscosidade do material está inversamente relacionada com a capacidade de penetração, isto é, quanto mais viscoso o material menor o coeficiente de penetração (Paris et al., 2007b). A avaliação do grau de penetração pode ser indiretamente mensurada pelo ângulo de contato do material com a superfície do esmalte. O ângulo de contato é definido como um ângulo formado entre um plano tangente a uma gota de um líquido e um plano contendo a superfície onde o líquido se encontra depositado (Luz et al., 2008).

Assim, o ângulo de contato formado entre uma gota de um líquido com uma tensão superficial conhecida e uma superfície sólida depende da relação entre as forças adesivas (que fariam a gota se espalhar sobre a superfície) e as forças coesivas do líquido (que tentam contrair a gota, tendendo a formação de uma esfera com uma superfície mínima) (Neumann e Kwok, 1999). Quando o ângulo de contato situa-se entre 0° e 90°, diz-se que o líquido molha a superfície do sólido; se estiver entre 90° e 180° considera-se que o líquido não molha o sólido (Neumann e Kwok, 1999; Anusavice e Phillips, 2005; Luz et al., 2008).

Além de apresentar um alto índice de penetração no corpo da lesão cariosa o material resinoso deveria apresentar propriedade bactericida ou pelo menos bacteriostática; ser biocompatível com os tecidos bucais; apresentar baixos níveis de degradação no meio ambiente bucal, oferecer suporte mecânico e ser esteticamente aceitável (Robinson et al., 1976; Paris et al., 2006; Paris et al., 2007b).

O infiltrante Icon® (DMG, Hamburgo, Alemanha), é um composto a base de metacrilatos com baixa viscosidade e alto coeficiente de penetração, segundo o fabricante. A utilização deste material foi testada em estudo *in vitro* que demonstrou penetrar em 73 e 77% em profundidade nas lesões ICDAS escores 2 (opacidade notável na presença de unidade) e 3 (cavitação localizada apenas em esmalte) respectivamente (Paris et al., 2011), e apresentou penetração homogênea e uniforme nas lesões de cárie de escore 1 (opacidade notável após secagem de 5 segundos) em todas as amostras (Araújo et al., 2013).Em estudos *in vivo* o infiltrante Icon® apresentou resultados promissores e pode ser considerado como procedimento minimamente invasivo para tratamento de manchas hipoplásicas (Muñoz et al., 2013),

fluorose (Muñoz et al., 2013; Wang et al., 2013; Tirlet et al., 2013) e esmalte hipomineralizado (Crombie et al., 2013).

No que se refere à estética, após aplicação do infiltrante Icon®, estudos *in vivo* (Paris e Meyer-Lueckel, 2009; Glazer, 2009; Shivanna e Shivakumar, 2011; Kim et al., 2011; Gugnani et al., 2012; Hammad et al., 2012; Lasfargues et al., 2013; Knösel et al. 2013) e *in vitro* (Rocha Gomes Torres et al., 2011; Torres et al., 2012; Paris et al., 2013a; Paris et al., 2013b; Subramaniam et al., 2014; Gugnani et al., 2014; Torres e Borges 2015; Araújo et al., 2015) demostraram alto índice de sucesso no mascaramento de lesões de mancha branca.

Quanto à efetividade do infiltrante Icon® comparada com medidas preventivas de higiene bucal, estudos clínicos randomizados foram realizados em dentes permanentes com acompanhamento de 18 meses (Paris et al., 2010) e 3 anos (Meyer-Lueckel et al., 2012). Nos dois períodos, os resultados confirmaram que o infiltrante é um método eficaz para reduzir a progressão das lesões proximais não cavitadas com extensão da lesão da metade interna do esmalte até o terço externo da dentina.
Quando o infiltrante Icon® foi comparado com selamento, estudo *in situ* demonstrou que o infiltrante foi mais efetivo após desmineralização por desafio cariogênico que o selante de fissuras Clinpro Sealant da 3M ESPE (Paris e Meyer-Lueckel, 2010). Da mesma forma, Martignon et al. (2012), em estudo clínico randomizado com pacientes adultos mostraram que a infiltração das lesões iniciais com o infiltrante Icon® apresentaram maior efeito terapêutico que o uso de sistema adesivo (Prime Bond NT®; Dentsply) e o placebo. Entretanto, foi evidenciado que mesmo com os tratamentos realizados houve progressão das lesões após três anos de acompanhamento (clínico e radiográfico). A avaliação da eficácia mostrada por meio de radiografias após 3 anos de acompanhamento mostrou a eficácia de 68% para a infiltração e 60% para o selamento. Se compararmos com a eficácia de 46% do verniz com flúor em estudo de meta-análise (Marinho et al., 2013) pode-se inferir que o uso dos infiltrantes é promissor. Recentes revisões de literatura (Altarabulsi et al., 2014; Doméjean et al., 2015; Dorri et al., 2015) e meta-análise (Ammari et al., 2014) discutem sobre o método microinvasivo utilizados pela aplicação do Icon® em lesões de mancha branca com resultados satisfatórios, mas evidenciam que mais estudos de longo prazo são necessários.

Embora os satisfatórios resultados observados quando do uso do infiltrante

Icon®, nota-se que ainda existem controvérsias com relação às propriedades físicas e químicas do infiltrante, como a solubilidade e rugosidade de superfície. Quanto à solubilidade do infiltrante, Taher (2013) demonstrou em estudo *in vitro* alta solubilidade do material após imersão em água durante 6 meses. No entanto, numa análise da estrutura do esmalte após uso do infiltrante em lesões de mancha branca e após desgaste por escovação, Icon® apresentou uma menor solubilidade que o adesivo Scotchbond® 1XT, 3M ESPE (Belli et al., 2011). Entretanto quanto à rugosidade de superfície, estudos *in vitro* demonstraram que a superfície infiltrada, mesmo após o polimento, não apresentou-se homogênea (Mueller et al., 2011; Yang et al.,2012).

Ainda, é sabido que a superfície não homogênea e rugosa de um material pode propiciar maior acúmulo de biofilme dental, causando biodegradação superficial e, consequentemente, comprometendo a vida útil de restaurações (Santos et al., 2007; Nolasco, 2007; de Fúcio et al., 2009; de Paula, 2011; de Paula et al., 2011; Correr et al., 2012) podendo ser extrapolado para outros materiais resinosos com superfície exposta na cavidade bucal, como os infiltrantes (Ulrich et al., 2015).

Uma vez que o uso da terapêutica de agentes não fluoretados como o infiltrante tem sido indicado como coadjuvante ao tratamento de pacientes com alta atividade de cárie, a adição de agente antimicrobiano na composição do material poderia minimizar o acúmulo de biofilme na superfície do material e regiões adjacentes (Imazato, 2003). O uso de agentes antimicrobianos em materiais odontológicos como compósitos, sistemas adesivos e cimentos ionoméricos tem demonstrado efeito positivo em relação a atividade antimicrobiana dos materiais (Damon et al., 1997; Bishara et al., 1998; Riggs et al., 2000; Leung et al., 2005; Anusavice et al., 2006; Cacciafesta et al., 2006; Ribeiro et al., 2008; Hiraishi et al., 2008; Sacramento et al., 2008; Mehdawi et al., 2009; Castilho, 2010; Hiraishi et al., 2010; de Castilho et al., 2012; Tüzüner et al., 2011). Dentre os agentes antimicrobianos, o diacetato de clorexidina (CHX) tem sido frequentemente estudado na literatura odontológica, como coadjuvante de tratamentos para controle do biofilme ou adicionadas aos materiais odontológicos como forma de se obter um efeito mais duradouro, com a liberação lenta do antimicrobiano (Riggs et al., 2000; Hiraishi et al. 2008; Cadenaro et al., 2009; Hiraishi et al., 2010). Possui propriedades catiônicas, capacidade de se ligar à hidroxiapatitia do esmalte dentário, à película adquirida na superfície dentária, às proteínas salivares e às proteínas extracelulares de origem bacteriana; possuindo dessa forma, um amplo

espectro de ação contra cepas gram-positivas, gram-negativas, fungos, anaeróbios facultativos e aeróbios (Fardal e Turnbull, 1986).

Num estudo realizado por Inagaki (2012), a incorporação de 0,1% e 0,2% de diacetato de clorexidina em infiltrantes experimentais baseados em TEGDMA, UDMA e BisEMA mostrou bons resultados quanto ao efeito antimicrobiano dos materiais sem interferir negativamente nas propriedades como o grau de conversão e dureza de superfície dos mesmos. Corroborando com estes resultados, Stanislawczuk et al. (2014), utilizaram as mesmas concentrações adicionadas em sistemas adesivos e observaram que não houve alteração quanto ao grau de conversão. Entretanto, estudos demonstraram que a adição de CHX pode interferir negativamente em propriedades físico/química, como diminuindo o grau de conversão (Hiraishi et al., 2008) e aumentando a solubilidade com lixiviação do material (Riggs et al., 2000; Leung et al., 2005; Anusavice et al., 2006).

Contudo o ambiente bucal pode levar a uma degradação dos polímeros, pois é constantemente exposto a saliva, alimentos e bebidas bem como as bactérias, estes podem afetar diretamente a dureza e rugosidade das superfícies intrabucais duras (dentes e materiais restauradores) comprometendo a longevidade destes (Yap et al., 2001; Yip et al., 2003).

Apesar do propósito dos infiltrantes não ser restaurar lesões cariosas e sim, promover a paralisação de lesões iniciais não cavitadas preenchendo os poros de esmalte, testes de resistência à degradação química, à biodegradação são importantes, uma vez que esses materiais estarão em contato direto com a região superficial do esmalte (faces interproximais, vestibulares e oclusais) e expostos a desafios químicos e físicos da cavidade bucal.

De acordo com Archegas et al. (2008) e Sideridou et al. (2011), a interação de materiais resinosos com as condições úmidas da cavidade bucal provoca redução das propriedades químicas, pois os compostos poliméricos utilizados nas superfícies dentais tendem a absorver água (mecanismo de sorção que causa aumento de massa) e liberar monômeros não polimerizados (lixiviação de partículas residuais que leva a uma redução de massa).

Apesar de terem sido publicados vários estudos sobre o infiltrante Icon® (Paris e Meyer-Lueckel, 2009; Paris e Meyer-Lueckel, 2010; Paris et al., 2010; Ekstrand et al., 2010; Shivanna e Shivakumar, 2011; Meyer-Lueckel et al., 2011; Kim et al., 2011;

Rocha Gomes Torres et al., 2011; Mueller et al., 2011; Torres et al., 2012; Martignon et al., 2012; Hammad et al., 2012; Paris et al., 2013 a,b; Altarabulsi et al., 2014), dentre estes nenhum estudo demostrou as características do desempenho do material à degradação polimérica.

Diante dos trabalhos científicos consultados, a adição de antimicrobianos como a clorexidina na composição do infiltrante pode melhorar a efetividade desse material quanto à atividade antimicrobiana, principalmente em relação aos microrganismos cariogênicos presentes nas lesões cariosas incipientes, e ainda diminuir a colonização bacteriana do biofilme sobre a área infiltrada.

Sendo assim, esta tese[1] tem por objetivo avaliar a influência da adição de diacetato de clorexidina ao infiltrante comercial Icon® sobre as propriedades biológicas, físicas e químicas do material.

1 Esta tese foi apresentada no formato alternativo de acordo com as normas estabelecidas pela deliberação 001/2015 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas.

2 ARTIGO

CAPÍTULO 1

Effect of chlorhexidine-adding commercial infiltrant on antimicrobial activity, physical and chemical properties[2]

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Abstract

Objectives. To evaluate the addition effect of chlorhexidine diacetate (CHX) to commercial infiltrant on antimicrobial activity, and physical/chemical properties.

Methods. CHX was to Icon®-DMG (w/w). Groups were set up as follows: Icon®-Infiltrant (Icon), Icon+0.1%CHX (0.1i), Icon+0.2%CHX (0.2i). Antimicrobial activity was performed concerning against *Streptococcus mutans* and *Lactobacillus acidophilus* using agar diffusion test (AD) (n=3) and biodegradation test was performed biofilm of S. *mutans*, concerning roughness (Ra) (n=10), and surface morphology. Physical/chemical properties were evaluated by degree of conversion (DC) (n=10), elastic modulus (EM) (n=12), Knoop hardness (KH) (n=10), softening (S) (n=10), sorption (Wsp) (n=5)/solubility (Wsl) (n=5), contact angle (CA) (n=10). Specific specimens concerning dimensions were accomplished for different assays, using the lightcuring unit for 60 seconds. Data from Ra and CA were subjected to two-way ANOVA and Tukey tests (α=5%). Data from AD, DC, EM, KH, S, Wsp and Wsl were subjected ANOVA and Tukey tests (α=5%).

Results. There was no antimicrobial activity for Icon group, but 0.1i and 0.2i showed similar antimicrobial effect ($p>0.05$). After biodegradation test 0.1i and 0.2i showed lower Ra than Icon group ($p<0.05$). There was no significant difference on DC, S and CA values for all studied groups. 0.1i and 0.2i groups showed higher EM, KH and Wsp values than Icon ($p<0.05$). However, Icon showed higher values Wsl than 0.1i and 0.2i ($p<0.05$).

Significance. CHX-adding Icon despite of improving the physical/chemical Icon properties, shows antibacterial effect, reducing material degradation and can be useful for preventing enamel caries lesions progression.

Keywords: resin infiltrant, antimicrobial, chlorhexidine.

1. Introduction

The promising no drilling treatment for white spot lesion called resin infiltration, allow the low viscosity resin materials, with infiltrating capacity, became an alternative to a less invasive dentistry approach, mainly for score 3 ICDAS caries lesions (Paris et al., 2011). *In vivo* studies using Icon® showed promising results. Also the resin infiltrant and can be considered a minimally invasive procedure, even so for hypoplasia stains (Muñoz et al., 2013), fluorosis (Muñoz et al., 2013; Wang et al., 2013) and hypomineralised enamel (Crombie et al., 2013).

Several *in vivo* (Paris & Meyer-Lueckel, 2009; Glazer, 2009; Shivanna & Shivakumar, 2011; Kim et al., 2011; Gugnani et al., 2012; Hammad et al., 2012; Lasfargues et al., 2013; Knösel et al. 2013) and *in vitro* studies (Rocha Gomes Torres et al., 2011; Torres et al., 2012; Paris et al., 2013a; Paris et al., 2013b; Araújo et al., 2015) has showed a high success rate in masking the white spot lesions. Two randomized clinical trials that Icon® infiltrant effectiveness compared to preventive oral hygiene, after 18 months (Paris et al., 2010) and 3 year follow-up (Meyer-Lueckel et al., 2012), showed that infiltration of proximal caries lesions could be effective on inhibit the lesion progression. However, Martignon et al. (2012), in a randomized clinical trial (3 years follow-up) showed that the lesions have progressed in 32.4% for Icon® and 40.5% for sealing (Prime Bond NT®; Dentsply).

Despite the good clinical performance showed by Icon® as previously described on literature, there are controversies regarding its physical and chemical properties. The stability and performance of a resinous material in oral environment is related with its physical/chemical properties. It has been showed that when Icon is submitted to usual drinking solutions as water, wine and tea, quickly it became stained (Paris et al., 2013b; Araújo et al., 2015). The resin material staining can be related to its water sorption and solubility coefficients, and also surface roughness, with conversion degree and softening. Concerning solubility, the infiltrant showed high solubility after 6 months immersion into water (Taher, 2013). In addition, *in vitro* studies showed that the infiltrated lesion, even after polishing, showed high surface roughness (Mueller et al., 2011; Yang et al., 2012).

In addition, a non-homogeneous and rough surface of a material may provide greater accumulation of dental biofilm, leading to surface degradation and

consequently, affecting the lifetime of restorations (Santos et al., 2007; de Fúcio et al., 2009; de Paula et al., 2011; Correr et al., 2012).

In spite of those deficiencies noted on infiltrant material, and since the monomer infiltration can be useful for treating white spot lesions, for high caries activity patients, antimicrobial agent added to this material would help to control the biofilm accumulation on material surface and adjacent regions. It may control the caries progression (Imazato, 2003).

Among antimicrobial agents, chlorhexidine diacetate (CHX) has been the most studied in the dental literature, as coadjuvant treatment to control biofilms or added to dental materials in order to obtain a lasting effect, with slow antimicrobial agent release (Riggs et al., 2000, Hiraishi et al., 2010). Studies have been conducted with chlorhexidine-added restorative dental materials showed an increased clinical effectiveness due to its antibacterial activity (Damon et al., 1997; Bishara et al., 1998; Riggs et al., 2000; Leung et al., 2005; Anusavice et al., 2006; Cacciafesta et al., 2006; Hiraishi et al., 2008; Mehdawi et al., 2009; Hiraishi et al., 2010; Tüzüner et al., 2011, de Castilho et al., 2012). However, adding CHX into the polymer can alter the material properties as water sorption and solubility, concentration dependent CHX (Hiraishi et al., 2008; Stanislawczuk et al., 2014), degree of conversion and elastic modulus (Cadenaro et al., 2009; Stanislawczuk et al., 2014).

This study aims to evaluate the effect of addition of chlorhexidine to Icon® infiltrant on its antimicrobial activity, biodegradation and physical/chemical properties. Therefore, this study hypothesized that the addition of CHX in the infiltrant material inhibiting the growth of *Streptococcus mutans* and *Lactobacillus acidophilus* and the S. *mutans* biofilm degradation, and maintaining the physics and chemical properties.

2. Material and methods

2.1. CHX-adding to Icon®

Table 1 shows the materials composition, manufacturers and batch number of the materials used in this study. The material preparation was done adding CHX into Icon® using 0.1 and 0.2% (w/w) the concentrations were tested in a pilot study. Icon® was conditioned in an amber glass bottles with light protection and CHX was added after weighted in a precision scale (0.0000 gF) and were blended with hand stirring. Two vials containing 0.1 and 0.2% CHX were prepared and stored (8° to 10°C). Then, it was accomplished three groups: Icon®-Infiltrant (Icon), Icon® + 0.1% CHX (0.1i), Icon® +0.2% CHX (0.2i).

Table 1. Composition, manufacturers and batch number of the materials used in this study.

Materials	Composition	Manufactures	Batch number
Icon®- Infiltrant	**Methacrylate-based resin matrix, initiators, additives**	**DMG, Hamburg, Germany**	**662769**
CHX	**chlorhexidine diacetate salt hydrate CAS-No.56-95-1**	**Sigma –Aldrich, Saint Louis, MO, USA**	**083K0014**

2.2. Antimicrobial test - Agar diffusion test

Stock cultures of *Streptococcus mutans* (UA159) and *Lactobacillus acidophilus* (LYO50DCU-S) strains were obtained from the Microbiology and Immunology Laboratory of Piracicaba Dental School – University of Campinas, Piracicaba, São Paulo, Brazil. Briefly, for each experiment, bacteria were cultured freshly from frozen stock on brain-heart infusion broth (BHI; DIFCO Laboratories, Detroit, MI, USA) for 24 hours at 37°C in a 10% CO_2 incubator. Cultures were grown in BHI for 18–24 hours at 37°C and adjusted to a concentration of 10^6 cells/mL to obtain an inoculum for subsequent testing.

The agar diffusion test was performed in triplicate for all group. 1 mL of the inoculum adjusted for each bacterial strain was mixed with 50 mL of BHI agar, and dispensed into sterile Petri dish (140 mm x 15 mm). After the agar solidified, five wells

with 5 mm diameter were made in each plate and completely filled with nonpolimerized material from each group. Then, materials were photoactivated using VALO - Broadband LED Curing Light (Ultradent Products Inc., South Jordan, UT, USA) for 60 seconds, with power density of 1000 mW/cm^2. Chlorhexidine digluconate 0.12% was used as a positive control for antibacterial activity expected and sterile distilled water was used as a negative control.

The plates were kept for 2 hours at room temperature for the diffusion of the materials and were then incubated at 37°C for 24 hours and 10%CO_2 supplemented environment. After incubation, the diameters of inhibition halos around the materials were measured using a digital caliper (Digimatic caliper, Mitutoyo Co., Kawasaki, Japan). The values of inhibition halo were calculated by subtracting the diameter of the well from the average measurements of the inhibition halo (vertical and horizontal) (Fig. 1).

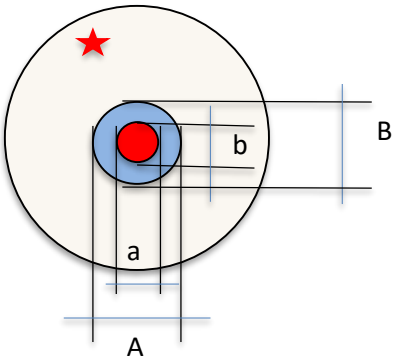

Fig. 1- Ilustration of the representative measurement of inhibition halo. Red star - petri plate; Red circle - well; Blue circle - inhibition halo; A - horizontal diameter of the inhibition halo; B - vertical diameter of the inhibition halo; a - horizontal diameter of the well; b - vertical diameter of the well. Inhibition halo was obtained from (A+B)/2 - (a+b)/2.

2.3. Biological test - Biodegradation

2.3.1. Specimen preparation

Ten cylindrical specimens (5 mm in diameter x 1 mm thick) of each material (Icon, 0.1i and 0.2i) were prepared using polyvinilsiloxane mold (Express^{TM} XT Light body, 3M ESPE, Seefeld, Germany). In order to obtain a smooth, flat surface with the minimum oxygen inhibition layer present, the mold was filled completely with the infiltrant and a polyester strip (Airon, Maquira Dental Products Industry, Maringá, PR, Brazil) was placed over it and after it was covered with a glass slide until light curing process. Each specimen was light cured for 60 seconds with LED (VALO - Ultradent Products Inc., South Jordan, UT, USA) light curing system with power density of 1000 mW/cm^2.

2.3.2. Biofilm growth

After preparation, all discs were stored in 100% relative humidity at 37°C for 24 hours, and the initial reading of roughness taken place. The specimens were sterilized in ethylene oxide process performed by a specialized company (ACECIL – Commercial and Industry Sterilization Center Ltda, Campinas, SP, Brazil).

Streptococcus mutans UA159 strain was obtained from the culture of the Department of Microbiology and Immunology, Piracicaba Dental School, University of Campinas. To prepare the inoculum, S. *mutans* was first grown on *Mitis Salivarius* agar (Difco Laboratories, Sparks MD, MI, USA) plates at 37ºC for 48 hours in an environment supplemented with 10% CO_2. Subsequently, single colonies were inoculated into 5 mL of brain heart infusion (BHI) broth (Difco Laboratories) and incubated at 37°C for 18 hours. Each material disc was placed in each well of polystyrene plates (TPP® 24-well, Schaffhausen, Switzerland) with 25 µL of S. *mutans* inoculum adjusted to an optical density of 0.6 at 550 nm (approximately 8 x 10^{11}CFU/mL) using a UV-Visible Spectrophotometer Evolution 260 Bio (Thermo Scientific, Waltham, MA, USA) under static. Then, 2 mL of sterile, fresh BHI broth supplemented with 1% sucrose (wt/vol) was added in each well. The bacterial accumulation occurred at 37ºC in an environment supplemented with 10% CO_2, developing 7-day-old biofilms (de Paula et al., 2007). The medium was renewed at 24 hours intervals. At the end of the experimental period, specimens were ultrasonically washed in deionized water for 10 minutes and analyzed for surface roughness.

2.3.3. Surface roughness (n=10)

Before and after biodegradation, discs were submitted to analysis the surface roughness using a Surfcorder SE1700 surface roughness-measuring instrument (Kosaka Corp, Tokyo, Japan) with speed of 0.1 mm/s; 0.25 mm cut off and 125 mm of reading length. The values of roughness (Ra) were obtained after three successive readings take in different directions starting from the center of specimen surface. An arithmetic average from each disc was obtained and data were submitted to statistical analysis.

2.3.4. Surface morphology assessment - Scanning electron microscopy (SEM) (n=2)

Two specimens from each group were accomplished, as described above, and no submitted to biodegradation, and two representative specimens of each group after biodegradation were submitted to SEM evaluation. All specimens were fixed on aluminum stubs using double-sided adhesive carbon tape in order to describe the effect of biodegradation on the material surfaces. The samples were sputter-coated with gold under vacuum (Balzers-SCD 050 sputter coater, Balzers, Liechtenstein) and examined with a Model JEOL JSM 5600 LV scanning electron microscopy (Tokyo, Japan) operating a 1000x and 3000x magnification.

2.4. Evaluation of physical and chemical properties

2.4.1. Specimen preparation

All physical/chemical assays used cylindrical specimens of each material for each test, except for elastic modulus test (rectangular) were accomplished as described above in biodegradation. Then, degree of conversion, polymer softening, Knoop hardness, Elastic modulus and water sorption and solubility evaluations were performed.

2.4.2. Degree of conversion (n=10)

Ten cylindrical specimens (5 mm in diameter x 1 mm thick) were prepared and stored in 100% relative humidity at 37°C for 24 hours. Degree of conversion was measured by Fourier Transform Infrared Spectroscopy (FTIR) (Spectrum 100, Perkin Elmer, Beaconsfield, UK) and was determined from the aliphatic C=C and carbonyl

C=O peaks for nonpolymerized and polymerized resin, according to the standard baseline technique (Rueggeberg et al., 1990). The remaining unconverted double bonds were determined by comparing the ratio of the aliphatic C=C absorption peak at 1638 cm^{-1} to the carbonyl group C=O peak at 1716 cm^{-1} between the polymerized and nonpolymerized materials (Atai et al., 2004). The absorption of the carbonyl C=O stretching band served as an internal standard, as it remains constant during the polymerization reaction. The monomer conversion was determined by subtracting the percentage of residual aliphatic C=C bonds from 100% (Borges et al., 2012). The degree of conversion - was calculated using the following equation: DC (%) =100 x [1 - (R polymerized/R nonpolymerized)] where R represents the ratio between the absorbance peak at 1638 cm^{-1} and 1716 cm^{-1}.

2.4.3. Polymer softening and Knoop hardness (n=10)

Ten cylindrical specimens (5 mm in diameter x 1 mm thick) of each material were prepared and stored in at 37°C for 24 hours. Knoop hardness number measurements were taken on the irradiated surface using an indenter (Microhardness Test FM-100 FUTURE-TECH CORP., Kawasaki-City, Japan), under a load of 10 gF for 5 seconds. Five readings were performed for each specimen. The initial Knoop hardness number (KHN 1) was the average of the five indentations. Thereafter, the specimens were stored in 1 mL of absolute ethanol (EMSURE®, Merck KGaA, Darmstadt, Germany) for 24 hours at room temperature, and hardness was again determined (KHN 2). The rate of polymer softening was determined using following equation: 100 - [(KHN 2/KHN 1) x 100]. This softening test is considered an indirect estimation of the cross-link density (Araújo et al., 2013).

2.4.4. Sorption and solubility (n=5)

The specimens for water sorption and solubility tests were prepared as described previously and following the ISO 4049/2009 specification (Internacional Standards Organization, 2009), except the specimens dimension (7 mm in diameter x 1 mm thick), changing the size was to favor homogenize the polymerization, since 7 mm was the LED tip diameter. Five specimens of each group were prepared, both the thickness and diameter of specimens were measured with a digital caliper, and the volume (V, in mm^3) was calculated. After curing, the specimens were transferred to a

desiccator maintained at 37°C. After 22 hours, specimens were removed and stored in a second desiccator maintained at 25°C for 2 hours and weighed on an analytical balance (JK-180: Chyo, Tokyo, Japan) accurate to 0.1 mg. This cycle was repeated until a constant mass (M1) was obtained. The specimens were immersed in distilled water at 37°C for 7 days. Afterwards, the discs were removed and dried with absorbent paper and weigh 1 minute after removal from the water. The excess water was removed by blotting with absorbent paper and the specimens were reweighed (M2). After this weighing, the specimens were reconditioned to constant dry mass (M3) in the desiccators using the cycle described above for M1. Water sorption (Wsp) and solubility (Wsl), given in micrograms per cubic millimeter (µg /mm^3), were calculated as follows:

$$W_{sp} = \frac{M2-M3}{V} \qquad W_{sl} = \frac{M1-M3}{V}$$

2.4.5. Elastic modulus (n=12)

Bar specimens (7 mm x 2 mm x 1 mm) from each group were prepared and stored in 100% relative humidity for 24 hours at 37°C (Araújo et al., 2013).

The three point-bending flexural test was performed to assess elastic modulus in a universal testing machine (INSTRON, model 4111, Instron Corp., OH, USA). The test was performed with a crosshead speed of 0.5 mm/min and a cell load of 500 N until fracture. The distance between supports was 5 mm. Elastic Modulus was calculated using Bluehill 2 software (Illinois Tool Works, Inc., IL, USA) coupled to the universal testing machine.

2.4.6. Contact Angle – Wettability Measurement (n=10)

The contact angles of each material were measured on rough and smooth surfaces. In this way, two types of microscope glass slides with 25.4 mm width, 76.2 mm long and 1.2 mm thick (Bioslide, Walnut, CA, USA) was used: (1) Smooth surface=the regular polished glass; (2) Rough surface=the frosted glass. The smooth glass slide showed 0.101 µm mean roughness (Ra) and it was selected in order to evaluate the contact angle in an ideal situation for liquid spreading on the solid surface. The rough glass slide showed 0.553 µm mean roughness (Ra) and it was selected to

understand how materials could spread in a condition closer to clinical application, considering the acid etching previously the infiltrant application.

Wettability of experimental resin infiltrant was evaluated by contact angle measurements. The sessile drop method was performed using Digidrop GBX goniometer (Labometric Lda, Leiria, Portugal) with distinct glass surfaces (smooth and rough). Briefly, each material was loaded into a 3 mL syringe (Luer-Lok™ Tip, BD, Franklin Lakes, NJ, USA) and coupled to the goniometer. Droplets (≅ 4 µL) were careful applied on the different glass surfaces using a 22-gauge needle (Injex Ltda, São Paulo, SP, Brazil). Ten drops of each material were dispensed on the glass surface. The measurement of contact angle was accomplished immediately after the infiltrant drop has formed on a glass slide. The test was accomplished at room temperature and the drop images captured without external lights interferences. Images were frozen by PixeLink system (Barrington, IL, USA) and the measurements were made by the GBX Digidrop Windrop software (GBX Company, Bourg de Péage, France). The focus of camera used to capture the images was adjusted in relation to the position of the table with glass slide surface and the needle tip. The right and left angles were measured in degrees of the contact angle and average automatically calculated by GBX Digidrop software (GBX Company, Bourg de Péage, France). The average obtained from each specimen and from each group was submitted to statistical analysis.

2.5. Statistical analysis

First, data were checked by equality of variances and normal distribution, except for Solubility evaluation where it was used a log transformation of the data. Then, data from agar diffusion test was submitted to one-way ANOVA and Tukey's test for post hoc comparisons between groups. Data from Biodegradation was submitted to repeated-measures two-way ANOVA and Tukey's test for post hoc comparisons between groups, study factors were material and time. The analyses from degree of conversion, polymer softening, knoop hardness, elastic modulus, and water sorption and solubility were subjected to one-way ANOVA and Tukey's test for post hoc comparisons between groups. Additionally, data from contact angle was subjected to two-way ANOVA followed by Tukey's test, study factors were material and surface. Statistical analyses were performed by GraphPad Prism 6.00 software (San Diego, CA, USA) and the significance level was set at 5%.

3. Results

3.1. Antimicrobial test - Agar diffusion test

The results of antimicrobial activity are described in Table 2. Icon showed no antimicrobial effect against *S. mutans* and *L. acidophilus* being similar the negative control ($p>0.05$). However, CHX-added groups showed antimicrobial effect against *S. mutans* and no significant difference between them was identified. In addition, there was no significant difference between 0.2% of CHX-adding Icon and positive control ($p>0.05$). Concerning *L. acidophilus*, there was no significant difference between CHX added groups ($p>0.05$), but they showed a lower value significant difference from positive control group ($p<0.01$). Fig. 2 shows the inhibition halo obtained by studied groups and negative and positive controls, used only for method control.

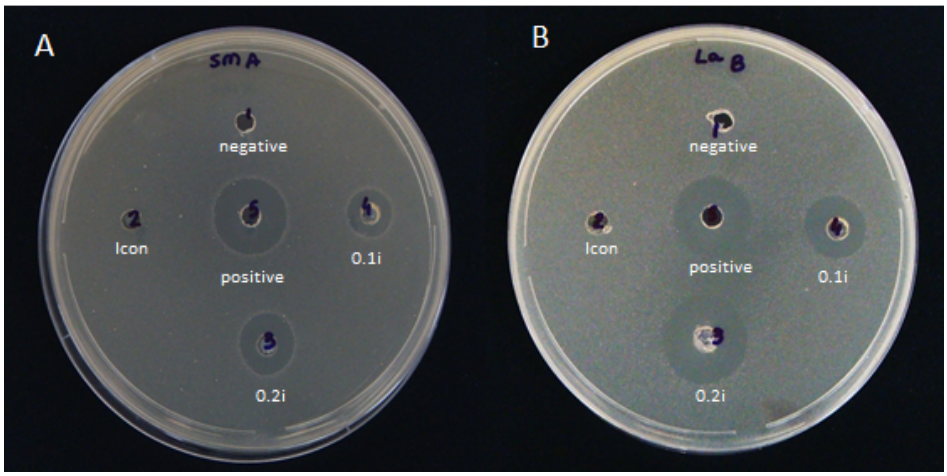

Fig. 2- Plates showing the antibacterial effect of the materials used in this study: Icon; 0.1i; 0.2i; positive control and negative control. A. S. *mutans*; B. L. *acidophilus*. The inhibition halo around the materials can be seen in both pictures. It can be noticed that there was no inhibition halo for Icon.

Table 2. Mean and standard deviations of inhibition halo (mm) obtained from agar diffusion test for S. *mutans* and L. *acidophilus*.

Materials	S. *mutans*	L. *acidophilus*
Deionized water - negative control	0 c	0 c
Icon	0 c	0 c
0.1i	14.04±1.10 b	12.2±2.23 b
0.2i	17.3±2.58 ab	13.95±2.01 b
CHX digluconate 0.12% - positive control	19.31±0.60 a	18.64±0.41 a

Similar lowercase letters (column) do not show statistically significant differences between groups regardless bacterial strain.

3.2. Biological test - Biodegradation

Table 3 shows the surface roughness values obtained before and after S. *mutans* biofilm biodegradation. The statistical analysis showed significant interaction of study factors (material and time) ($p<0.0001$). All the groups showed no significance difference on roughness before biodegradation. After *S. mutans* biofilm degradation, Icon presented the highest values of surface roughness ($p<0.0001$). CHX added groups showed no significant difference on surface roughness between before and after biodegradation, and showed the lowest surface roughness values. ($p<0.0001$).

Table 3. Surface roughness values ra (µm), mean and standard deviations of infiltrants submitted to S. *mutans* biofilm degradation.

Materials	Before	After
Icon	0.14±0.03 Ba	0.85±0.40 Aa
0.1i	0.09±0.02 Ba	0.28±0.11 Bb
0.2i	0.11±0.02 Ba	0.16±0.05 Bb

Similar lowercase letters (column) do not show statistically significant differences between groups materials studied.
Similar uppercase letters (line) do not show statistically significant differences between groups time studied.

Morphological analysis of materials surface can be observed on Fig. 3, the SEM micrograph showed a smooth surface before biodegradation to all studied groups (Figs. 3a, 3c and 3e). However, after biodegradation Icon group showed an accumulation of biofilm, which can be clearly observed on the higher magnification (Figs. 3b and 3b1). S. *mutans* colonies adhered on surface can be observed and

provided a rougher surface to Icon than 0.1i and 0.2i. The CHX added groups after biodegradation (Figs. 3d and 3f) showed no change in surface compared to before (Figs. 3c and 3e). In addition, no biofilm on surface was observed for both groups, 0.1i and 0.2i.

3.3. Evaluation of physical and chemical properties

The results of properties of the CHX added groups and Icon are presented in Table 4. There was no significant difference between studied groups concerning degree of conversion, polymer softening and contact angle. Concerning contact angle, there was no significant interaction between the studies factors (material and surface) ($p>0.05$). Icon showed lower Knoop hardness, water sorption and elastic modulus values than CHX added groups ($p<0.0001$). However, Icon showed the higher solubility values than CHX added groups ($p<0.0001$).

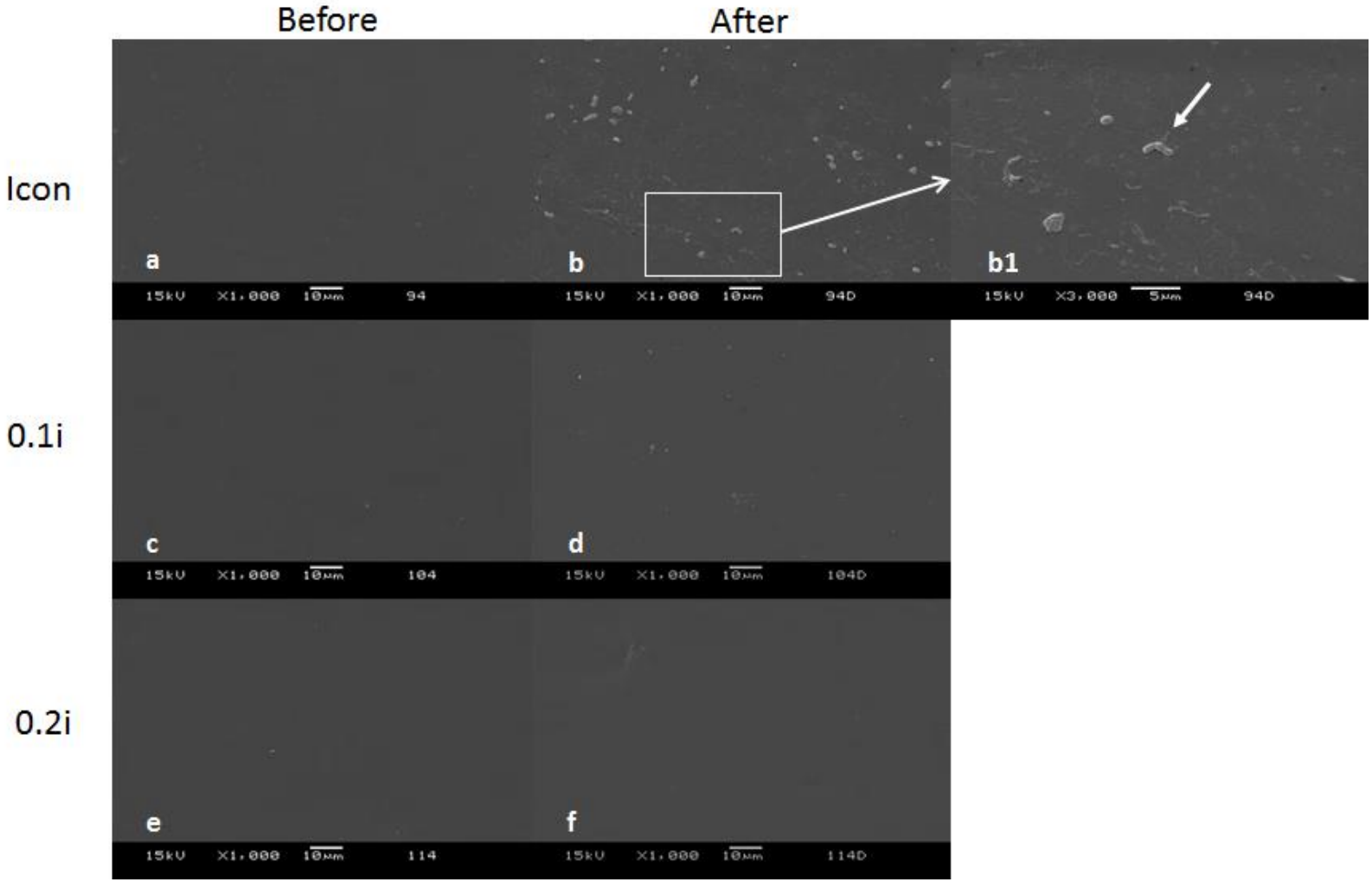

Fig. 3 - Representative SEM images of materials surfaces before (a, c and e) and after (b, b1, d and f) biodegradation, at an original magnification of 1000x, except b1 of 3000x. b and b1 SEM micrographics revealed the presence of S. *mutans* biofilm on surface of Icon after the biodegradation (b1-arrow).

Table 4. Mean values and standard deviations of degree of conversion (%), polymer softening (%), contact angle on smooth and rough surface(º), Knoop hardness (KHN), elastic modulus (GPa), sorption (µg /mm^3) and solubility (µg /mm^3) to studied groups (Icon, 0.1i and 0.2i).

Materials	Degree of conversion	Polymer softening	Contact Angle smooth surface	Contact Angle rough surface	Knoop Hardness	Elastic modulus	Sorption	Solubility
Icon	**59.40 ± 3.45 a**	**44.27 ± 10.61 a**	**14.78 ± 1.49 a**	**16.13 ± 3.93 a**	**4.66 ± 1.74 b**	**0.46 ± 0.14 b**	**3.54±0.38 b**	**5.76±1.15 a**
0.1i	**62.71 ± 6.71 a**	**44.18 ± 7.60 a**	**16.06 ± 2.65 a**	**17.47 ± 4.18 a**	**12.40 ± 1.10 a**	**0.95 ± 0.12 a**	**5.54±0.38 a**	**0.26±0.15 b**
0.2i	**56.37 ± 10.94 a**	**38.67 ± 3.54 a**	**15.25 ± 1.72 a**	**18.88 ± 5.18 a**	**12.18 ± 1.80 a**	**1.01 ± 0.12 a**	**5.81±0.29 a**	**0.33±0.13 b**

Different lowercase indicates statistically significant difference between groups, considering independently tests conducted ($p < 0.05$).

4. Discussion

The hypothesis of this study was proved, once the addition of CHX in the infiltrant material inhibited the S. *mutans* and L. *acidophilus* growth and the S. *mutans* biofilm degradation, and also maintaining or improving the physics and chemical properties. The CHX added to Icon improved some material properties as surface hardness, elastic modulus and maintained the contact angle, degree of conversion and polymer softening. In addition, it increased water sorption but decreased solubility. Those properties can be directly related with the material performance on the oral environment, since the material can become resistant to chemical challenges.

It is a challenge to modify any kind of materials. Addition of solvents, filler particles and other components can compromise the performance of the original material, besides some positive effects can bring about. An important surface property of the infiltrant is it high penetration coefficient (Paris et al. 2007b) due to necessity of resin infiltrant penetrate into white spot lesion; this property is directly related to low viscosity and high wettability. The wettability can be measured by the contact angle (Yilgor et al., 2012). It is known that the lower contact angle, the higher wettability. Contact angles lower than 90° indicate that the liquid has spontaneous capacity to wet the solid surface; the closer to zero contact angles, the greater the surface energy and the wettability property between the interface solid/liquid (Long et al., 2005). Despite of the ability of an infiltrant to penetrate into the tiny porosities of the enamel caries lesions can be affected by the components addition, in this study, all groups showed a very low contact angle in both smooth and rough surfaces about 16° (Table 4), close to zero. Then, the addition of CHX in the infiltrant composition did not affect the Icon wettability.

In addition, other properties are essential for a good material performance and longevity as the conversion degree and polymer softening. They can predict how the material will perform. The addition of CHX to the materials did not jeopardize on the polymeric chain formation of the Icon infiltrant, since the degree of conversion and polymer softening results was not different from Icon group. These results corroborate other study that found CHX added adhesives did not interfere on the degree of conversion (Stanislawczuk et al., 2014; da Silva et al., 2015). It may be due to the CHX addition cannot affect the propagation of the polymerization reaction and remaining entrapped between linear chains after polymer network formation. Concerning the

degree of conversion of Icon, the results found in our study were similar to those found by Rahiotis et al. (2015) who observed 57.4 ± 1.5% conversion degree. However, the results were quite different from Araujo et al. (2013) who found 98% of Icon conversion degree. This difference can be attributed to modifications introduced into the material composition by manufacturers. Those statements are based on the new patents from Icon (Neffgen and Neander, 2013 Jan 29). Adding a solvent to monomer blends can prompt the formation of microgels near the centers of initiation, which become sites of low mobility of radicals, thus decreasing polymerization. These chains form a heterogeneous distribution of chain mobility (Araújo et al., 2013). Therefore, other changes can be noticed as cross-link density and elastic modulus as a consequence of the solvent addition (Ye et al., 2007).

In addition, a higher hardness was observed for CHX added groups than Icon®, probably after de addition and blended the CHX there was evaporation of the Icon solvent, increasing the hardness surface and the elastic modulus (Table 4). The hardness and elastic modulus decreased with an increased the concentration of solvent (Araújo et al., 2013). The elastic modulus of enamel there was the 1.3±0.3 GPa (Chun and Lee 2014) similar the added 0.2 CHX group (1.01±0.12) (Table 4). Therefore, this finding is very important for improving chemical property, since this reinforcement can enrich of fragile structure of demineralized enamel.

The water sorption analysis showed higher values for the added CHX groups than Icon® group. The CHX particles were not physically or chemically bound to the polymer network, could form microvoids into the resin monomer, which provides channel/pathway for water molecules (Hiraishi et al., 2010); since the CHX particles have water attraction, that have leaning formation of water droplets around the CHX particles, modify the clustering formation of the parent system, and can increase the water attraction leading polymer water sorption increased (Riggs et al., 2006). Once the water comes in the polymer matrix, it can have greater difficulty output of matrix. In addition, TEGDMA has hydrophilic property, which suggest an interaction with oral environment, mainly water, since a high affinity for water molecules.

However, CHX added to Icon showed decreased solubility values, regardless its concentration. Despite the higher water sorption provided by CHX adding in the Icon®, regardless concentration added, they showed lower solubility values. Anusavise et al., 2006 showed that CHX has limited solubility in basic pH, and maybe can have contributed to decrease the 0.1i and 0.2i solubility. Since TEGDMA is the Icon main

component and its hydrophilicity can favor to polymer hydrolysis (Delaviz et al., 2014) due to high water sorption, CHX can contribute to decrease the polymer degradation. Instead, Icon with its high solubility can contribute to degradation and can jeopardize physical/chemical properties of resin monomer decreases the lifetime.

Unfriendly environment can be found on the oral cavity made by presence of water, by-products from food, drink and mainly bacteria metabolism and they can degrade the restorative materials as Icon, maybe by hydrolysis and acid pH breaking polymer chains (Williams and Zhong 1994). Then, the incorporation of CHX as an antimicrobial agent was effective for inhibiting the growth of S. *mutans* and L. *acidophilus.* While both CHX concentrations used in this study were effective against bacteria strains studied, 0.2% CHX was more effective to S. *mutans* by agar diffusion test. Other studies (Takahashi et al., 2006; Korkmaz et al., 2013; Mittal et al., 2015) adding high concentration of CHX to glass ionomer cement showed potential antimicrobial effect on S. *mutans* and L. *casei*. However, Hiraishi et al. (2008) used the 0.1 and 0.2% concentration of CHX added at resin blends and no inhibiting halo were observed, being effective only from 1% of CHX. The difference between studies can be attributed to different methodology used and resin blend. Hiraishi et al. (2008) studied polymerized monomer blend discs immersed in deionized water and were retrieved from water storage at 24 hours; in our study, the material was inserted into the wells before polymerization, which may have spread the CHX into the agar. However, a lower concentration of CHX in infiltrant material showed satisfactory results for inhibiting the growth bacteria when compared with control group Icon.

In spite of the surface roughness analysis before biodegradation showed similar values of roughness between groups, the addition of CHX did not alter the roughness initial and after biodegradation. However, after biodegradation, the roughest surface was found to Icon. Therefore, it can be noticed that instead of CHX been released from resin monomer in a water environment (Leung et al., 2005), there was a lower biodegradation associated CHX addition to Icon infiltrant. The higher roughness the Icon group can be associated with the higher solubility of the infiltrant material (Taher, 2013), as showed in our study (Table 4), which facilitated increased S. *mutans* adhesion and provided a rougher surface. It is known that there is an association between biofilm accumulation and higher roughness (Quirynen et al., 1990) and this accumulation causes polymer matrix degradation and consequently, affecting the lifetime of material (Santos et al., 2007; de Fúcio et al., 2009; de Paula et al., 2011;

Correr et al., 2012). In addition, Bollen et al. (1997) showed that a roughness higher than 0.2 µm is an appropriate substrate to biofilm accumulation. Therefore, after S. *mutans* biofilm degradation, Icon showed the highest roughness surface, four times more than that pointed out by Bollen et al. (1997) as a harmful surface. However, when to the concentration of 0.2 CHX observed the value 0.16 µm after biodegradation. It is a concerning situation, since clinically Icon remains as a surface directly affected by oral environment as a dental biofilm. The SEM images after biodegradation showed a S. *mutans* adhesion on material surface for Icon group which could confirm no antimicrobial effect found on agar diffusion test.

Therefore, when observed the discs submitted to biofilm degradation, although the all groups have showed biofilm growth on the sample, the CHX added groups the biofilm was no strongly adhered on surface. Therefore, SEM images showed no biofilm on those surfaces, contrary to those observed by Icon group. Maybe, it was occurred due to the antimicrobial action of CHX released to an external environment and on surface that inhibited the biofilm aggregation (Hiraishi et al., 2008).

At first looking, the results found in this study seem contradictory. If on one hand CHX adding to Icon brought about satisfactory results, as antibacterial effect and biofilm control, decreased solubility, increased surface hardness, and did not affect the conversion degree, contact angle and polymer softening, by the other hand increased the elastic modulus and water sorption. However, elastic modulus and water sorption alterations cannot be harmful to the stability of the infiltration technique, since the material will be entrapped into the white spot lesion and would have no clinical significance. Thereby, further studies are necessary to analyze the behavior of the infiltrant material added of CHX in the oral stress situation over time, since this study *in vitro* allows the characterization of the properties the modified resin infiltrant that will serve as a basis for clinical studies in the future.

5. Conclusions

Based on the results obtained, it can be concluded that the CHX added groups showed be promising results, since it was provided increased biological properties, as S. *mutans* and L. *acidophilus* inhibition and biodegradation resistance. In addition physics and chemical properties were maintained and improved; except to water sorption.

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Conflict of Interest

None

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3 CONCLUSÕES

Baseando-se nos resultados obtidos, pode-se concluir que a adição de diacetato de clorexidina ao infiltrante comercial Icon®, independente das concentrações testadas, mostrou ter resultados promissores, uma vez que promoveu efeito antimicrobiano contra *Streptococcus mutans* e *Lactobacillus acidophilus,* produziu menor rugosidade de superfície após a biodegradação com *S. mutans*. Além de manter ou melhorar as propriedades físicas e químicas, exceto a sorção de água.

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APÊNDICE 1

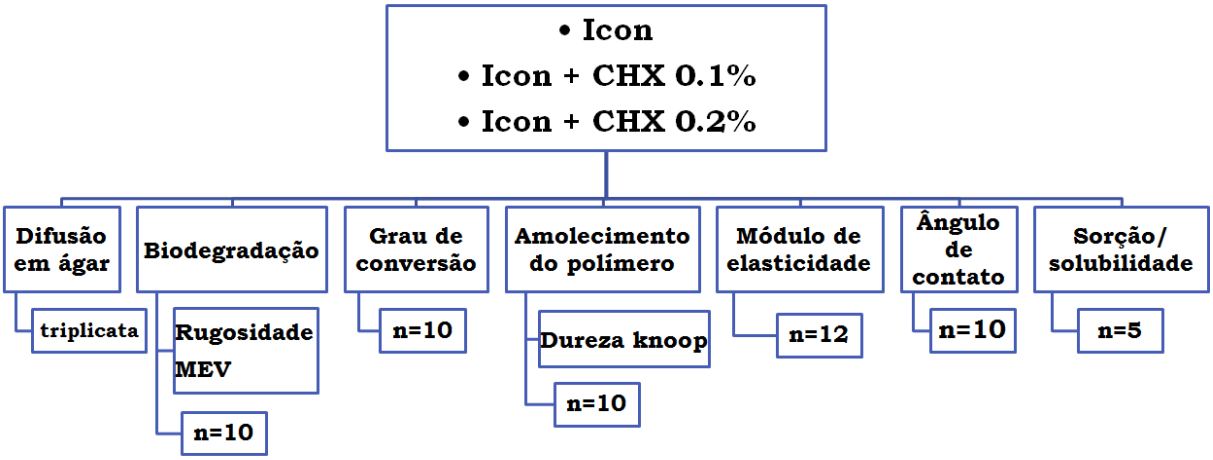

Figura 1. Organograma.

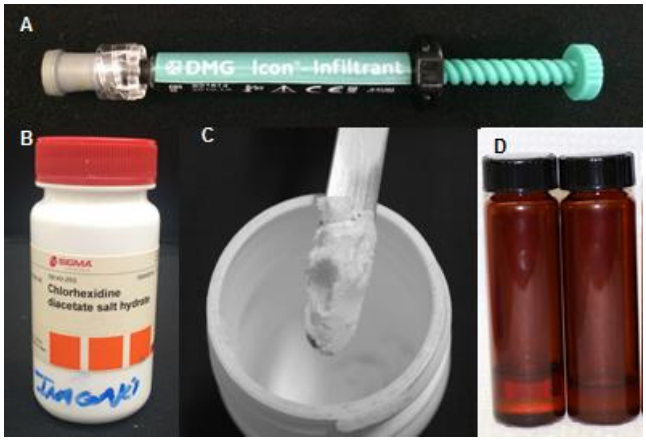

Figura 2. A. Infiltrante Icon®. B. Diacetato de clorexidina (CHX) (Sigma). C. Aspecto do pó de CHX. D. Frasco âmbar com proteção de luz para armazenamento da mistura de Icon® e CHX.

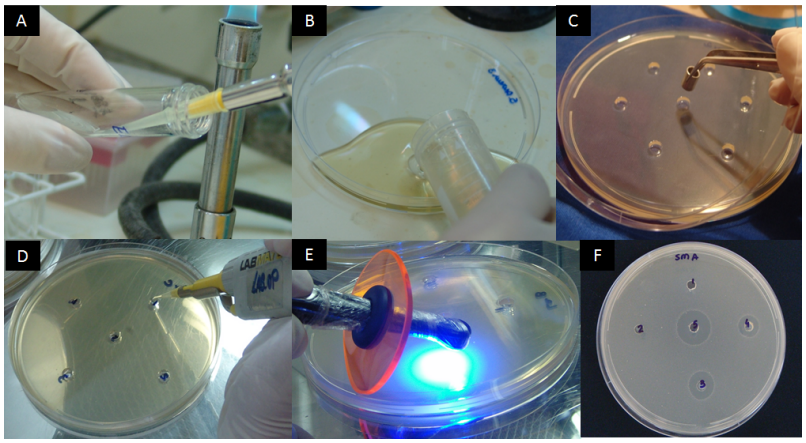

Figura 3. Teste de difusão em ágar. A. preparo do inóculo. B. preenchimento da placa de Petri com ágar inoculado. C. preparo dos poços com molde cilíndrico de 5 mm esterilizado.

D. preenchimentos dos poços com os materiais estudados. E. Fotoativação dos materiais. F. Halos de inibição após 24 horas.

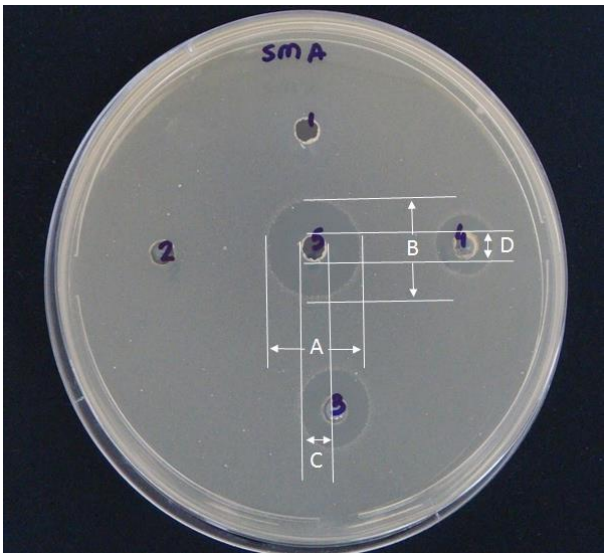

Figura 4. Medida do halo de inibição com paquímetro digital. Cálculo do halo de inibição (mm): [(A+B)/2] – [(D+C)/2].

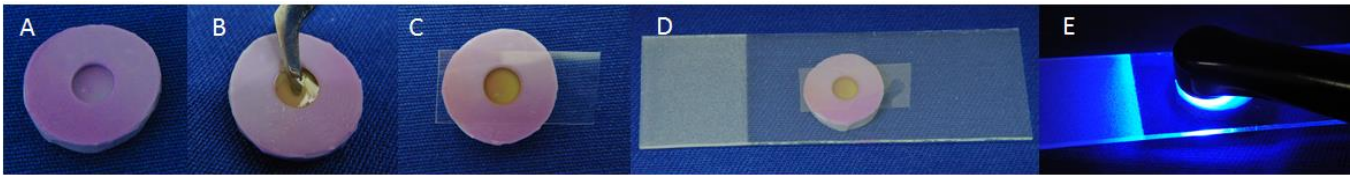

Figura 5. A. molde de silicone de adição leve. B. preenchimento do molde com o material infiltrante. C. colocação de tira matriz de poliéster. D. colocação de lâmina de vidro. E. fotoativação.

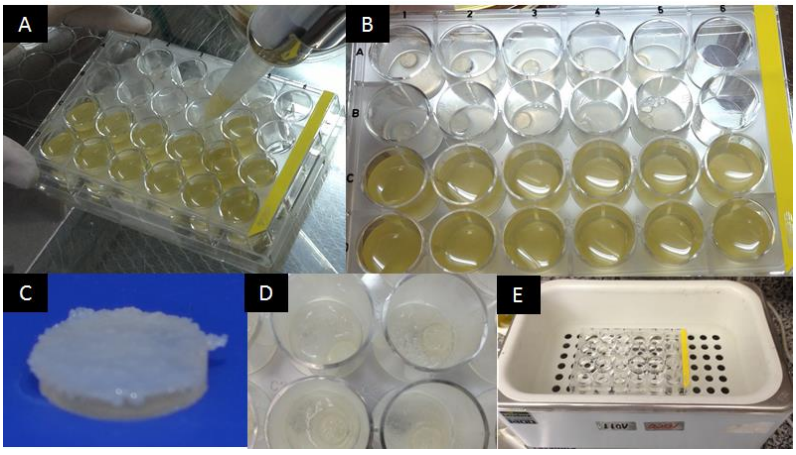

Figura 6. Biodegradação. A e B. preparo dos poços com as amostras dos materiais e preenchimento com BHI suplementado com 1% de sacarose, realizada troca diária por 7 dias. C e D. após 7 dias a formação do biofilme de *Streptococcus mutans*. E. lavagem com ultrassom das amostras com água deionizada por 10 minutos.

Figura 7. A. Espectrocospia Transformada de Fourier – FTIR. B. análise do material infiltrante não polimerizado. C. análise do material infiltrante polimerizado.

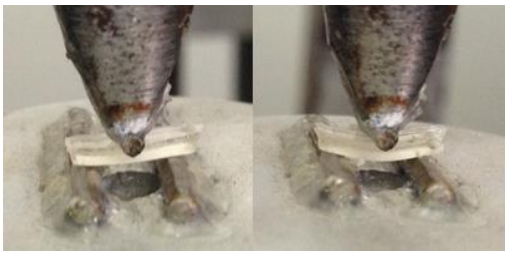

Figura 8. Teste de módulo de elasticidade.

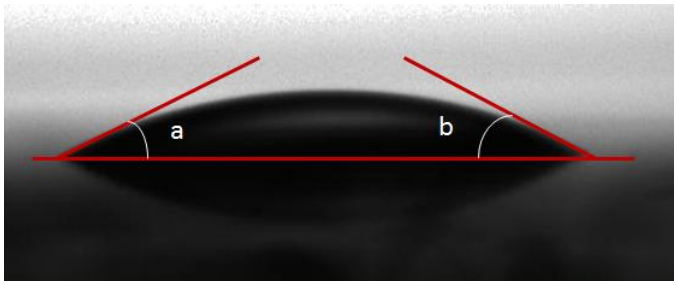

Figura 9. Análise do ângulo de contato. Cálculo do ângulo de contato (º): (a+b)/2.

ANEXOS 1

De: **Dental Materials** (dentistry.dentmatj@manchester.ac.uk)
Enviada: quarta-feira, 10 de fevereiro de 2016 13:05:58
Para: vanb09@hotmail.com

Dear Dr. Vanessa Dainezi,

You have been listed as a Co-Author of the following submission:

Journal: Dental Materials
Corresponding Author: Regina Puppin-Rontani
Co-Authors: Vanessa B Dainezi, DDS, MS, PhD Student; Luciana T Inagaki, DDS, MS, PhD; Andreia B de Paula, DDS, MS, PhD; Fernanda M Pascon, DDS, MS, PhD;
Title: Effect of chlorhexidine-adding commercial infiltrant on biological, physical and chemical properties

If you did not co-author this submission, please contact the Corresponding Author of this submission at rmpuppin@fop.unicamp.br;rmpuppin@gmail.com; do not follow the link below.

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Piracicaba, 18 de novembro de 2015.

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