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NADINE LUÍSA GUIMARÃES ALBUQUERQUE

EFEITO DA INCORPORAÇÃO DE MICROPARTÍCULAS POLIMÉRICAS CARREGADAS COM CATEQUINA NAS PROPRIEDADES FÍSICO-QUÍMICAS DE UM SISTEMA ADESIVO

FORTALEZA
2015

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

Orientador: Prof. Dr. Sérgio Lima Santiago.
Coorientadores: Profa. Dra. Monica Yamauti e Prof. Dr. Francisco Fábio Oliveira de Sousa.

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Aprovada em: ___/___/_____.

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RESUMO

O objetivo desse estudo *in vitro* foi avaliar o efeito da incorporação de micropartículas poliméricas carregadas com Epigalocatequina-3-galato (EGCG) nas propriedades físico-químicas de sistema adesivo convencional de 2 passos. Primeiramente, realizou-se o grau de conversão (%GC) pelo Espectrofotômetro FT-IR e ensaio de liberação dos adesivos para avaliar o desempenho das micropartículas poliméricas carregadas com EGCG (Experimento 1). Para o ensaio de liberação, alíquotas de cada amostra foram coletadas e quantificadas em termos de liberação de EGCG por meio do Espectrofotômetro UV-Vis. No Experimento 2, quarenta e cinco terceiros molares humanos foram divididos em 5 grupos (n=9) de acordo com a solução de pré-tratamento utilizada (água destilada, solução aquosa de EGCG a 0,1% e solução aquosa de micropartículas carregadas com EGCG (PLGA50:50/EGCG) a 1,0%) e sistema adesivo Adper Single Bond 2 (3M ESPE) aplicado (contendo EGCG puro a 0,1%, micropartículas carregadas com EGCG a 1,0% ou na sua forma original como controle). Cinco incrementos de 1 mm de espessura de resina composta foram aplicados e fotoativados individualmente por 40 s. Os dentes foram armazenados em água destilada a 37ºC por 24h. Após armazenamento, foram seccionados longitudinalmente em ambos os sentidos para obter espécimes em forma de palitos com a área de secção transversal de aproximadamente 1 mm^2. Cada espécime foi tracionado a uma velocidade de 0,5 mm/min em uma máquina de ensaios universais. Os valores de resistência de união e %GC foram avaliados estatisticamente por ANOVA, com nível de significância de 5%. Não houve diferença estatística significante entre as médias do grau de conversão após a incorporação de micropartículas poliméricas carregadas com EGCG no sistema adesivo ($p>0,05$). Em relação ao ensaio de liberação dos adesivos, o grupo PLGA50:50/EGCG a 1,0% apresentou melhores resultados, alcançando a maior liberação em termos quantitativos, sendo o escolhido para ser usado no teste de resistência de união (Experimento 2). Após 24 h de armazenamento, nenhuma diferença estatisticamente significante foi encontrada entre as médias dos valores de resistência de união dos grupos testados ($p>0,05$). Concluindo, a incorporação das micropartículas poliméricas carregadas com EGCG não interferiu no grau de conversão dos adesivos. O sistema adesivo com micropartículas carregadas com EGCG incorporado em sua composição foi capaz de liberar EGCG. Porém, o flavonóide Epigalocatequina-3-galato (EGCG) não teve efeito quando incorporado ao sistema adesivo convencional ou aplicado como pré-tratamento da dentina, de forma pura e microencapsulada, na resistência de união imediata.

Palavras-chave: adesivos, camellia sinensis, polímeros, catequina, metaloproteinases da matriz.

ABSTRACT

The aim of this *in vitro* study was to evaluate the performance of polymeric microparticles loaded with Epigallocatechin-3-gallate (EGCG) on the physicochemical properties of a two-step etch-and-rinse adhesive system. First, the degree of conversion (%DC) was evaluated by FT-IR spectrophotometry and release assay of adhesives to evaluate the performance of EGCG loaded PLGA microparticles was realized (Experiment 1). For release assay, aliquots were collected of each samples and quantified in terms of EGCG release at pre-defined times by means of UV-Vis Spectrophotometer. In Experiment 2, forty-five molars were divided into 5 groups (n=9) according to the rewetting solution used (distilled water, 0.1% EGCG aqueous solution and 1.0% microparticles aqueous solution (PLGA50:50/EGCG)) and the Adper Single Bond 2 adhesive system used (containing 0.1% free EGCG, 1.0% EGCG loaded PLGA microparticles or in original form as control). Five 1-mm-thick increments of composite resin were build up and light-cured for 40 s individually. The teeth were stored in distilled water at 37ºC for 24 h. After storage, they were longitudinally sectioned in both directions to obtain bonded sticks with a cross-sectional area of approximately 1.0 mm^2. Each bonded stick was testing to a tensile force of 0.5 mm/min in the universal testing machine. %DC and μTBS values were statistically analyzed with ANOVA, with significance level of 5%. There was no statistically significant difference between the DC means after PLGA-microparticles loaded with EGCG incorporated ($p>0.05$). In relation to release assay, the 1.0% PLGA50:50/EGCG group presented better results, achieving the highest release in quantitative terms, being the elect to be used in bond strength test (Experiment 2). After 24 h of storage, there was no statistically significant difference between the mean bond strength values of the tested groups ($p>0.05$). The incorporation of the polymeric microparticles loaded with EGCG did not interfere in the adhesive degree of conversion. The adhesive system loaded microparticles EGCG incorporated in its composition was able to release EGCG. However, the flavonoid epigallocatechin-3-gallate (EGCG) had no effect when incorporated into etch-and-rinse adhesive system or applied as dentin pretreatment, on free and microencapsulated forms, in the immediate bond strength.

Keywords: adhesives, camellia sinensis, polymers, catechin, matrix metalloproteinases.

SUMÁRIO

1 INTRODUÇÃO GERAL

Os sistemas adesivos têm demonstrado resultados promissores na prática clínica diária. Entretanto, apesar de todo o arsenal que temos à disposição para uma adequada adesão, ainda existe uma dificuldade para se conseguir união entre os materiais restauradores e as estruturas dentárias em virtude da heterogeneidade dos substratos envolvidos (PASHLEY, TAY, IMAZATO, 2011). O esmalte é considerado um substrato com maior conteúdo mineral e de morfologia homogênea possibilitando uma adesão efetiva, enquanto a dentina é mais complexa, apresentando menor conteúdo mineral, de natureza heterogênea e intrinsecamente úmida (VAN MEERBEECK *et al*., 2003). Essa umidade tem papel importante na degradação da interface adesiva e na redução das propriedades mecânicas da adesão (PEREIRA *et al*., 1999; CARRILHO *et al*., 2004). Além disso, ainda existem outros desafios para uma boa adesão como a nanoinfiltração que é a diferença entre a zona de dentina desmineralizada pelo ácido e a zona infiltrada pelo adesivo durante a formação da camada híbrida, gerando fibras colágenas expostas e desprotegidas tornando-as susceptíveis à ação dos fluidos orais (SANO *et al*., 1995; WANG e SPENCER, 2003; DE MUNCK *et al*., 2005); e a permeabilidade dos adesivos dentinários (TAY, PASHLEY, YOSHIYAMA, 2002).

Shono *et al*., em 1999, publicaram um dos primeiros estudos que avaliou a durabilidade da união resina-dentina ao longo do tempo. Demonstraram, através de microscopia eletrônica de varredura, um aumento da porosidade no topo da camada híbrida e no interior da interface adesiva. A partir disso, vários estudos têm demonstrado perda da resistência de união ao longo do tempo (LOGUERCIO, STANISLAWCZUK, POLLI, 2009; HASHIMOTO, 2010; PASHLEY *et al*., 2011b; YIU *et al*., 2012). Esse fato tem sido atribuído principalmente à degradação da camada híbrida na interface dentina/resina (CARRILHO *et al*., 2007; DE MUNCK *et al*., 2009; DE MUNCK *et al*., 2010; ZOU, JESSOP, ARMSTRONG, 2010) como resultado da degradação hidrolítica do adesivo resinoso e da proteólise das fibrilas colágenas (BURROW, TAGAMI, HOSODA, 1993; HASHIMOTO, OHNO, KAGA, 2000; OKUDA *et al*., 2002; OSORIO *et al*., 2005; BRESCHI *et al*., 2008; ERHARDT, OSORIO, TOLEDANO, 2008; OSORIO *et al*., 2011).

Essa degradação das fibrilas colágenas expostas na interface adesiva se dá pelas metaloproteinases de matriz (MMPs), enzimas endógenas presentes na dentina, responsáveis pela organização e mineralização da matriz dentinária (CHAUSSAIN-MILLER *et al*., 2006; CARRILHO *et al*., 2007; STANISLAWCZUK *et al*., 2009; BRESCHI *et al*., 2010a; YIU *et al*., 2012). Estas se encontram inativas e são ativadas em baixo pH, ou seja, quando é realizado o condicionamento ácido dos sistemas adesivos convencionais ou na aplicação dos adesivos autocondicionantes (TURK *et al*., 1995; TJÄDERHANE *et al*., 1998; VAN STRIJP *et al*., 2003; VISSE, NAGASE, 2003; PASHLEY *et al*., 2004; NISHITANI *et al*., 2006; TERSARIOL *et al*., 2010; LIU *et al*., 2011; TJÄDERHANE *et al*., 2013). Alguns estudos confirmaram a presença da MMP-2, MMP-3, MMP-8 e MMP-9 em dentina humana desmineralizada (PASHLEY *et al*., 2004; NISHITANI *et al*., 2006; MAZZONI *et al*., 2007; SULKALA *et al*., 2007 STANISLAWCZUK *et al*., 2009; BRESCHI *et al*., 2010b). A MMP-2, também conhecida como Gelatinase A, degrada colágeno tipo I, principal colágeno encontrado na dentina e a MMP-9, ou Gelatinase B, degrada o colágeno tipo IV, que é o principal componente do colágeno desnaturado (MORGUNOVA *et al*., 1999; CHAUSSAIN-MILLE *et al*., 2006). Já a MMP-8, também denominada de colagenase-2, foi identificada por Sulkala *et al*., em 2007, como a principal enzima colagenolítica da dentina humana. Mais recentemente, outro grupo de enzimas proteolíticas foi identificado na dentina humana indicando que a atividade colagenolítica da dentina não se dá apenas pela presença das MMPs, mas também pela atividade das cisteínas catepsinas (TERSARIOL *et al*., 2010; NASCIMENTO *et al*., 2011).

Portanto, durante os últimos anos, o entendimento dos mecanismos que envolvem a degradação proteolítica da interface adesiva tem ganhado imensa atenção e rapidamente vêm crescendo o interesse da comunidade científica em desenvolver estratégias para aumentar a longevidade clínica das restaurações adesivas. Assim, o uso de inibidores da atividade enzimática tem sido aceito como uma estratégia eficaz para melhorar a longevidade das restaurações adesivas (LOGUERCIO, STANISLAWCZUK, POLLI, 2009; OSORIO *et al*., 2011; YIU *et al*., 2012; SANTIAGO *et al*., 2013).

Uma das substâncias que tem sido apontada como potencial inibidora de metaloproteinases é o digluconato de clorexidina (HEBLING *et al*., 2005; CARRILHO

et al., 2007; LOGUERCIO, STANISLAWCZUK, POLLI, 2009; OSORIO *et al*., 2011). A clorexidina é uma molécula sintética que se liga a várias proteínas por meio de um mecanismo quelante e pode inibir a ação dessas enzimas mesmo em baixas concentrações, desacelerando assim o processo de degradação da interface de união (CARRILHO *et al*., 2007; LOGUERCIO, STANISLAWCZUK, POLLI, 2009). O primeiro estudo que constatou a benéfica ação do digluconato de clorexidina usado como pré-tratamento da dentina foi realizado por Hebling *et al*., em 2005. Seus resultados mostraram uma diminuição da degradação de colágeno da camada híbrida se comparado à técnica original de condicionamento ácido e posterior aplicação do adesivo. Em seguida, diversos estudos apontaram a clorexidina como potente inibidor das metaloproteinases seja como solução para pré-tratamento da dentina (CARRILHO *et al*., 2007; LOGUERCIO, STANISLAWCZUK, POLLI, 2009) ou incorporada ao sistema adesivo (YIU *et al*., 2012). No entanto, há uma preocupação na aplicação de digluconato de clorexidina em tecidos humanos no que se refere à segurança biológica, sendo necessária a procura por inibidores mais biocompatíveis (FARIA, CARDOSO, LARSON, 2009).

Na busca por outras substâncias viáveis para uso odontológico, com a característica de inibição da atividade enzimática, o flavonóide Epigalocatequina-3-galato (EGCG) mostrou-se altamente eficaz na inibição da expressão e ação das MMPs -2 e -9 (GARBISA, BIGGIN, CAVALLARIN, 1999; DELL'AICA *et al*., 2007) além de diminuir a atividade das cisteínas catepsinas (DEVIKA, PRINCE, 2008; KATO *et al*., 2012). O EGCG é o principal polifenol encontrado no chá verde (*Camellia sinensis*) e apresenta ação antioxidante, antimicrobiana, antimutagênica, anticancerígena e anti-inflamatória, sendo considerado pouco tóxico, mesmo em altas concentrações (ISBRUCKER *et al*., 2006; HIRASAWA, TAKADA, 2003; RASHEED *et al*., 2009). O uso de EGCG como uma solução de pré-tratamento dentinário, associado ao sistema adesivo convencional de 2 passos (SANTIAGO *et al*., 2013) e autocondicionante de 1 passo (dados não publicados) resultou na manutenção da resistência de união à dentina após 6 meses de armazenamento. Du *et al*. (2012) avaliaram o efeito da incorporação do EGCG em um sistema adesivo convencional de 2 passos e obteve a manutenção da resistência de união à dentina após 6 meses de armazenamento. Adicionalmente, foi demonstrado que a incorporação desse inibidor de MMPs, em baixas concentrações

(abaixo de 0,2%), não promoveu alterações significativas no grau de conversão dos monômeros resinosos (DU *et al*., 2012; PALLAN *et al*., 2012). Embora, a incorporação de EGCG aos sistemas adesivos tenha demonstrado resultados promissores, há uma preocupação em relação à disponibilidade da catequina dentro da camada híbrida. Sendo o EGCG uma molécula solúvel em água (solubilidade 5mg/ml) (Sigma-Aldrich, St. Louis, MO, USA) foi observada uma alta taxa de liberação nas primeiras 24 horas de exposição à agua destilada, seguida de uma redução significativa na taxa de liberação até o 28° dia (PALLAN *et al*., 2012). Portanto, o tempo de permanência do EGCG na camada híbrida pode ser curto devido à ação da água (PALLAN *et al.*, 2012). Contudo, esse inconveniente pode ser contornado a partir de métodos de liberação controlada de fármacos (LIANG, WONG, BURT, 2005; GAIGNAUX *et al*., 2012).

Para isso, buscou-se a utilização de micropartículas poliméricas, produzidas a partir de alguns polímeros, como o ácido poli láctico-co-glicólico (PLGA), padrão-ouro na liberação controlada de fármacos, devido sua incomparável biodegradação e biocompatibilidade (PRIOR *et al*., 2000; BLANCO-PRIETO *et al*., 2002; GRAVES *et al*., 2004; SCHNIEDERS *et al*., 2006). A incorporação de micropartículas poliméricas carregadas com EGCG ao sistema adesivo convencional pode ser uma estratégia eficaz se comparado à incorporação de EGCG puro ao sistema adesivo. Além de uma liberação mais prolongada e controlada, apresenta vantagem em relação à proteção do fármaco frente aos processos fisiológicos degradativos e à bioabsorção, visto que a ocorrência desses fenômenos acarretaria na perda da atividade farmacológica (PRIOR *et al*., 2000; BLANCO-PRIETO *et al.*, 2002; GRAVES *et al*., 2004; SCHNIEDERS *et al*., 2006).

Diante do exposto, faz-se necessário que as técnicas adesivas sejam reformuladas ao longo do tempo justificando-se o estudo do flavonóide Epigalocatequina-3-galato como agente inibidor da atividade enzimática conseguindo assim, a preservação da camada híbrida e consequentemente, maior durabilidade das restaurações adesivas.

Dessa forma, o objetivo do presente estudo foi realizar testes físico-químicos como, grau de conversão e ensaio de liberação, dos adesivos incorporados com micropartículas poliméricas carregadas com EGCG e comparar o efeito do flavonóide Epigalocatequina-3-galato (EGCG), na forma pura ou microencapsulada, incorporado

ao sistema adesivo convencional ou aplicado como agente de pré-tratamento dentinário, na resistência de união à dentina.

2 PROPOSIÇÃO

2.1 Objetivo Geral

Avaliar o efeito da incorporação de micropartículas poliméricas carregadas com Epigalocatequina-3-galato (EGCG) nas propriedades físico-químicas de sistema adesivo convencional de 2 passos.

2.2 Objetivos Específicos

- Avaliar o grau de conversão dos monômeros em polímeros dos adesivos em sua composição original e nas formulações contendo as micropartículas carregadas com EGCG.
- Analisar a liberação do princípio ativo a partir do adesivo convencional contendo micropartículas carregadas com EGCG.
- Avaliar o efeito do EGCG na resistência de união imediata à dentina, através de ensaio de microtração.

3 CAPÍTULO

Esta dissertação está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de um estudo envolvendo dentes humanos, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em pesquisa da Universidade Federal do Ceará, tendo sido aprovado, conforme o parecer consubstanciado nº 459.659 de 14 de novembro de 2013 (ANEXO B).

Assim sendo, esta dissertação é composta de um capítulo contendo um artigo científico que será submetido ao periódico Dental Materials (ANEXO A) conforme descrito na sequência:

Adhesive system containing polymeric microparticles loaded with catechin: Physicochemical characterisation.

Albuquerque NLG, Neri JR, Yamauti M, Sousa FFO, Santiago SL.

CAPÍTULO 1

Adhesive system containing polymeric microparticles loaded with catechin: Physicochemical characterisation.

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ABSTRACT

Objective. Evaluate the performance of polymeric microparticles loaded with Epigallocatechin-3-gallate (EGCG) on degree of conversion and release assay of adhesives and examine resin–dentin bond strength with different EGCG application modes.

Methods. The degree of conversion (%DC) was evaluated by FT-IR spectrophotometry and release assay of adhesives containing different proportions of EGCG loaded PLGA microparticles was performed. Aliquots were collected of each samples and quantified in terms of EGCG release at pre-defined times by means of UV-Vis Spectrophotometer (Experiment 1). After that, forty-five molars were divided into 5 groups (n=9) according to EGCG different application modes. The teeth were prepared to bonding and testing in the universal testing machine (Experiment 2). %DC and μTBS values were statistically analyzed with ANOVA.

Results. There was no statistically significant difference between the DC means after PLGA-microparticles loaded with EGCG incorporated ($p>0.05$). The 1.0% PLGA50:50/EGCG group presented better results, achieving the highest release in quantitative terms, being the elect to be used in bond strength test. After 24 h of storage, there was no statistically significant difference among the mean bond strength values of the tested groups ($p>0.05$).

Significance. EGCG can be effective in improving the longevity of adhesive restorations therefore, their permanence time in the hybrid layer can be short due to its solubility in water. Hence, the strategy to produce polymeric microparticles loaded with EGCG to achieve a more controlled and prolonged release.

Keywords: adhesives, camellia sinensis, polymers, catechin, matrix metalloproteinases.

1. Introduction

Studies have shown that adhesive systems lose their bond to dentin over time, and there is a consensus that the hybrid layer created by current adhesive systems is imperfect, susceptible to degradation and, could negatively affect the bond strength [1,2]. The decrease of bond strength is related to a hydrolytic degradation of the polymers of the adhesive systems and the proteolysis of collagen matrix of the hybrid layer [3]. Transmission electron microscopy analyses showed that almost 70% of collagen from the adhesive interface disappears after 44 months water storage [4]. Host-derived proteases (matrix metalloproteinases and cysteine cathepsins) with collagenolytic activity in hybrid layers are the mainly responsible by degradation of collagen fibrils reducing the longevity of clinically applied resin-based restorations [2,5,6].

Therefore, studies have focused their research on the modification of dental adhesives to improve the durability of bonding to dentin of resin-based restorations [1,2]. The use of protease inhibitors on dentin surface after acid etching or incorporation into the adhesive system has been well accepted [7-11]. Chlorhexidine (CHX) was the first protease inhibitor proposed for preserve the hybrid layer through the inhinition of MMPs [12] and cysteine cathepsins [13] but recently other inhibitors has received increased attention from researchers.

Epigallocatechin-3-gallate (EGCG) is a natural substance, the main polyphenol found in green tea (*Camellia sinensis*), potential MMP-inhibitory with low toxicity and anti-inflammatory properties [14]. Du and others [10] incorporated EGCG in different concentrations into etch-and-rinse adhesive system and evaluated the antimicrobial effect and the physicochemical properties of dentin bonding over time. Similarly, Neri and others [15] evaluated the influence of EGCG incorporation on the physicochemical properties of a self-etch dental adhesive and observed that the addition of EGCG to adhesive reduced the solubility without affecting degree of conversion and flexural strength. Santiago and others [11] evaluated the effect of dentin pretreatment with EGCG solutions in the preservation of the adhesive interface with etch-and-rinse adhesive systems. All the studies showed that the use of epigallocatechin-3-gallate

flavonoid is effective in improving the longevity of adhesive procedures independent of adhesive system.

In attempt to achieve a release of EGCG in a more controlled and prolonged as possible, sought the use of polymeric microparticles produced by polymers as Poly (D-L lactide-co-glycolide) acid (PLGA), one of the main polymers used in the development of release systems. It considered gold standard in controlled drug delivery, mostly due to its superior biocompatibility [16-19]. Besides be used in the development of release systems, this polymer has others applications as: prevention of postsurgical adhesions through of application of nanofibers of poly (lactic-co-glycolic acid) (PLGA) loaded with epigallocatechin-3-O-gallate (EGCG) [20] and enhance wound healing by accelerating cell infiltration, re-epithelialization and angiogenesis by eletroctrospum membranes composed of PLGA containing 1 wt% EGCG [21]. Thus, the use of polymeric microparticles loaded with EGCG can be an effective strategy in comparison to free EGCG due to the protection of the drug from the physiological degradation and bioadsorption, avoiding the loss of pharmalogical activity. In addition, the controlled drug delivery systems could contribute to a prolonged effect of the drug in the specific therapeutic site.

Therefore, the aim of this study was to evaluate the effect of different EGCG application modes on the resin-dentin bonds. To achieve this objective, the study was divided into two experiments. The objective of the first experiment was to evaluate the performance of polymeric microparticles loaded with EGCG on degree of conversion and release assay of adhesives and the second experiment was to examine early resin–dentin bond strength with two EGCG formulations applied as pretreatment or incorporated into etch-and-rinse adhesive system. The null hypotheses were: (1) incorporation of polymeric microparticles loaded with EGCG have no effect on adhesive degree of conversion; (2) the incorporation of PLGA-microparticles loaded with EGCG into adhesive will not be able to release EGCG; (3) the use of EGCG in free form or loaded into PLGA-microparticles as dentin pretreatment or incorporated into the adhesive does not affect the immediate bond strength.

2. Materials and Methods

2.1. Epigallocatechin-3-gallate microparticles used

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

Epigallocatechin-3-gallate (EGCG) (Sigma Aldrich, St. Louis, MO, USA) was added to different formulations of microparticles using two types of Poly (D-L lactide-co-glycolide) acid (PLGA, Sigma Aldrich, St. Louis, MO, USA): Resomer® RG502H (PLGA 50:50) and Resomer® RG756S (PLGA 75:25) by means of spray-drying technique [22].

Formulations were prepared in the ratio PLGA:EGCG 16:1. A blank formulation (PLGA only) from each polymer was obtained and used as a reference. Due to differences in solubility among the drugs and polymers, an emulsification process was proposed. Briefly, PLGA was dissolved in dichloromethane (DCM) and EGCG was dissolved in ethyl acetate in another vial. The solutions were mixed in a disperser system (Ultraturrax IKA T10B; IKA/Works, Inc. NC, USA) and immediately spray dried in a laboratory spray drier (Mini Spray Drier Buchi 290, Buchi, Flawil, Switzerland).

After the procedure, microparticles were collected, weighted to determine the yield of production and stored into clean sealed glass vials. Particle size, morphology, encapsulation efficacy and drug loading were also determined (unpublished data).

Table 1 – Materials and chemicals used in the study.

Material (/Manufacturer)	Batch number	Basic formulation
Adper Single Bond 2* (3M/ESPE®, St. Paul, MN, EUA)	1312201025	Etchant: 35% phosphoric acid (batch #1219600378). Bis-GMA, HEMA, dimethacrylates, silica nanofiller (5 nm), polyalquenoic acid copolymer, initiators, water and ethanol.
Resin Filtek Z250 XT (3M/ESPE®,St. Paul, MN, EUA)	37277	Bis-EMA, Bis-GMA and UDMA. Filled to 60% by volume with zirconia silica filler, average.particle size = 0.6 μm
Poly (D-L lactide – co – glycolide acid) Resomer® RG502H / Sigma Aldrich, St. Louis, MO, USA	STBD2887V	----------
Poly (D-L lactide – co – glycolide acid) Resomer® RG756S / Sigma Aldrich, St. Louis, MO, USA	STBC6378V	----------
Epigallocatechin-3-gallate (Sigma Aldrich, St. Louis, MO, USA)	SLBL1959V	----------

* This brand name is the same product as Adper Scotchbond 1XT, Adper Single Bond Plus and Adper Single Bond 1 XT.

2.2. Preparation of adhesive formulations

The adhesive formulations were prepared by incorporating PLGA microparticles loaded with EGCG into Adper Single Bond 2 adhesive system (3M/ESPE®, St. Paul, MN, EUA) by manual blending. The amount of microparticles incorporated into the adhesives ranged between 0.5, 1.0 and 2.0% (w/w). Each adhesive formulation (Table 2) briefly mixed in a vortex (Biomixer QL-901, SP, Brazil) for 1 min at reduced ambient light.

Table 2 – Description of the experimental groups of Experiment 1 (Degree of conversion and Release assay of adhesives containing microencapsulated EGCG).

GROUPS	MATERIAL	MICROENCAPSULATED FORMS OF EGCG
Control	Adper Single Bond 2	Control
0.5% PLGA50:50/EGCG		Containing 0.5% (w/w) of microparticles (PLGA50:50) of EGCG
0.5% PLGA75:25/EGCG		Containing 0.5% (w/w) of microparticles (PLGA75:25) of EGCG
1.0% PLGA50:50/EGCG		Containing 1.0% (w/w) of microparticles (PLGA50:50) of EGCG
1.0% PLGA75:25/EGCG		Containing 1.0% (w/w) of microparticles (PLGA75:25) of EGCG
2.0% PLGA50:50/EGCG		Containing 2.0% (w/w) of microparticles (PLGA50:50) of EGCG
2.0% PLGA75:25/EGCG		Containing 2.0% (w/w) of microparticles (PLGA75:25) of EGCG

Experiment 1

2.3. Characterization of the formulations

In order to establish the maximum microparticles loading into the adhesive system, a study was conducted to determine the degree of conversion and release assay of adhesives containing different proportions of EGCG loaded PLGA microparticles.

2.3.1. Degree of conversion

The degree of conversion (DC) of the adhesive resins was assessed by Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer Spectrum 100, Perkin Elmer, Shelton, CT, USA). Each adhesive system was dispensed into a small agate mortar and thoroughly mixed with potassium bromide (KBr), at a ratio of 4:100. The pellets of KBr/adhesive solution were prepared with a hand press (Hand Press Kit 161-1100, PIKE Technologies, Madison, WI, USA). FTIR spectrum of the uncured adhesive was obtained from each sample using 32 scans in a range of 4000-400 cm^{-1}, at 4 cm^{-1} resolution in transmission mode.

The adhesive system was light-activated for 40 s using the light source (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA). Additional FTIR spectra were obtained immediately after light curing. The analyses were performed at 25 °C with 70% relative humidity. Tree specimens per group (n=3) were tested. The rate of unreacted carbon-carbon double bonds (C=C) was determined from the ratio of absorbance intensities of aliphatic C=C (peak at 1636 cm^{-1}) against an internal standard (aromatic carbon-carbon bond peak at 1608 cm^{-1}) before and after curing. Degree of conversion was determined by subtracting the C=C from 100%.

2.3.2. Release assay of adhesives containing microencapsulated EGCG

In view of the difference in hydrophobicity and also the biodegradation rates of the two PLGA (PLGA 50:50 and PLGA 75:25) used in this study, there was a release assay in order to observe their performance into the adhesive system (Adper Single Bond 2).

A calibration curve was used to quantify the drug from a series of reference solutions ranging from 2.5 to 40 ppm, resulting in a linear relationship between absorbance peak height and drug concentration. A UV-Vis Spectrophotometer (DU-730; Beckman Coulter, Fullerton, CA, USA) was used to evaluate and confirm the absorbance peak of EGCG at 275 nm.

Individual Teflon matrix (6.0 mm diameter x 1.0 mm thick) was used to enclose the adhesives formulations, which were used in triplicate. The matrix was filled

with each adhesive formulation, a mylar strip was placed in the top of it and glass slide was placed to perform the light-curing process. The material was light-cured (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA) for 40 s at 600 mW/cm^2. Samples were stored in individual vials containing 1 mL of distilled water at 37ºC stored until 2904 h.

Experiment 2

2.4. Microtensile Bond Strength Test

For the second experiment, forty-five (45) extracted, caries-free human third molars were used. The teeth were collected after the patient's informed consent had been obtained under a protocol reviewed and approved by the local Research and Ethics Committee. The selected teeth were stored for about one month after extraction in 0.01% (w/v) thymol solution.

The occlusal enamel of each tooth was removed using a slow-speed diamond saw (IsoMet; Buehler, Lake Bluff, IL, USA) under water-cooling in order to expose a flat coronal dentin surface. The enamel-free, exposed dentin surfaces were further polished on wet #600-grit SiC paper for 60 s to standardize the smear layer.

The teeth were divided into 5 groups (n=9) according to different EGCG application modes (rewetting solution (RS) or incorporated adhesive system used). The type (PLGA 50:50 or PLGA 75:25) and concentration of microparticles of EGCG/PLGA (0.5%, 1.0% or 2.0%) were established in the Experiment 1. The concentration of free EGCG was in accordance with previous studies [11,15]. The exposed dentin surfaces of all teeth were etched with 35% phosphoric acid gel (Scotchbond Phosphoric Acid Etchant; 3M ESPE, St. Paul, MN, USA) for 15 s, rinsed for 30 s with distilled water, and dried with oil-water-free air for 30 s. The teeth were then treated with 20 µL of one of the following rewetting solutions: distilled water; 0.1% epigallocatechin-3-gallate aqueous solutions (EGCG) and 1.0% microparticles aqueous solution (PLGA50:50-EGCG) (Table 3). The solutions were left in contact with the tooth surface for 60 s, and excess was removed with absorbent paper, leaving the dentin surface visibly moist.

Table 3 – Description of the experimental groups of Experiment 2 (Bond strength test).

GROUPS	DENTIN PRETREATMENT	ADHESIVE SYSTEM
Control	Distilled water	Adper Single Bond 2
SB+0.1% free EGCG	Distilled water	Containing 0.1% (w/w) of free EGCG.
SB+1.0% PLGA50:50/ EGCG	Distilled water	Containing 1.0% (w/w) of microparticles (PLGA50:50-EGCG)
RS 0.1% free EGCG	Epigallocatechin-3-gallate aqueous solutions (0.1% EGCG)	Adper Single Bond 2
RS 1.0% PLGA50:50/ EGCG	Microparticles aqueous solution (1.0% PLGA50:50/EGCG)	Adper Single Bond 2

The etch-and-rinse adhesive system Adper Single Bond 2 (3M ESPE®) was then applied according to the manufacturer's instructions. After light curing the adhesive (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA), five 1-mm-thick increments of composite resin were build up (Filtek Z250 XT - 3M/ESPE®). Each increment was light cured (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA) for 40 s at a power density of 600 mW/cm². The bonded teeth were stored in distilled water at 37ºC for 24 h.

After storage, they were longitudinally sectioned in both 'x' and 'y' directions across the bonded interface using a diamond saw (Isomet), under a water cooling to obtain bonded sticks with a cross-sectional area of approximately 1.0 mm². The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm and recorded for subsequent calculation of the bond strength. One third of he bonded sticks were tested immediately. For testing, each bonded stick was attached to a jig in the universal testing machine (Emic, São José dos Pinhais, PR, Brazil) with cyanoacrylate resin (SuperBonder® flex gel, Loctite, Itapevi, SP, Brazil) and subjected to a tensile force of 0.5 mm/min. The load of fracture, expressed in MPa, was used to calculate the bond strength (μTBS).

The failure modes were evaluated at 80X magnification (StereoZoom Leica S8 APO, Leica Microsystems; Wetzlar, Hesse, Germany) and classified as cohesive (failure exclusively within the dentin or composite; C), adhesive (A) when failure occurred at the dentin/adhesive interface, ou mixed (M) when two modes of failure occurred simultaneously.

2.5. Statistical Analysis

DC and μTBS values were submitted a Shapiro-Wilk test to analyze the normal distribution of errors. As normal distribution was confirmed, data were analyzed using a statistical analysis of variance one-way ANOVA. Statistical procedures were performed with the SigmaStat 3.5 for Windows statistical program software (Systat Software, San Jose, California, USA). The significance level was set at $p < 0.05$ for all tests.

3. Results

3.1. Degree of conversion

Table 4 showed the DC means and standard deviations for dental adhesives. There was no statistically significant difference between the DC means after PLGA-microparticles loaded with EGCG incorporated ($p > 0.05$).

Table 4 - Mean and standard desviation of the tested groups.

EGCG microencapsulated forms incorporated in SB	Mean (SD)
Control	58.32 (0.38)
0.5% PLGA50:50/EGCG	57.29 (0.73)
0.5% PLGA75:25/EGCG	57.64 (0.73)
1.0% PLGA50:50/EGCG	58.11 (0.32)
1.0% PLGA75:25/EGCG	58.10 (0.89)
2.0% PLGA50:50/EGCG	59.07 (0.57)
2.0%PLGA75:25/EGCG	57.61 (0.50)

3.2. Release assay of adhesives containing microencapsulated EGCG

EGCG release profiles of adhesive system are shown in Fig. 1. In groups 0.5% PLGA50:50/EGCG and 1.0% PLGA50:50/EGCG a controlled release was observed reaching the total release (100%) during the assayed period. In contrast, among the PLGA75:25 groups, the highest release obtained did not reach more than 60% of the overall drug content (0.5% PLGA75:25/EGCG).

In all groups, except from 1.0% PLGA75:25/EGCG and 2.0% PLGA75:25/EGCG was observed a pulsatile release profile, characterized by moments of cessation and subsequent rapid increase of release at 300, 600 and 900 h. In 1.0% PLGA75:25/EGCG and 2.0% PLGA75:25/EGCG groups this perfomance was more discreet and less perceived in Fig. 1.

The 1.0% PLGA50:50/EGCG group showed the best results, achieving the highest release in quantitative terms, reaching the highest released mass (77.30 mcg), among all studied groups and completing the full release at the end of 90 days.

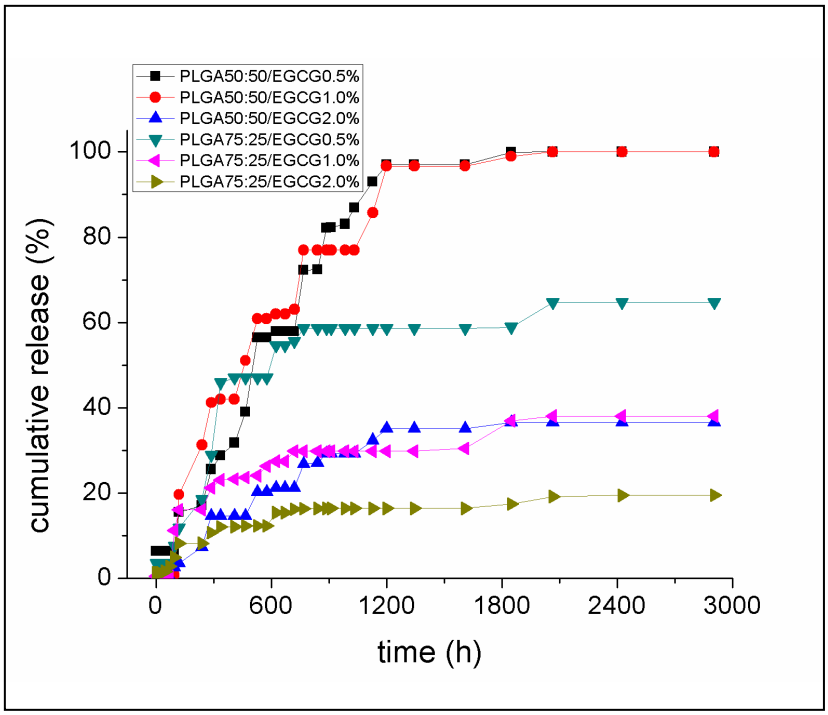

Fig. 1 - Cumulative EGCG release (%) from adhesive system in aqueous medium during the entire evaluation period (2904 h).

3.3. Bond strength

Mean μTBS values were calculated and are expressed in Table 5. After 24 h of storage, there was no statistically significant difference between the mean bond strength values of the tested groups ($p > 0.05$).

Table 5 - Mean μTBS values and standard desviation (MPa) of the tested groups.

Groups	Mean (SD)
Control	35.12 (7.80)
SB+0.1% free EGCG	38.97 (5.41)
SB+1.0% PLGA50:50/EGCG	35.92 (5.45)
RS 0.1% free EGCG	33.15 (6.93)
RS 1.0% PLGA50:50/EGCG	36.93 (5.25)

4. Discussion

One of the main properties of epigallocatechin-3-galate related to adhesive dentistry is prevent dentin collagen degradation by MMP inhibition [23] differing from others MMP-inhibitors to be a natural product, extracted from green tea (*Camellia sinensis*). Thus, it can be used in cavities of any depth because of its low toxicity and anti-inflammatory properties [14].

Dentin treatment using EGCG significantly improved the mechanical properties of demineralized dentin, which suggests potential collagen cross-linking [24]. These positive data support the introduction of EGCG in dental practice. However, drugs are released more quickly when uncoated [25-27] for that reason, polymeric microparticles produced by polymers as Poly (D-L lactide-co-glycolide) acid (PLGA) has been extensively used in several applications concerning the controlled release of drugs [22,28,29] include in Endodontics and Periodontics [22,30].

The association between EGCG and polymeric materials aimed to obtain a long-acting drug delivery system, which could be applied in dental therapeutics. It has not been studied, especially when it concerns the physical and/or chemical properties of the molecules and the resulting effect on the release ability from the material. The present study evaluated the effect of two types EGCG solution, free and microencapsuled, applied as dentin pretreatment or incorporated into etch-and-rinse adhesive system on dentin bond strength. In order to establish the better PLGA microparticles loaded with EGCG formulations was conducted degree of conversion and release assay of adhesives containing microencapsulated EGCG (Experiment 1) and after, teeth were prepared to subsequent bond strength test (Experiment 2).

Result of FTIR analysis verified that no difference between the degrees of conversion among different concentrations of microencapsuled EGCG. In this case, the first null hypothesis, incorporation of polymeric microparticles loaded with EGCG have no effect on adhesive degree of conversion, was accepted. Du and others (2012) speculated that higher concentrations of epigallocatechin-3-gallate, eg, 0.5%, could interfere in the formation of linear polymer chains but in the present study there was no interference since the higher EGCG concentration used was 0.08% (2.0% PLGA50:50/EGCG and 2.0% PLGA 75:25/EGCG groups). Neri and others [15] also

demonstrated that the incorporation of EGCG in low concentrations (0.01 and 0.1%) into a specific one-step self-etch adhesive did not cause any detrimental effect on the DC.

Concerning the EGCG release, the results showed different cumulative release profile between the groups (Fig. 1). Thus, the second null hypothesis, the incorporation of PLGA-microparticles loaded with EGCG into adhesive will not be able to release EGCG was rejected. It was observed a controlled release profile in all the groups but, PLGA 50:50 groups reached a total release (100%) during the assay period while, among PLGA75:25 groups which released more just reached 60% of the total (0.5% PLGA75:25/EGCG). We speculated that this behavior could be explained by polymer hydrophobicity that suits better to hydrophobic adhesive jeopardizing the drug diffusion. Futhermore, in all PLGA 50:50 groups was observed a pulsatile release profile characterized by forming a plateau momentary immediately followed by an increase in release. This support the cases where there are required concentration peaks, such as metabolic disease and/or in which the effect is directly dependent on the minimum plasma level to achieve the pharmacological effect.

In this study, the objective is *in situ* controlled release with the drug remain covered by the polymer, securing their efficacy over time. The pulsatile release profile enable that higher doses are reached in individual and gradual moments, encouraging the effects related to inhibition enzymatic. Therefore, considering all factors, the 1.0% PLGA50:50/EGCG group was presented better results, being the elect to be used in Experiment 2.

The results of this study revealed that EGCG has not effect on the immediate bond strength independent of the application mode since that there was no statistically significant difference between the mean bond strength values of the tested groups ($p > 0.05$). Thus, the third null hypothesis, that the use of EGCG in free form or loaded into PLGA-microparticles as dentin pretreatment or incorporated into the adhesive does not affect the immediate bond strength, must be accepted.

Although it was not a statistically significant difference, our results confirm those from previous studies, which have shown that after EGCG incorporated, the immediate mean bond strength of the dental adhesives was higher than the SB (Control Group) [10]. In relation that dentin pretreatment with free EGCG, the immediate mean

bond strength was lower than Control Group unlike the findings of Santiago and others [11] at the same EGCG concentration (0.1%) (Table 5).

In the present study, we speculate that only immediate bond strength test may not have been sufficient to detect the effects of hydrolytic degradation of the adhesive interface. A study by Kiyomura et al. [31] reported that storage times between 2 and 4 years were required to detect the effects of hydrolytic degradation. Therefore, complementary studies are being conducted to confirm the potential of this catechin in preservation of collagen and maintenance of bond strength and evaluate the influence of the polymeric microparticles loaded with EGCG on the physicochemical properties of a commercial etch-and-rinse adhesive system.

5. Conclusion

- The addition of microencapsulated EGCG did not affect the degree of conversion of etch-and-rinse adhesive system, independent of concentration.
- Adhesive system incorporated with PLGA-microparticles loaded with EGCG were able to release EGCG, making these systems viable for dental applications.
- EGCG did not adversely affect the early resin-dentin bond strength when two EGCG formulations, free or microencapsulated, were applied as pretreatment or incorporated into etch-and-rinse adhesive system.

REFERENCES

[1] Hashimoto M, Ohno H and Kaga M. *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000;79:1385-1391.

[2] Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, et al. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116-135.

[3] Manso AP, Bredan-Russo AK, Suh B, Pashley DH, Carvalho RM. Mechanical stability of adhesives under water storage. Dent Mater 2009;25:744-749.

[4] Armstrong SR, Vargas MA, Chung I, Pashley DH, Campbell JA, Laffoon JE, et al. Resin-dentin interfacial ultrastructure and microtensile dentin bond strength after five-year water storage. Oper Dent 2004;29:705-712.

[5] Chaussain-Miller C, Fioretti F, Goldberg M and Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. J Dent Res 2006;85:22-32.

[6] Reis A, Carrilho M, Breschi L and Loguercio AD. Overview of clinical alternatives to minimize the degradation of the resin-dentin bonds. Oper Dent 2013;38:1-25

[7] Carrilho MR, Carvalho RM, De Goes MF, Di Hipólito V, Geraldeli S, Tay FR, et al. Chlorhexidine preserves dentin bond in vitro. J Dent Res 2007;86:90-94.

[8] Loguercio AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD, Reis A. Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. Eur J Oral Sci 2009;117:587-596.

[9] De Munck J, Mine A, Van Den Steen PE, Van Landuyt KL, Poitevin A, Opdenakker G, et al. Enzymatic degradation of adhesive–dentin interfaces produced by mild self-etch adhesives. Eur J Oral Sci 2010;118:494–501.

[10] Du X, Huang X, Huang C, Wang Y and Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent 2012;40:485-492.

[11] Santiago SL, Osorio R, Neri JR, Carvalho RM and Toledano M. Effect of the Flavonoid Epigallocatechin-3-Gallate on Resin-Dentin Bond Strength. J Adhes Dent 2013;15:535-540.

[12] Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83:216-221.

[13] Scaffa PM, Vidal CM, Barros N, Gesteira TF, Carmona AK, Breschi L, et al. Chlorhexidine inhibits the activity of dental cysteine cathepsins. J Dent Res 2012;91:420-425.

[14] Perdigão J, Reis A and Loguercio AD. Dentin Adhesion and MMPs: A comprehensive review. J Esthet Rest Dent 2013;25:219:241.

[15] Neri JR, Yamauti M, Feitosa VP, Pires APM, Araújo RS and Santigo SL. Physicochemical Properties of a methacrylate-based dental adhesive incorporated with epigallocatechin-3-gallate. Braz Dent J 2014;25:528-531.

[16] Prior S, Gamazo C, Irache JM, Merkle HP and Gander B. Gentamicin encapsulation in PLA/PLGA microspheres in view of treating Brucella infections. Int J Pharm 2000;25:115-125.

[17] Blanco-Prieto MJ, Lecaroz C, Renedo MJ, Kunkova J and Gamazo C. In vitro evaluation of gentamicin released from microparticles. Int J Pharm 2002;21:203-226.

[18] Graves RA, Pamujula S, Moiseyev R, Freeman T, Bostanian LA and Mandal TK. Effect of different ratios of high and low molecular weight PLGA blend on the characteristics of pentamidine microcapsules. Int J Pharm 2004;11:251-262.

[19] Schnieders J, Gbureck U, Thull R and Kissel T. Controlled release of gentamicin from calcium phosphate - poly(lactic acid-co-glycolic acid) composite bone cement. Biomaterials 2006;27:4239-4249.

[20] Shin YC, Yang WJ, Lee JH, Oh JW, Kim TW, Park JC, et al. PLGA nanofiber membranes loaded with epigallocatechin-3-O-gallate are beneficial to prevention of postsurgical adhesions. Int J Nanomedicine. 2014;22:4067-4078.

[21] Kim HL, Lee JH, Kwon BJ, Lee MH, Han DW, Hyon SH, et al. Promotion of full-thickness wound healing using epigallocatechin-3-O-gallate/poly (lactic-co-glycolic acid) membrane as temporary wound dressing. Artif Organs. 2014;38:411-417.

[22] Sousa FFO, Luzardo-Alvarez A, Pérez-Estévéz A, Seoane-Prado R and Blanco-Méndez J. Development of a novel AMX-loaded PLGA/zein microsphere for root canal disinfection. Biomed Mater 2010;5:055008.

[23] Kato MT, Leite AL, Hannas AR, Calabria MP, Magalhães AC, Pereira JC, et al. Impact of protease inhibitors on dentin matrix degradation by collagenase. J Dent Res 2012;91:1119-1123.

[24] Hiraishi N, Sono R, Sofiqul I, Yiu C, Nakamura H, Otsuki M, et al. *In vitro* evaluation of plant-derived agents to preserve dentin collagen. Dent Mater 2013;29:1048-1054.

[25] Huang J, Wong HL, Zhou Y, Wu XY, Grad H and Komorowski R. *In vitro* studies and modeling of a controlled-release device for root canal therapy. J Cont Rel 2000;67:293-307.

[26] Guse C, Koennings S, Kreye F, Siepmann F, Goepferich A and Siepmann J. Drug release from lipid-based implants: Elucidation of the underlying mass transport mechanism. Int J Pharm 2006;314:137-144.

[27] Sousa FFO, Blanco-Méndez J, Pérez-Estévéz A, Seoane-Prado R and Luzardo-Álvarez A. Effect of zein on biodegradable inserts for the delivery of tetracycline within periodontal pockets. J Biomater Appl 2011;27:187-200.

[28] Heling I, Sommer M, Steinberg D, Friedman M and Sela MN. Microbiological evaluation of the efficacy of chlorhexidine in a sustained-release device for dentine sterilization. Int Endod J 1992;25:15-19.

[29] Lee DY, Spangberg LS, Bok YB, Lee CY and Kum KY. The sustaining effect of three polymers on the release of chlorhexidine from a controlled release rug device for root canal disinfection. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:105-111.

[30] De Sousa FFO, Blanco-Méndez J, Pérez-Estévez A, Seoane-Prado R and Luzardo-Álvarez A. Effect of zein on biodegradable inserts for the delivery of tetracycline within periodontal pockets. J Biomater Appl 2012;27:187-200.

[31] Kiyomura M. Bonding strength to bovine dentin with 4-META/ MMA-TBB resin long-term stability and influence or water. J Jpn Soc Dent Mater Dev 1987;6:860-872.

4 CONCLUSÃO GERAL

- A incorporação das micropartículas poliméricas carregadas com EGCG não interferiu no grau de conversão dos adesivos.
- O sistema adesivo com micropartículas carregadas com EGCG incorporado em sua composição foi capaz de liberar EGCG.
- O flavonóide Epigalocatequina-3-galato (EGCG) não teve um efeito imediato significante quando incorporado ao sistema adesivo convencional ou aplicado como pré-tratamento da dentina, de forma pura e microencapsulada, na resistência de união. Portanto, ele não afetou negativamente a técnica adesiva podendo aumentar a durabilidade da interface de união ao longo do tempo.

REFERÊNCIAS GERAIS

BLANCO-PRIETO, M. J.; LECAROZ, C.; RENEDO, M.J; KUNKOVA, J; GAMAZO, C. In vitro evaluation of gentamicin released from microparticles. **International Journal of Pharmaceutics**, v. 21, n. 242, p. 203-226, 2002.

BRESCHI, L.; MAZZONI, A.; RUGGER, A; CADENARO, M; DI LENARDA, R; DE STEFANO DORIGO, E. Dental adhesion review: Aging and stability of the bonded interface. **Dental Materials**, v. 24, n. 1, p. 90-101, 2008.

BRESCHI, L.; MARTIN, P.; MAZZONI, A; NATO, F; CARRILHO, M; TJÄDERHANE, L. *et al.* Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. **Dental Materials**, v. 26, n. 6, p. 571-578, 2010a.

BRESCHI, L.; MAZZONI, A.; NATO, F; CARRILHO, M; VISINTINI, E; TJÄDERHANE, L. *et al.* Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. **Dental Materials**, v. 26, n. 4, p. 320-325, 2010b.

BURROW, M. F.; TAGAMI, J.; HOSODA, H. The long-term durability of bond strength to dentin. **The Bulletin of Tokyo Medical and Dental University**, v. 40, n. 4, p. 173-191, 1993.

CARRILHO, M. R.; CARVALHO, R. M.; TAY, F. R; PASHLEY, DH. Effects of storage media on mechanical properties of adhesive systems. **American Journal of Dentistry**, v. 17, n. 2, p. 104-108, 2004.

CARRILHO, M. R.; CARVALHO, R. M.; DE GOES, M. F; DI HIPÓLITO, V; GERALDELI, S; TAY, F. R. *et al*. Chlorhexidine preserves dentin bond in vitro. **Journal of Dental Research**, v. 86, n. 1, p. 90-94, 2007.

CHAUSSAIN-MILLER, C.; FIORETTI, F.; GOLDBERG, M; MENASHI S. The role of matrix metalloproteinases (MMPs) in human caries. **Journal of Dental Research**, v. 85, n. 1, p. 22-32, 2006.

DE MUNCK, J.; VAN LANDUYT, K.; PEUMANS, M; POITEVIN, A; LAMBRECHTS, P; BRAEM, M. *et al*. A Critical Review of the durability of adhesion to tooth tissue: Methods and results. **Journal of Dental Research**, v. 84, n. 2, p. 118-132, 2005.

DE MUNCK, J.; VAN DEN STEEN, P. E.; MINE, A; VAN LANDUYT, K.L.; POITEVIN, A; OPDENAKKER, G. *et al*. Inhibition of enzymatic degradation of adhesive-dentin interfaces. **Journal of Dental Research**, v. 88, n. 12, p. 1101-1106, 2009.

DE MUNCK, J.; MINE, A.; VAN DEN STEEN, P. E.; VAN LANDUYT, K.L.; POITEVIN, A.; OPDENAKKER, G. *et al*. Enzymatic degradation of adhesive–dentin

interfaces produced by mild self-etch adhesives. **European Journal of Oral Science**, v. 118, n. 5, p. 494–501, 2010.

DELL'AICA, I.; CANIATO, R.; BIGGIN, S; GARBISA, S. Matrix proteases, green tea, and St. John's wort: Biomedical research catches up with folk medicine. **Clinica Chimica Acta**, v. 381, n. 1, p. 69–77, 2007.

DEVIKA, P.T.; PRINCE, P.S. Preventive effect of (-)epigallocatechin-gallate (EGCG) on lysosomal enzymes in heart and subcellular fractions in isoproterenol-induced myocardial infarcted Wistar rats. **Chemico-Biological Interactions**, v. 172, n. 3, p. 245-252, 2008.

DU, X.; HUANG, X.; HUANG, C.; WANG, Y.; ZHANG, Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. **Journal of Dentistry**, v. 40, n. 6, p. 485-492, 2012.

ERHARDT, M. C. G.; OSORIO, R.; TOLEDANO, M. Dentin treatment with MMPs inhibitors does not alter bond strengths to caries-affected dentin. **Journal of Dentistry**, v. 36, n. 1, p. 1068-1073, 2008.

FARIA, G.; CARDOSO, C. R.; LARSON, R. E. Chlorhexidine-induced apoptosis or necrosis in L929 fibroblasts: A role for endoplasmic reticulum stress. **Toxicology and Applied Pharmacology**, v. 234, n. 2, p. 256-265, 2009.

GAIGNAUX, A.; RÉEFF, J.; SIEPMANN , F.; SIEPMANN, J.; DE VRIESE, C.; GOOLE, J. *et al*. Development and evaluation of sustained-release clonidine-loaded PLGA microparticles. **International Journal of Pharmaceutics**, v. 437, n. 1-2, p. 20-28, 2012.

GARBISA, S.; BIGGIN, S.; CAVALLARIN, N. Tumor invasion: molecular shears blunted by green tea. **Nature Medicine**, v. 5, n. 11, p. 1216, 1999.

GRAVES, R.A.; PAMUJULA, S.; MOISEYEV, R.; FREEMAN, T.; BOSTANIAN, L.A.; MANDAL, T.K. Effect of different ratios of high and low molecular weight PLGA blend on the characteristics of pentamidine microcapsules. **International Journal of Pharmaceutics**, n. 11, v. 270, p. 251-262, 2004.

HASHIMOTO, M.; OHNO, H.; KAGA M. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. **Journal of Dental Research**, v. 79, n. 6, p. 1385-1391, 2000.

HASHIMOTO, M. A review – micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions. **Journal of Biomedical Materials Research: Part B, Applied Biomaterials**, v. 92, n. 1, p. 268-280, 2010.

HEBLING, J.; PASHLEY, D. H.; TJÄDERHANE, L.; TAY, F.R. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. **Journal of Dental Research**, v. 84, n. 8, p. 741-746, 2005.

HIRASAWA, M.; TAKADA, K. Multiple effects of green tea catechin on the antifungal activity of antimycotics against Candida albicans. **Journal of Antimicrobial Chemotherapy**, v. 53, n. 2, p. 225–229, 2003.

ISBRUCKER, R. A.; BAUSCH, J.; EDWARDS, J. A.; WOLZ, E. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 1: genotoxicity. **Food and Chemical Toxicology**, v. 44, n. 5, p. 626–635, 2006.

KATO, M.T.; LEITE, A.L.; HANNAS, A.R.; CALABRIA, M.P.; MAGALHÃES, A.C.; PEREIRA, J.C. Impact of protease inhibitors on dentin matrix degradation by collagenase. **Journal of Dental Research,** v. 91, n. 12, p. 1119-1123, 2012.

LIANG, L.S.; WONG, W.; BURT, H.M. Pharmacokinetic study of methotrexate following intra-articular injection of methotrexate loaded poly(L-lactic acid) microspheres in rabbits. **Journal of Pharmaceutical Science**, v. 94, n. 6, p. 1204-1215, 2005.

LIU, Y.; TJÄDERHANE, L.; BRESCHI, L; MAZZONI, A.; LI, N.; MAO, J. *et al*. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. **Journal of Dental Research**, v.90, n. 8, p. 953-968, 2011.

LOGUERCIO, A. D.; STANISLAWCZUK, R.; POLLI, L. G.; COSTA, J.A.; MICHEL, M.D.; REIS, A. Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. **European Journal of Oral Science**, v. 117, n. 5, p. 587-596, 2009.

MAZZONI, A.; MANNELLO, F.; TAY, F. R.; TONTI, G.A.; PAPA, S.; MAZZOTTI, G. *et al*. Zymographic analysis and characterization of MMP-2 and -9 isoforms in human sound dentin. **Journal of Dental Research**, v. 86, n. 5, p. 436-440, 2007.

MORGUNOVA, E.; TUUTTILA, A.; BERGMANN, U.; ISUPOV, M.; LINDQVIST, Y.; SCHNEIDER, G. *et al.* Structure of human pro-matrix metalloproteinase-2: Activation mechanism. **Revealed Science**, v. 284, n. 4, p. 1667-1670, 1999.

NASCIMENTO, F.D.; MINCIOTTI, C.L.; GERALDELI, S.; CARRILHO, M.R.; PASHLEY, D.H.; TAY, F.R. *et al*. Cysteine cathepsins in human carious dentin. **Journal of Dental Research**, v. 90, n. 4, p. 506-11, 2011.

NISHITANI, Y.; YOSHIYAMA, M.; WADGAONKAR, B.; BRESCHI, L.; MANNELLO, F.; MAZZONI, A. *et al.* Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. **European Journal of Oral Sciences**, v.114, n. 2, p. 160-166, 2006.

OKUDA, M.; PEREIRA, P.N; NAKAJIMA, M.; TAGAMI, J.; PASHLEY, D.H. Long-term durability of resin dentin interface: nanoleakage vs. microtensile bond strength. **Operative dentistry**, v.27, n.3, p. 289-296, 2002.

OSORIO, R.; ERHARDT, M.C.G.; PIMENTA, L.A.F.; OSORIO, E.; TOLEDANO, M. EDTA treatment improves resin-dentin bonds' resistance to degradation. **Journal of Dental Research**, v. 84, n. 8, p. 736-740, 2005.

OSORIO, R.; YAMAUTI, M.; OSORIO, E.; ROMÁN, J.S.; TOLEDANO, M. Zinc-doped dentin adhesive for collagen protection at the hybrid layer. **European of Journal Oral Science**, v.119, n. 5, p. 401-410, 2011.

PALLAN, S.; FURTADO, M.V.A.; CILLI, R.; PRAKKI, A. Mechanical properties and characteristics of developmental copolymers incorporating catechin or chlorhexidine. **Dental Materials**, v. 28, n. 6, p. 687-694, 2012.

PASHLEY, D. H.; TAY, F.R.; YIU, C.; HASHIMOTO, M.; BRESCHI, L.; CARVALHO, R.M. *et al.* Collagen degradation by host-derived enzymes during aging. **Journal of Dental Research**, v. 83, n. 3, p. 216-221, 2004.

PASHLEY, D. H.; TAY, F. R.; IMAZATO S. How to increase the durability of resin-dentin bonds. **Compendium of Continuing Education in Dentistry**, v. 32, n. 7, p. 60-64, 2011.

PASHLEY, D.H.; TAY, F. R.; BRESCHI, L.; TJÄDERHANE, L.; CARVALHO, R.M.; CARRILHO, M. *et al*. State of the art etch-and-rinse adhesives. **Dental Materials**, v. 27, n. 1, p. 1-16, 2011.

PEREIRA, P.N.; OKUDA, M.; SANO, H.; YOSHIKAWA, T.; BURROW, M.F.; TAGAMI, J. Effect of intrinsic wetness and regional difference on dentin bond strength. **Dental Materials**, v. 15, n. 1, p. 46-53, 1999.

PRIOR, S.; GAMAZO, C.; IRACHE, J. M.; MERKLE, H.P.; GANDER, B. Gentamicin encapsulation in PLA/PLGA microspheres in view of treating Brucella infections. **International Journal of Pharmaceutics**, v. 25, n. 196, p. 115-125, 2000.

RASHEED, Z.; ANBAZHAGAN, A.N.; AKHTAR, N.; RAMAMURTHY, S.; VOSS, F.R.; HAQQI, T.M. Green tea polyphenol epigallocatechin-3-gallate inhibits advanced glycation end products-induced expression of tumor necrosis factor-alpha and matrixmetalloproteinase-13 in human chondrocytes. **Arthritis Research & Therapy,** v. 11, n. 3, 2009.

SANO, H.; YOSHIYAMA, M.; EBISU, S.; BURROW, M.F.; TAKATSU, T.; CIUCCHI, B. *et al*. Comparative SEM and TEM observations of nanoleakage within the hybrid layer. **Operative Dentistry**, v.20, n. 4, p. 160-167, 1995.

SANTIAGO, S. L.; OSORIO, R.; NERI, J.R.; CARVALHO, R.M.; TOLEDANO, M. Effect of the Flavonoid Epigallocatechin-3-Gallate on Resin-Dentin Bond Strength. **Journal of Adhesive Dentistry**, v. 15, n. 6, p. 535-540, 2013.

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

SHONO, Y.; TERASHITA, M.; SHIMADA, J.; KOZONO, Y.; CARVALHO, R.M.; RUSSELL, C.M. *et al*. Durability of resin-dentin bonds. **Journal of Adhesive Dentistry**, n. 1, v. 3, p. 211-218, 1999.

STANISLAWCZUK, R.; AMARAL, R. C.; ZANDER-GRANDE, C.; GAGLER, D.; REIS, A.; LOGUERCIO, A.D. Chlorhexidine-containing acid conditioner preserves the longevity of resin-dentin bonds. **Operative Dentistry**, v. 34, n. 4, p. 481-490, 2009.

SULKALA, M.; TERVAHARTIALA, T.; SORSA, T.; LARMAS, M.; SALO, T.; TJÄDERHANE, L. Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin. **Archives of Oral Biology**, v. 52, n. 2, p. 121-127, 2007.

TAY, F.R.; PASHLEY, D. H.; YOSHIYAMA, M. Two modes of nanoleakage expression in single-step adhesives**. Journal of Dental Research**, v. 81, n. 7, p. 472-476, 2002.

TERSARIOL, I. L.; GERALDELI, S.; MINCIOTTI, C. L.; NASCIMENTO, F.D.; PÄÄKKÖNEN, V.; MARTINS, M.T. *et al*. Cysteine cathepsins in human dentin-pulp complex. **Journal of Endodontic**, v. 36, n. 3, p. 475-481, 2010.

TJÄDERHANE, L.; LARJAVA, H.; SORSA, T.; UITTO, V.J.; LARMAS, M.; SALO, T. The activation and function of host matrix metalloproteinase in dentin matrix during breakdown in carious lesions. **Journal of Dental Research**, v.77, n. 8, p. 1622-1629, 1998.

TJÄDERHANE, L.; NASCIMENTO, F. D.; BRESCHI, L.; MAZZONI, A.; TERSARIOL, I.L.; GERALDELI, S. *et al*. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. **Dental Materials**, v. 29, n. 1, p. 116-135, 2013.

TURK, B.; BIETH, J.G.; BJÖRK, I.; DOLENC, I.; TURK, D.; CIMERMAN, N. *et al*. Regulation of the activity of lysosomal cysteine proteinases by pH-induced inactivation and/or endogenous protein inhibitors, cystatins. **Biological Chemistry Hoppe Seyler**, v. 376, n. 4, p. 225-230, 1995.

VAN MEERBEECK, B.; DE MUNCK, J.; YOSHIDA, Y.; INOUE, S.; VARGAS, M.; VIJAY, P. *et al*. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. **Operative Dentistry**, v. 28, n. 3, p. 215-235, 2003.

VAN STRIJP, A. J.; JANSEN, D. C.; DEGROOT, J.; TEN CATE, J.M.; EVERTS, V. Host-derived proteinases and degradation of dentine collagen in situ. **Caries Research**, v. 37, n. 1, p. 58-65, 2003.

VISSE, R.; NAGASE, H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases. Structure, function, and biochemistry. **Circulation Research**, v. 92, n. 8, p. 827-839, 2003.

WANG, Y.; SPENCER, P. Hibridization efficiency of the adhesives/dentin interface with wet bonding. **Journal of Dental Research**, v. 82, n. 2, p. 141-145, 2003.

YIU, C.K.; HIRAISHI, N.; TAY, F.R.; KING, N.M. Effect of chlorhexidine incorporation into dental adhesive resin on durability of resin-dentin bond. **Journal of Adhesive Dentistry**, v. 14, n. 4, p. 355-362, 2012.

ZOU, Y.; JESSOP, J.L.; ARMSTRONG, S.R. In vitro enzymatic biodegradation of adhesive resin in the hybrid layer. **Journal of Biomedical Materials Research Part A**, v.94, n. 1, p. 187–192, 2010.

APÊNDICE

TERMO DE DOAÇÃO DE DENTES

Pelo presente instrumento que atende às exigências legais, o Sr(a) _____, após ter tomado conhecimento do protocolo da pesquisa "EFEITO DA INCORPORAÇÃO DE MICROPARTÍCULAS POLIMÉRICAS CARREGADAS COM CATEQUINA NAS PROPRIEDADES FÍSICO-QUÍMICAS DE UM SISTEMA ADESIVO." que tem como objetivo comparar o efeito da aplicação do flavonóide Epigalocatequina-3-galato (EGCG) como pré-tratamento da dentina ou incorporado ao sistema adesivo convencional, de forma pura e microencapsulada, na resistência de união, através de ensaios de microtração, vem na melhor forma de direito DOAR à cirurgiã-dentista Nadine Luísa Guimarães Albuquerque __ dentes (terceiros molares), declarando, sob as penas da lei, que os dentes objeto da presente doação foram extraídos por indicação terapêutica, cujos históricos circunstanciados fazem parte dos prontuários dos pacientes de quem se originam.

Data:___/___/___

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ANEXO

ANEXO A - NORMAS DO PERIÓDICO DENTAL MATERIALS

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Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

This must be presented in a structured format, covering the following subjects, although actual subheadings should not be included:

- succinct statements of the issue in question;
- the essence of existing knowledge and understanding pertinent to the issue (reference);
- the aims and objectives of the research being reported relating the research to dentistry, where not obvious.

Materials and methods

- describe the procedures and analytical techniques.
- only cite references to published methods.
- include at least general composition details and batch numbers for all materials.
- identify names and sources of all commercial products e.g.

"The composite (Silar, 3M Co., St. Paul, MN, USA)..."
"... an Au-Pd alloy (Estheticor Opal, Cendres et Metaux, Switzerland)."

- specify statistical significance test methods.

Results

- refer to appropriate tables and figures.
- refrain from subjective comments.
- make no reference to previous literature.
- report statistical findings.

Discussion

- explain and interpret data.
- state implications of the results, relate to composition.
- indicate limitations of findings.
- relate to other relevant research.

Conclusion (if included)

- must NOT repeat Results or Discussion
- must concisely state inference, significance, or consequences

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

- ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract (structured format)

• 250 words or less.
• subheadings should appear in the text of the abstract as follows: Objectives, Methods, Results, Significance. (For Systematic Reviews: Objectives, Data, Sources, Study selection, Conclusions). The Results section may incorporate small tabulations of data, normally 3 rows maximum.

Graphical abstract

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Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords

Up to 10 keywords should be supplied e.g. dental material, composite resin, adhesion.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

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Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

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As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

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Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

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ANEXO

ANEXO B – PARECER CONSUBSTANCIADO DO CEP

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DA INCORPORAÇÃO DE CATEQUINA EM SISTEMA ADESIVO CONVENCIONAL NA RESISTÊNCIA DE UNIÃO À DENTINA

Pesquisador: Nadine Luisa Soares de Lima Guimarães

Área Temática:

Versão: 1

CAAE: 22468813.0.0000.5054

Instituição Proponente: Departamento de Odontologia Restauradora

Patrocinador Principal: Departamento de Odontologia Restauradora

DADOS DO PARECER

Número do Parecer: 459.659

Data da Relatoria: 14/11/2013

Apresentação do Projeto:

Os materiais adesivos são bastante utilizados na odontologia e têm demonstrado excelentes resultados na prática clínica diária. Atualmente, os sistemas adesivos são classificados em convencionais e autocondicionantes.O presente estudo como objetivo comparar o efeito do flavonóide Epigalocatequina-3-galato (EGCG) incorporado ao sistema adesivo convencional ou como pré-tratamento da dentina na resistência de união, através de ensaios de microtração imediatos e após armazenagem por 6 e 12 meses.

Serão utilizados 40 terceiros molares humanos hígidos. Após remoção do esmalte e dentina oclusal, utilização da lixa de carbeto de silício (SIC) Nº 100 acoplada em uma politriz elétrica para completa remoção de esmalte e a de Nº 600 durante 1 minuto para gerar um padrão de smear layer em todos os espécimes, os espécimes serão distribuídos aleatoriamente em 5 Grupos (n=8): Grupo I (Água destilada + Adper¿ Single Bond 2®); Grupo II (Água destilada + EGCG puro incorporado ao Adper¿ Single Bond 2®); Grupo III (EGCG em cápsula incorporado ao Adper¿ Single Bond 2®); Grupo IV (Solução aquosa de EGCG + Adper¿ Single Bond 2®) e; Grupo V (Solução de EGCG cápsula + Adper¿ Single Bond 2®). Primeiramente,

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Bairro: Rodolfo Teófilo **CEP:** 60.430-270
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

será feito condicionamento das superfícies dentárias com ácido fosfórico por 15 segundos, em seguida essas superfícies serão lavadas com spray
ar/água por 15 segundos, secas por 30 segundos, e reumidecidas com as soluções de limpeza de acordo com cada Grupo (15 ¿L, ativamente por
60s). O excesso será removido com papéis absorventes, deixando a superfície dentinária visivelmente úmida. Na sequência, será aplicado o sistema
adesivo Adper¿ Single Bond 2® de formulação específica para cada Grupo, de acordo com as recomendações do fabricante. A porção coronária
será reconstruída com 4 incrementos (1 mm cada) de resina composta (Filtek Z250XT®). Após estocagem em água destilada a 37 °C por 24h,
espécimes em forma de palito (constituídos de resina e dentina unidas pela interface adesiva) serão obtidos por meio de cortes seriados dos dentes.
Após a obtenção dos palitos, estes serão submetidos a uma força de tração em uma máquina de ensaios universais à velocidade de 0,5 mm/ minuto
até que ocorra a fratura. Os palitos que irão ser testados após 6 meses e 1 ano ficarão armazenados em em água destilada. Serão avaliados os
modos de fratura com o auxílio de uma Lupa Estereoscópica e classificadas em: 1) Falha coesiva em resina composta (FCR); 2) Falha coesiva em
dentina (FCD); 3) Falha adesiva (FA); e 4) Falha mista (FM). A partir dos dados obtidos em Mpa será realizado teste de normalidade e, se
constatada a presença de distribuição normal das amostras, os dados serão submetidos ao teste ANOVA (Análise de Variância) para comparação
entre os grupos e em seguida, eventuais diferenças entre os grupos serão analisadas por teste de comparação múltipla. No caso dos resultados
falharem no teste de normalidade, será aplicado teste Kruskal-Wallis. O nível de significância será de p0,05 em todas as situações.

Objetivo da Pesquisa:

Comparar o efeito do flavonóide Epigalocatequina-3-galato (EGCG) incorporado ao sistema adesivo convencional ou como pré-tratamento da
dentina na resistência de união, através de ensaios de microtração imediatos e após armazenagem por 6 e 12 meses.

Avaliação dos Riscos e Benefícios:

Riscos:

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Não há riscos envolvidos pois trata-se de uma pesquisa em laboratório com dentes humanos extraídos por motivos que não envolvem este estudo.

Benefícios:

De acordo com os resultados obtidos, será possível propor novas técnicas e materiais a serem usados na odontologia restauradora.

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante na área de dentística com metodologia clara e objetiva e compatível com o objetivo do trabalho

Considerações sobre os Termos de apresentação obrigatória:

Apresentou: folha de rosto, dispensa do TCLE, termo de doação de dentes, orçamento detalhado, currículo Lattes, carta de apreciação ao COMEPE, cronograma, autorização do local onde será realizada a pesquisa, declaração de concordância, declaração de vínculo da aluna no mestrado, declaração de custeio da pesquisa, projeto com anexos.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Não se aplica.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

FORTALEZA, 18 de Novembro de 2013

Assinador por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador)

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