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CECÍLIA ATEM GONÇALVES DE ARAÚJO COSTA

EFEITO DA EPIGALOCATEQUINA-3-GALATO NAS PROPRIEDADES BIOLÓGICAS E NO DESEMPENHO CLÍNICO DE UM SISTEMA ADESIVO UNIVERSAL: ESTUDOS *IN VITRO* E *IN VIVO*

Fortaleza

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, como requisito parcial para a obtenção do título de Doutor em Odontologia. Área de concentração: Clínica Odontológica.

Orientador: Prof. Dr. Sérgio Lima Santiago
Coorientador: Prof. Dr. Juliano Sartori Mendonça

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Aprovada em: ___/___/_____.

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RESUMO

A epigalocatequina-3-galato (EGCG) é um flavonoide que foi recentemente introduzido na pesquisa odontológica devido a vários benefícios, como o efeito antibacteriano, a inibição de metaloproteinases e a capacidade de melhorar as propriedades biomecânicas do colágeno. Essas características sugerem que o EGCG pode ser um composto promissor a ser associado a procedimentos adesivos, visando melhorar o prognóstico do tratamento restaurador e evitar a degradação da união resina-dentina. O objetivo deste estudo foi investigar a citotoxicidade do EGCG em células-tronco dentárias, a atividade antibacteriana de sistemas adesivos comerciais incorporados com EGCG no biofilme de *Streptococcus mutans* e avaliar o desempenho clínico após 2 anos do pré-tratamento da dentina com EGCG em restaurações de lesões cervicais não cariosas (LCNC) usando um adesivo universal em duas diferentes estratégias adesivas. No primeiro capítulo, células-tronco de polpa dental humana foram usadas para a avaliação de citotoxicidade transdentinal com pré-tratamento com solução aquosa de EGCG a 0,1%. Para análise antibacteriana, o pó de EGCG foi incorporado em sistemas adesivos comerciais a 0,1% e espécimes de adesivo polimerizado serviram de substratos para o teste antimicrobiano de contato direto e para formação de biofilme. Para o ensaio clínico randomizado, descrito no segundo e terceiro capítulos, 33 voluntários foram selecionados e 156 LCNCs foram restauradas variando as estratégias adesivas, ou seja, condicionamento total (CT) ou autocondicionante (AUTO), com a presença (CT-ECGC) ou ausência (AUTO-EGCG) do pré-tratamento da dentina com solução aquosa de EGCG a 0,1%. Dois avaliadores, "cegos" para os tratamentos realizados, avaliaram as restaurações no *baseline*, e após 6, 12, 18 e 24 meses, utilizando os critérios FDI (Federação Odontológica Mundial) para descoloração marginal, retenção, adaptação marginal, cárie e sensibilidade pós-operatória. Moldagens foram feitas das LCNCs e das restaurações para criar réplicas de resina epóxica para observação sob microscopia eletrônica de varredura. Os dados de todos os testes foram submetidos à análise estatística ($\alpha = 0,05$). Os resultados *in vitro* mostraram que o pré-tratamento dentinário com EGCG não foi citotóxico para células dentais, mas a incorporação de EGCG em adesivos dentais não mostrou atividade antibacteriana em biofilme de *Streptococcus mutans*. Na avaliação clínica, após 12 meses, as taxas de retenção foram 97,37% para CT e AUTO, 100% para CT-EGCG e 94,6% para AUTO-EGCG, enquanto que, aos 24 meses, as taxas de

retenção foram 97,14% para CT, 94,44% para AUTO, 97,14% para CT-EGCG e 94,59% para AUTO-EGCG, sem diferença estatística entre os grupos nos dois períodos. Não houve diferenças estatisticamente significantes entre os grupos para todos os outros critérios em 12 e 24 meses, nem entre os mesmos grupos quando os resultados de *baseline* e 12 meses foram comparados. Para adaptação marginal, entretanto, uma diferença significativa foi detectada para o grupo AUTO entre *baseline* e 24 meses ($p = 0,0313$). Como conclusão, a incorporação de 0,1% de EGCG em adesivos comerciais não demonstrou atividade antibacteriana, e o pré-tratamento dentinário não foi citotóxico para células dentais. A retenção clínica do adesivo universal após 12 e 24 meses não dependeu da estratégia de adesão utilizada bem como do pré-tratamento da dentina com EGCG.

Palavras-chave: adesivos, biofilme, catequina, citotoxicidade e ensaio clínico randomizado.

ABSTRACT

Epigallocatechin-3-gallate (EGCG) is a flavonoid that has been recently introduced in dental research because of several benefits such as the antibacterial effect, inhibition of metalloproteinase and ability to increase the biomechanical properties of collagen. These characteristics suggest that EGCG may be a promising compound to be associated with adhesive procedures aiming improve the prognosis of restorative treatment and avoid degradation of resin-dentin bonding. The objective of this study was to investigate the cytotoxicity of EGCG in dental stem cells; The antibacterial activity of commercial adhesive systems incorporated with EGCG on Streptococcus mutans biofilm; And to evaluate the clinical performance after 2 years of dentin pre-treatment with EGCG in restorations of non-carious cervical lesions (NCCL) using a universal adhesive in two different adhesive strategies. In the first chapter, human dental pulp stem cells were used for the evaluation of transdentinal cytotoxicity with pre-treatment with 0.1% EGCG aqueous solution. For antibacterial analysis, EGCG powder (0.1% w/v) was incorporated into commercial adhesive systems and the adhesive specimens were used as substrates for the antimicrobial direct contact test and biofilm formation. For the randomized clinical trial described in the second and third chapters, 33 volunteers were selected and 156 NCCLs were restored by varying the adhesive strategies: etch-and-rinse (ER) or self-etching (SE) with presence (ER-ECGC) or absence (SE-EGCG) dentin pretreatment with 0.1% EGCG aqueous solution. Two evaluators, blinded to the treatments, evaluated the baseline restorations and after 6, 12, 18 and 24 months, using the FDI criteria for marginal discoloration, retention, marginal adaptation, caries and postoperative sensitivity. Impressions were made from NCCLs and restorations to create epoxy resin replicas for observation under scanning electron microscopy. Data from all tests were submitted to statistical analysis ($\alpha = 0.05$). *In vitro* results showed that dentin pre-treatment with EGCG was not cytotoxic to dental cells, but the incorporation of EGCG into dental adhesives did not show antibacterial activity on Streptococcus mutans biofilm. After 12 months, the retention rates were 97.37% for ER and SE, 100% for ER-EGCG and 94.6% for SE-EGCG, whereas, at 24 months, retention rates were 97.14% for ER, 94.44% for SE, 97.14% for ER-EGCG and 94.59% for SE-EGCG, with no statistical difference between groups in both periods. There were

no statistically significant differences between the groups for all other criteria at 12 and 24 months, nor intragroup when the baseline and 12 months outcomes were compared. For marginal adaptation, however, a significant difference was detected for the SE group between *baseline* and 24 months ($p = 0.0313$). In conclusion, incorporation of 0.1% EGCG into commercial adhesives did not demonstrate antibiofilm activity, and dentin pretreatment was not cytotoxic to dental cells. The clinical retention of the universal adhesive after 12 and 24 months did not depend on the adhesive strategy used as well as the pretreatment of the dentin with EGCG.

Key words: adhesives, biofilm, catechin, cytotoxicity and randomized clinical trial.

SUMÁRIO

INTRODUÇÃO GERAL

1 INTRODUÇÃO GERAL

O conceito de camada híbrida, ou zona de interdifusão, encontra-se consolidado na literatura como o mecanismo básico da união dos materiais restauradores à estrutura dentária (NAKABAYASHI, KOJIMA, MASUHARA, 1982; VAN MEERBEEK *et al.,*1993). Assim, as pesquisas atuais em Odontologia adesiva estão direcionadas no sentido de melhorar a qualidade da formação dessa interface adesiva e na busca pela compreensão dos seus mecanismos de degradação, gerando conhecimentos que possam proporcionar uma maior longevidade da união dos materiais restauradores à estrutura dentária (SANO *et al*., 1995; VAN MEERBEEK *et al*., 2003; HASHIMOTO, 2010; PASHLEY *et al*., 2011; LIU *et al*., 2011; CARVALHO *et al*., 2012; REIS *et al*., 2013).

A estabilidade da interface de união é essencial para a longevidade das restaurações. A degradação pode enfraquecer a união e conduzir a falhas entre a estrutura dentária e o material restaurador. A descoloração e a desadaptação marginal, por exemplo, apresentam-se como consequências relacionadas à durabilidade clínica de restaurações de resina composta (BRESCHI *et al*., 2008).

Em relação à degradação, o principal desafio para os sistemas adesivos parece ser a busca por uma união igualmente eficaz para esmalte e dentina (VAN MEERBEEK *et al*., 2011). A união resina-dentina permanece imperfeita (BRESCHI *et al*., 2008) e menos durável que a união resina-esmalte, o que pode ser explicado por características como a heterogeneidade histológica, a umidade e a permeabilidade do substrato dentinário (PEREIRA *et al*., 1999; CARVALHO *et al.*, 2004; PERDIGÃO, 2010; VAN MEERBEEK *et al*., 2011).

A dentina hibridizada apresenta-se como uma região com características híbridas de colágeno e polímeros resinosos resultante da difusão e polimerização *in situ* de monômeros sobre a superfície dentinária desmineralizada (NAKABAYASHI & PASHLEY, 1998). A degradação hidrolítica constitui um dos principais mecanismos de degradação dessa interface (TJÄDERHANE *et al*., 2013) e foi comprovada pela preservação da resistência de união e da integridade da camada híbrida, quando armazenada em óleo mineral (CARRILHO *et al*., 2004). Nesse contexto, a suscetibilidade dos adesivos à sorção de água/fluidos orais está, portanto, diretamente relacionada à hidrólise dos componentes resinosos (SADEK *et al*., 2008), à hidrólise do silano associado ao desprendimento das partículas de carga (VAN LANDUYT *et al*., 2010; BRACKETT *et al*., 2011) e à hidrólise da matriz orgânica: fibrilas de colágeno desmineralizadas que não foram recobertas por material resinoso, ou que foram expostas pela hidrólise do polímero (SANO *et al*.,1995).

Pashley e colaboradores, em 2004, entretanto, sugeriram que enzimas hospedeiras da saliva e dentina humanas, conhecidas como metaloproteinases da matriz (MMPs), também seriam responsáveis pela degradação (proteolítica) da matriz orgânica. Elas seriam ativadas durante os procedimentos adesivos, acelerando a degradação da matriz orgânica desmineralizada e não infiltrada pelo sistema adesivo (HEBLING *et al*., 2005; NISHITANI *et al*., 2006; TAY *et al*., 2006; CHAUSSAIN-MILLE *et al.*, 2006; CARRILHO *et al*., 2007a; ZHANG *et al*., 2009).

Outras enzimas foram recentemente descobertas, tais como as cisteínas catepsinas (CC), presentes em dentina humana, com capacidade de degradar a maior parte das proteínas da matriz extracelular, como o colágeno, sendo detectada forte correlação positiva entre CC e atividade de MMPs (TERSARIOL *et al*., 2010). Estas

descobertas sugerem que, além de MMPs, CC podem também contribuir para a atividade proteolítica endógena na dentina, resultando na degradação do colágeno dentro da camada híbrida e influenciando na durabilidade da união à dentina. (LIU, *et al*., 2011; TJADEHRANE, *et al*., 2013).

Recentes estudos, portanto, têm dado ênfase ao papel das MMPs e CC na degradação da adesão à dentina (LIU, *et al*., 2011; TJADEHRANE, *et al*., 2013; PERDIGÃO *et al.*, 2013; MAZZONI *et al.*, 2015). Dessa forma, além da eliminação da água a partir da interface (para retardar ou eliminar a degradação hidrolítica dos componentes da camada híbrida), o uso de inibidores enzimáticos (separadamente ou incorporados aos sistemas adesivos) e o aumento da resistência do colágeno à degradação enzimática têm sido aceitos como estratégias eficazes para manter a integridade da camada híbrida e para melhorar a resistência de união à dentina ao longo do tempo (CARRILHO *et al*., 2007a; CARRILHO *et al.,* 2007b; LOGUERCIO, STANISLAWCZUK, POLLI, 2009; STANISLAWCZUC *et al*., 2009; KOMORI *et al*., 2009; BRESCHI *et al*., 2010; YIU *et al*., 2012; SCAFFA *et al.*, 2012; SANTIAGO *et al*., 2013).

A clorexidina é potencial inibidora de MMPs e CC, (GENDRON *et al*., 1999; SCAFFA *et al.*, 2012; SABATINI, 2013) e diversos estudos laboratoriais (HEBLING *et al*., 2005; CARRILHO *et al*., 2007a; LOGUÉRCIO *et al*., 2009; STANISLAWCZUC *et al*., 2009; KOMORI *et al*., 2009; BRESCHI *et al*., 2010; YIU *et al*., 2012; SCAFFA *et al.*, 2012) e clínicos (CARRILHO *et al*., 2007b; BRACKETT *et al*., 2009; RICCI *et al*., 2010) concluíram que, quando associada aos procedimentos adesivos (seja como pré-tratamento da dentina, incorporada ao ácido fosfórico ou ao adesivo), resulta na desaceleração do processo de degradação da interface de união

adesiva, embora ainda não haja consenso em relação a esse benefício nas diferentes estratégias adesivas (condicionamento total e autocondicionante) (CAMPOS *et al*., 2009; CELIK *et al*., 2012; WANG *et al*., 2013; NISHITANI *et al*., 2013; ARAÚJO *et al*., 2015).

Outros inibidores enzimáticos, principalmente produtos naturais sem efeitos colaterais, têm despertado o interesse da comunidade científica. Os polifenóis encontrados no chá verde, em especial a epigalocatequina-3-galato (EGCG), com comprovada capacidade de inibir a expressão e a ação das MMP-2 e MMP-9 (DEMEULE *et al*., 2000) têm sido recentemente estudados em associação aos procedimentos adesivos. Estudos laboratoriais demonstraram que o uso de EGCG é eficaz na preservação da resistência de união quando incorporada ao adesivo dentinário (DU *et al.*, 2012) ou quando utilizada como pré-tratamento dentinário em adesivos de condicionamento total (SANTIAGO *et al.*, 2013) e autocondicionantes (NERI *et al.*, 2016), bem como não interferiu no grau de conversão e na resistência flexural quando incorporada a um adesivo autocondicionante (NERI *et al.*, 2014).

A EGCG apresenta ainda efeitos positivos nas propriedades mecânicas e nas propriedades de estabilização de colágeno contra a degradação proteolítica. Um recente estudo (VIDAL *et al.,* 2014) mostrou que catequinas monoméricas com radical galoil, como a EGCG, são mais eficazes para aumentar o módulo de elasticidade e reduzir as taxas de biodegradação do colágeno. O estudo mostrou o efeito de catequinas na modificação da matriz de dentina, com a formação de ligações cruzadas, *cross-links*, em uma estrutura já altamente reticulada, concluindo ser a EGCG a catequina monomérica mais potente com capacidade de ligação cruzada em dentina.

Além das propriedades de inibição enzimática e de formação de ligações cruzadas em dentina, a atividade antibacteriana da ECGC também foi comprovada por estudos laboratoriais (JEON *et al*., 2014) incluindo bactérias cariogênicas *Streptococcus mutans* e *Lactobacillus acidophilus* (ANITA *et al*., 2015), e periodontopatogênicas *Eikenella corrodens* (MATSUNAGA *et al*., 2010). O potencial antimicrobiano do EGCG também tem sido demostrado através de estudos com a incorporação dessa catequina em ionômero de vidro (HU *et al*., 2013) e sistemas adesivos (DU *et al.*, 2012; MANKOVSKAIA, LÉVESQUE, PRAKKI, 2013).

Portanto, na busca por agentes bioativos que possam apresentar, ao mesmo tempo, propriedades antimicrobianas e de melhoria na resistência de união adesiva dos materiais restauradores à estrutura dental (CHAI *et al*, 2011; FEITOSA *et al*., 2014), a associação de procedimentos adesivos ao EGCG pode surgir como alternativa para o desenvolvimento de novas técnicas ou materiais com propriedades importantes capazes de melhorar o prognóstico do tratamento restaurador (IMAZATO, 2009; IMAZATO, *et al*, 2014).

Entretanto, apesar dos resultados laboratoriais demonstrarem os benefícios na resistência de união imediata e a longo prazo de sistemas adesivos associados à EGCG (DU *et al.*, 2012; SANTIAGO *et al.*, 2013; NERI *et al*., 2016), sabe-se que ainda há um questionamento sobre a falta de correlação entre os resultados dos testes *in vitro* e a realidade clínica as quais as restaurações adesivas são expostas.

Portanto, os benefícios já publicados em estudos laboratoriais com EGCG, somando-se ao fato de ser um produto natural muito abundante e um recurso sustentável, justificam a aplicação desse produto em avaliações clínicas de longa

duração, uma vez que ainda não há estudos clínicos publicados avaliando os benefícios dessa aplicação.

Assim, o objetivo do presente estudo foi avaliar a epigalocatequina-3-galato quanto à citotoxicidade, à atividade antibacteriana na formação e desenvolvimento do biofilme de *Streptococcus mutans* quando incorporada a sistemas adesivos comerciais, e quanto à influência do pré-tratamento dentinário no comportamento clínico de um sistema adesivo universal através de um estudo clínico randomizado.

PROPOSIÇÃO

2 PROPOSIÇÃO

2.1. Objetivo geral :

Avaliar a epigalocatequina-3-galato quanto à citotoxicidade, à atividade antibacteriana na formação e desenvolvimento do biofilme de *Streptococcus mutans* quando incorporada a sistemas adesivos comerciais, e quanto à influência do seu uso como pré-tratamento dentinário no comportamento clínico de um sistema adesivo universal através de um estudo clínico randomizado.

2.2. Objetivos específicos:

1. Avaliar *in vitro* a citotoxicidade da EGCG em célula-tronco de polpa dentária humana, a atividade antibacteriana da incorporação direta de EGCG a 0.1% em sistemas adesivos comerciais (*Single Bond 2* e *Single Bond Universal - 3M ESPE)* na formação e desenvolvimento do biofilme de *Streptococcus mutans*;

2. Avaliar *in vivo* (estudo clínico randomizado) a influência do pré-tratamento dentinário com solução aquosa de EGCG a 0,1% em restaurações de lesões cervicais não cariosas realizadas com o sistema adesivo *Single Bond Universal - 3M ESPE,* utilizando-se duas estratégias adesivas (condicionamento total e autocondicionante), através de avaliação clínica de restaurações nos períodos imediato e após 6, 12, 18 e 24 meses.

CAPÍTULOS

3 CAPÍTULOS

REGIMENTO INTERNO

Esta tese está baseada no Artigo 46 do Registro Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, que regulamenta o formato alternativo para tese de doutorado, permitindo a inserção de artigos científicos de autoria e coautoria do candidato. Por se tratar de pesquisa envolvendo seres humanos, o projeto de pesquisa deste trabalho seguiu as Diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos. (Resolução 466/12) e foi submetido à apreciação do Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade Federal do Ceará via Plataforma Brasil, tendo sido aprovado sob o número CAAE - 40975514.0.0000.5054 (ANEXO A). O estudo foi também publicado no Registro Brasileiro de Ensaios Clínicos (ReBEC) sob número: RBR-2hr94r (ANEXO B). Dessa forma, a presente tese de doutorado é composta pelos seguintes capítulos:

- **Capítulo 1:** *The green tea catechin Epigallocatechin-3-gallate: cytotoxicity and effects on Streptococcus mutans biofilm.*

  Autores: COSTA, C.A.G.A.; PANARIELLO, B.H.D.P; QUISPE-SALCEDO, A.; SHIMIZU, E.; SANTIAGO, S.L.; DUARTE, S.

  Periódico: *Beverages**

- **Capítulo 2:** *One-year of Epigallocatechin-3-galate Dentin Pretreatment on Clinical Performance of a Universal Adhesive: A Randomized Clinical Trial*.

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CAPÍTULO 1

The green tea catechin Epigallocatechin-3-gallate: cytotoxicity and effects on *Streptococcus mutans* biofilm.

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The green tea catechin Epigallocatechin-3-gallate: cytotoxicity and effects on Streptococcus mutans biofilm.

Abstract

Epigallocatechin-3-gallate (EGCG) is the most active and abundant catechin present in green tea and has been associated with the improvement of the long-term bond strength to dentin when associated with dental adhesives. However, studies regarding the cytotoxicity of this catechin to dental cells and its effect on *Streptococcus mutans* biofilms are still scarce. The objective of this study was to investigate the EGCG cytotoxicity to dental cells and the antibacterial activity of commercial adhesive systems incorporated with EGCG. For cytotoxicity assay, dentin slices were divided into five test groups: G1: Dentin (control group); G2: Dentin with 100% Ethanol; G3: Dentin with excessive ethanol (negative control); G4: Control cells - no dentin; and G5: dentin with 0.1% EGCG aqueous solution. Human dental pulp stem cells (DPSCs) were incubated with or without dentin slices and the cells viability was analyzed on triplicate (n=6). For antibacterial analysis, EGCG powder was incorporated into Adper Single Bond 2 and Single Bond Universal (3M ESPE) at 0.1% w/v and adhesive light-cured specimens were used as substrate for direct contact antimicrobial test and for biofilm formation. The biofilms were evaluated by dry-weight and colony-forming unit (CFU) assays after 24 h (day 1), 48 h (day 3) and 72 h (day 5) of biofilm development (n=6). Statistical analyses were performed using Student's t test, one-way ANOVA with Tukey *post-hoc* test and independent samples Kruskall-Wallis test for non-parametric data, considering a significance level of 5%. EGCG did not inhibit cell proliferation compared to the control groups ($p>0.05$). Human DPSCs with EGCG were recovered

and revitalized at 72 h. The direct contact test showed that EGCG is not bactericidal, but has few antibactericidal effects. No statistical differences were observed for CFU and dry-weight between all groups in the days 1, 3 and 5 of biofilm development (p>0.05). In conclusion, although 0.1% (w/v) EGCG incorporation into dental adhesives did not show antibacterial activity, it was not cytotoxic for DPSCs and may be a promising biocompatible compound for bioactive dental materials.

Key words: Adhesives, biofilm, catechin, cytotoxicity, and dentin.

1. Introduction

The knowledge about the role of microbial etiology in caries disease [1] caused important changes in dental practice. Nowadays, minimally invasive restorative dentistry searches for strategies to avoid the removal of healthy hard tissues [2] and focus the de/remineralization balance, aiming to control bacterial activity in the dental biofilm. In this context, "bioactive" materials have emerged with antimicrobial and remineralizing properties that can improve the restorative treatment prognosis [3,4]. The use of bioactive materials aims to alter the cariogenicity of the biofilm, preventing the formation of secondary caries lesions [5]. In restorative dentistry, the expected effect is that this strategy may prevent bacterial colonization at the tooth-restoration interface and avoid remaining bacteria growth in the prepared cavity [5].

The incorporation of agents in adhesives systems may be a critical step. The possible beneficial effects of any product should not jeopardize the physico-chemical properties of the materials nor the bonding mechanism during restorative procedure [6]. The stability of the bonding interface is essential for the longevity of adhesive

restorations [7] and should not be impaired by the incorporation. Also, how the bioactive compounds affect the cytotoxicity of an adhesive interface is of concern [8].

Resin monomers associated with quaternary ammonium compounds (QAM) represent the main "antibacterial adhesives" and several studies have evaluated the antimicrobial potential of these materials against the cariogenic microorganisms [9-11] and against biofilm formation of *S. mutans* [12]. Recently, the incorporation of enzymatic inhibitors into adhesive systems has been accepted as effective strategies to maintain the integrity of the hybrid layer and to improve the bond strength to the long-term dentin [13]. Inhibitors incorporated into the adhesives can act in the inactivation of enzymes known as matrix metalloproteinases (MMPs) and cysteines cathepsins (CT). These enzymes are responsible for the degradation of the organic matrix in hybrid layer, influencing the durability of bonding to the dentin [14]. Thus, an ideal bioactive agent must have antimicrobial and/or remineralizing properties and should improve the bond strength of restorative materials to dental tissue, while inducing low cytotoxicity [6,11].

Chlorhexidine digluconate is the “gold standard” agent in controlling biofilm formation, and is a potential inhibitor of MMPs and CTs [15,16]. *In vitro* and *in vivo* studies have concluded that, when associated to adhesive systems, it enhances the bond of the adhesives to dentin [17,18], but its cytotoxic effects were demonstrated when applied directly on cells, with a dose-time dependent toxic effect [19] and when incorporated into glass ionomer cement [20]. De Castilho et al., 2013 [20] demonstrated that the 2.5% chlorhexidine digluconate affected two important properties of the material: the cytotoxicity on odontoblast-like cells and compressive strength of the cement.

In this field, natural products stand out when searching for more biocompatible inhibitors. The polyphenols, especially the epigallocatechin-3-gallate (EGCG), are the most active and abundant catechins present in green tea with a proven ability to inhibit the expression and action of MMP-2 and MMP-9 [21]. The use of EGCG is effective in the preservation of adhesive bond strength when incorporated into the dentin adhesive [22] or when used as a dentin pretreatment [23]. Neri et al, 2014 [24] showed that the incorporation of EGCG (0.01% and 0.1%) into a one-step self-etch adhesive did not affect its physicochemical properties, except the solubility and it may be useful for therapeutic adhesion to dental hard substrates, particularly to dentin, due to the potential of improving the longevity of adhesive procedures.

The antibacterial activity of green tea and ECGC was confirmed by laboratory studies [25] including cariogenic bacteria *Streptococcus mutans* and *Lactobacillus acidophilus* [26] and periodontal pathogenic *Eikenella corrodens* [27]. The antimicrobial mechanism of EGCG is mainly attributed to the influence on initial bacterial adhesion. EGCG represents a natural anti-cariogenic agent [28], exhibiting antimicrobial activity against *S. mutans*, and suppressing the specific virulence factors associated with its cariogenicity [29,30]. Nevertheless, to the best of the current authors' knowledge, there is a lack of data related to dentin cytotoxicity of EGCG to dental pulp cells and to the potential therapeutic effect of EGCG incorporation in adhesive systems on the formation and development of biofilm by *S. mutans* [22].

Hence, the purpose of this study was to assess if EGCG has toxicity in Human dental pulp stem cell, and the antibacterial activity of EGCG incorporated to commercial adhesive systems in the formation and development of the *S. mutans* biofilm.

2. Material and methods

2.1. Cytotoxicity test

2.1.1. Dentin specimen preparation

Twenty (20) extracted, caries-free human third molars were used. The teeth were collected after obtaining the patient's informed consent under a protocol reviewed and approved by the local Research and Ethics Committee. The roots and the occlusal enamel of each tooth were removed using a slow-speed diamond saw under water-cooling (IsoMet, Buehler, Lake Bluff, IL, USA). Flat dentin slices (0.5 mm thickness) were obtained from the mid-coronal dentin of each tooth and the exposed dentin surfaces were further polished on wet #320 and #600-grit SiC paper. Dentin slices were etched with 37% phosphoric acid for 15 seconds, rinsed with Milli-Q water for 15 seconds, dried with sterile absorbent paper and randomized in the experimental groups: G1: Dentin slice (control group); G2: Dentin with 100% Ethanol; G3: Dentin slice with excessive ethanol (negative control); G4: Control cells - no dentin; and G5: dentin treatment with 0.1% EGCG aqueous solution. Five (5) μL of each solution was applied on dentin slices and allowed to dry.

2.1.2. Cell culture

Human dental pulp stem cells (DPSCs) were purchased from Lonza and cultured in DPSC growth medium including DPSC Basal Medium and Single Quots Kit (Lonza) according to the manufacturer's instructions.

2.1.3. Cell metabolic activity - MTT assay

Human dental pulp stem cells (DPSCs) were placed into 24-well plates for 24 h before treatments. Dentin slices were set into columns, which could not attach the cells directly. DPSCs were incubated with or without dentin slices (columns) for 48 h and 72

h at 37°C in 5% CO_2, and the cells viability was analyzed on triplicate (n=6). The tetrazolium dye (MTT) solution (Vybrant® MTT Cell Proliferation Assay Kit #V-13154, Thermo Fisher Scientific) was added to medium in each well and incubated for 4 hours according to the manufacturer's instructions. Sodium dodecyl sulfate (SDS) was directly added into the medium in each well and incubated overnight. The absorbance signal was measured on a spectrophotometer at 562 nm.

2.2. Direct contact test against S. mutans

2.2.1. Experimental design

Commercial adhesive systems, adhesives systems incorporated with EGCG, aqueous and alcoholic EGCG solution were directly tested against *S. mutans* (UA159). Antibacterial contact activity was analyzed after 5 min, 30 min, 1 h and 24 h. The tested groups were: (a) adhesive discs: 40 µL of Single Bond (3M ESPE) and Single Bond Universal (3M ESPE) with and without 0.1 % w/v EGCG; (b) aqueous solutions: 0.1 % w/v EGCG/ H_2O; 0.5 % w/v EGCG/ H_2O; (c) alcoholic solutions: 0.5 % w/v EGCG/ 10% ethanol; 1.0 % w/v EGCG/ 10% ethanol; 1.5 % w/v EGCG/ 10% ethanol; (d) negative control: 0.89 % saline solution and (e) (positive controls): 0.2 % and 2 % chlorhexidine.

2.2.2. Adhesive incorporation and specimen preparation.

ECGC powder 0.1 % (w/v) was directly incorporated into adhesive systems. This concentration was based on previous in vitro studies [23]. EGCG powder was weighted on an analytical scale and transferred to a microcentrifuge tube covered with aluminum foil. The calculated amount of adhesive was added directly with a calibrated pipette and vortexed for immediate use. Adhesive discs (4 mm diameter x 1.5 mm thick) were confectioned using a pre-fabricated silicone mold. Forty (40) μl of the

incorporated adhesive were carefully dispensed into the mold with a calibrated pipet, avoiding air bubble incorporation. A plastic matrix strip was placed over the mold and a microscope glass slide was placed above them. The adhesive specimens were light-cured for 40 s (Poly Wireless, Kavo, São Paulo, SP, Brazil) in close contact with the glass to standardize the distance of light curing (intensity of 600 mW/cm^2 on each side of the specimen).

2.2.3. Bacterial Inoculum

The strain *S. mutans* (ATCC 700610 / UA159), a cariogenic pathogen, maintained as frozen stock at –80°C, was reactivated on tryptic soy agar plates with 5% sheep blood (TSA II 5% SB - BD, USA). To prepare the inoculum, colonies were inoculated on tryptic soy broth supplemented with 0.6% yeast extract (TSB+YE; Difco Laboratories, Detroit, MI, USA) and 1% glucose to grow overnight (18-24 h) under microareophilic conditions (37^{0}C, 5% CO_2). *S. mutans* inoculum was adjusted to 660 nm in a spectrophotometer to match the turbidity of 1.5 x 10^9 CFU/ mL.

2.2.4. Bactericidal activity assay [31]

Each adhesive disc and 40 µL of each solution (EGCG and controls) were placed into a well of 96-well cell culture plates, which contained 120 µL of bacterium inoculum. After 5 min, 30 min, 1 h and 24 h, 5 µL of microbial suspension from each well was plated and incubated to grow overnight (18-24h) under microarephilic conditions for 48h (37^{0}C, 5% CO_2). The experiment was performed in duplicate in three different experiments (n=6).

2.3. S. mutans biofilm development test

2.3.1 Biofilm formation.

The groups tested for direct contact were also tested for biofilm formation. The adhesive discs were coated with filter-sterilized clarified human whole saliva. Fresh saliva samples were collected from groups of 2-3 volunteers without active carious lesions, erosions or salivary dysfunctions. The subjects did not eat or smoke for 8 h before the sampling. The volunteer saliva was stimulated by chewing a paraffin film for 5 minutes. Saliva from the first minute of chewing was swallowed, and the remaining was collected and deposited into centrifuge tubes kept on ice. The saliva samples were centrifuged (10 min, 5000 x *g*, 4^0C) centrifuge. The clear fluid above the sediment was collected (clarified saliva) and filtered through 0.22 µm low protein binding filter (Milipore Co., Bedfrod, Mass, USA) to sterelize. Adhesive discs were submerged in 1 mL saliva solution and incubated at 37^0C for 1h on an orbital shaker. Saliva-coated adhesive discs were placed in *S. mutans* inoculated medium (3 mL) with TSB+YE supplemented with 1 % sucrose (37º, 5% CO_2) for 5 days [32]. The biofilms were kept undisturbed for 24 h to allow initial formation. The culture medium was replaced daily and the effects of the EGCG incorporation in the formation and development of biofilms were measured after 24, 72 and 120 hours. In each period, the biofilms were analyzed by CFU/mL (cfu) and dry-weight assays. The experiment was done in duplicate fot three independent experiments with the controls for disc contamination (disc plus medium without bacteria); medium contamination (only medium without bacteria) and bacterial growth (only inoculated medium).

2.3.2. Biofilm analysis

On each day of analysis, biofilms were processed for dry-weight and bacterial viability (CFU/mL). The adhesive discs were washed twice with 2 mL 0.89% NaCl and individually released into glass tubes with 1 mL 0.89% NaCl and more 1 mL was

added. The tubes were placed in ultrasonic bath and sonicated for 10 minutes on ice. Each disc was removed from its tube using a sterile spatula. The volume was completed to 5 mL with sterile 0.89% NaCl and the removed biofilms were subjected to sonication using three 15 s pulses at an output of 7 W (Fisher Scientific, Sonic Dismembrator model 100; USA). The homogenized suspension was used for analysis. For the dry-weight determination, three volumes of cold ethanol (-20ºC) were added to 1 mL biofilm suspension and the resulting precipitate was centrifuged (10,000 g for 10 min at 4ºC). The supernatant was discarded, and the pellet was washed with cold ethanol, and then lyophilized and weighed. Dry-weight is expressed in milligrams (mg). For bacterial viability, an aliquot (0.1 mL) of the homogenized suspension was diluted and plated on TSA II 5% SB plates in duplicate. The plates were incubated (37ºC, 5 % CO_2) for 48 h and the CFU/mL was determined.

2.4 Statistical Analysis

For cytotoxicity and direct contact tests, all results are expressed as means ± standard deviation (SD) of triplicate measurements with all experiments. Statistical analyses were carried out using Student's t test and one-way ANOVA using the Tukey HSD test (α=0.05). For CFU/mL and dry-weight, normal distribution of data was verified by Shapiro-Wilk test and homogeneity of variance was checked by Levene's test (α = 0.05). The quantitative data of CFU/mL were Log_{10} transformed prior to analysis. Data that met the assumptions of normality and homogeneity were submitted to analysis of variance (ANOVA), followed by post-hoc Tukey's test (α = 0.05). Data that did not meet the assumptions of normality were analyzed by the Kruskal-Wallis test (α = 0.05). Analyses were performed using the software SPSS (IBM® SPSS® Statistics, version 20, Chicago, IL, USA).

3. Results

3.1. MTT assay results

For 48 hours incubation with materials, dentin slice with ethanol or 0.1% EGCG aqueous solution significantly suppressed cell proliferation compared to dentin slice without ethanol (control group). The excessive ethanol with dentin slice was used as a negative control, which significantly inhibited cell proliferation compared to dentin slice with ethanol. The dentin slice with 0.1% EGCG aqueous solution also significantly suppressed cell proliferation compared to dentin slice with ethanol (Figure 1).

For 72 hours incubation with materials, dentin slice with excessive ethanol significantly suppressed cell growth compared to dentin slice without ethanol (positive control). However, the dentin slices with 0.1% EGCG aqueous solution did not inhibit cell proliferation compared to dentin slice with excessive ethanol, indicating that human DPSCs with 0.1% EGCG aqueous solution are recovered and revitalized at 72 hours (Figure 2).

3.2. Direct contact results

The results of bactericidal contact activity against *S. mutans* are depicted in Table 1. Results showed that only the positive controls (0.2% and 2% chlorhexidine solutions) were antibactericidal and eliminated the microorganisms. The negative control (0.89% NaCl solution) did not kill *S. mutans* at any tested time and the other test solutions showed few bactericidal effects, regardless EGCG incorporation.

3.3. Colony forming unit

Cell viability expressed in Log_{10} (CFU/mL) is presented in Table 2. At day 1, Log_{10} (CFU/mL) data did not meet the assumption of normality (p=0.025). Overall independent samples Kruskall-Wallis test did not show significant differences across

the samples (p=0.338) (Figure 3). For day 3, Log_{10} (CFU/mL) data did not meet the postulation of normality (p=0.004). Kruskall-Wallis overall test showed that there were no significant differences between the samples (p=0.670) (Figure 4). Log_{10} (CFU/mL) data from day 5 presented normal distribution (p=0.102) and homoscedasticity (p=0.465) of variances, so ANOVA one-way with Tukey *post-hoc* test was applied. Tests showed that there were no significant differences between groups (p=0.934) (Figure 5). Hence, there was no statistical differences between Single Bond, Single Bond + 0.1 % (w/v) EGCG, Single Bond Universal and Single Bond Universal+ 0.1 % (w/v) EGCG at day 1, day 3 and day 5.

3.4 Dry weight results

Dry-weight data for day 1 met the normality criteria (p=0.089), so data were analyzed by one-way ANOVA with Tukey *post-hoc* test (α=0.05). Tests showed that there were no statistical differences between Single Bond, Single Bond + 0.1 % (w/v) EGCG, Single Bond Universal and Single Bond Universal+ 0.1 % (w/v) EGCG at day 1 (p>0.396). On the other hand, data for day 3 did not meet the postulation of normality (p=0.05), so the Kruskal-Wallis test was applied (α=0.05). The test showed that there were no statistical significant differences across the samples (p=0.992) at day 3. Similarly, data for day 5 did not show normality of variances (p=0.000). Tests revealed that the distribution is the same across the samples (p=0.996), thus, no statistical significant differences occurs in the dry-weight of the studied groups after 1, 3 or 5 days of biofilm formation (Figures 6-8).

4. Discussion

After water, tea is the most popular and consumed drink in the world [33]. Epigallocatechin-3-gallate (EGCG) is a natural compound and an active polyphenolic

catechin that represents around 59% of the total catechins from the leaves of the green tea [34]. This main polyphenol of green tea has been demonstrated to have biological properties, like cytostatic properties for preserving cells, antibacterial and anti-inflammatory reactions, as well as antioxidant and anticarcinogenic effects [35-37].

EGCG has been reported to reduce the production of acidic compounds and to have antibacterial effects against oral streptococci via the non-reversible damage of the microbial cytoplasmic membrane [38-40]. Previous studies have verified that EGCG can exhibit a wide range of physiological effects on *S. mutans*, mainly on virulence factors associated with its acidogenicity and acidurity [29,30]. Besides the antibacterial effects EGCG has demonstrated ability to increase collagen resistance to enzymatic or physicochemical degradation [41] and to preserve the long-term dentin bond effectiveness [22,23,42].

Given the variety of biological benefits, EGCG has been reported as a promising bioactive substance to be associated with adhesive procedures. Thus, the aim of this study was to investigate this natural compound cytotoxicity to dental pulp cells and the antibacterial activity of commercial adhesive systems incorporated with EGCG in the concentration that it has been evaluated to achieve the dentin-bond effectiveness benefits [23,42].

To evaluate the propensity of ECGC in killing *S. mutans*, direct contact was performed before biofilm tests. Contrary to previous study that observed an inhibitory effect on the growth of *S. mutans* from 200 μg/mL and 300 μg/mL EGCG-incorporated dental adhesive [22], in the present study, EGCG in different concentrations [1.5% (w/v), 1.0% (w/v), 0.5% (w/v), 0.1% (w/v)] showed slight bactericidal effects, whether

incorporated to adhesives or not. Therefore, as antimicrobial effects were observed, ECGC incorporated in adhesive systems was tested in biofilms.

When incorporated in Single Bond and Single Bond Universal at the concentration of 0.1% (w/v), EGCG did not show significant effects in reducing the number of viable cells of *S. mutans* or the dry-weight of the biofilms after 24 h, 48 h and 72 h of biofilm formation. In a previous study, MTT assay revealed that EGCG at the concentration of 0.1% (w/w) incorporated into a conventional glass ionomer cement (Fuji IX) improved the antibacterial properties of the glass ionomer cement after 4 h of initial *S. mutans* adhesion to the specimens; however, after 24 h, antibacterial results were not observed [43].

During biofilm development, the adsorption and passive transportation of oral bacteria to tooth surfaces occurs in the initial 4 h [44]. In the next 24 h, the later colonizers adhere to the already attached early colonizers and grow [45]. Adherence of pathogenic microorganisms to host tissues begins the disease development [46]. The attached bacteria produce a diversity of extracellular polymers to construct the biofilm matrix that could disturb the diffusion of substances throughout the biofilm [47]. Thus, first of all, the presence of the matrix in the mature biofilms of 24 h, 48 h and 72 h might have difficulted the diffusion of the ECGC through the biofilms to reach the *S. mutans* cells, by way of microorganisms protected in the matrix are more resistant to antimicrobials [48]. Therefore, future studies should focus on disorganize the biofilm matrix before applying the ECGG to improve its antimicrobial aspects by increasing the bioavailability and maintenance of EGCG inside the biofilm.

Besides of the protective effect of the matrix, lack of significant results regarding direct contact, CFU and dry-weight might be explained by the oxidation of

EGCG, that starts already during direct contact with air [49]. EGCG is a catechin with a pyrogallol-type structure on the B-ring, a characteristic that leads to robust antioxidative activity and autooxidation to form reactive oxygen species that results in polymerization and decomposition [50]. Thus, during the different steps for direct contact test and biofilm formation, the contact with the air might have decomposed part of or whole ECGG; therefore, its mechanism of action might have been impaired. Moreover, EGCG degradation is enhanced by relative humidity and temperature [51]. As direct contact tests and biofilms were performed at 37º C and the humidity was not controlled, it might be supposed that the sum of these conditions may have contributed to enhance the oxidation with air contact. As the biofilm formation depends on specific conditions of temperature, an alternative to improve results might be to increase the concentration of ECGC, as the concentrations studied here did not exhibit cytotoxicity. Moreover, it is necessary to avoid excessive air contact during *in vitro* tests, and it can be achieved by working in an anaerobic chamber, for example.

Finally, there is a limitation in quantifying the release of EGCG from the adhesive system after light-cure. Therefore, the available final concentration of EGCG on both antimicrobial tests might be lower than the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values reported by [29] as 625 μg/ml Land >1,250 μg/mL, respectively for planktonic cells and as 312.5 μg/mL for $MBIC_{50}$ (the lowest flavonoid concentration that showed at least 50% inhibition of the formation of biofilms compared with control) [29]. Therefore, further studies are encouraged to assess the releasing of EGCG to more reliable quantified concentration.

The present study also investigated the epigallocatechin-3-gallate (EGCG) cytotoxicity to dental cells. The transdentinal method in the present study's MTT assay simulated the clinical situation, which the product is applied onto the dentin surface, where it can protect of protecting the underlying cells [8]. We observed that human DPSCs with EGCG were recovered and revitalized at 72 h.

In conclusion, although 0.1% (w/v) EGCG incorporation into dental adhesives did not show antibacterial activity, it has been shown to be biocompatible for DPSCs, and may be safe promising for bioactive dental materials as they did not reduce cell viability.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Tables

Table 1. Bactericidal contact activity against Streptococcus mutans.

	5 min	30 min	1 h	24 h
0.89 % NaCl (control)	+	+	+	+
0.2 % CHX	Ø	Ø	Ø	Ø
2 % CHX	Ø	Ø	Ø	Ø
0.1 % EGCG/H_20	+	+	+	+
0.5 % EGCG/H_20	+	+	+	+
0.5 % EGCG/ Ethanol	+	+	+	+
1.0 % EGCG/ Ethanol	+	+	+	+
1.5 % EGCG/ Ethanol	+	+	+	+
Single Bond	+	+	+	+
Single Bond + 0.1% EGCG	+	+	+	<
Single Bond Universal	+	+	+	<
Single Bond Universal + 0.1% EGCG	+	+	+	<<

The (+) signal indicates that there was normal bacterial growth at that specific time compared to the control group. The (ø) signal indicates that the treatment was antibacterial at that specific time compared to the control group. The (<) signal indicates that there was less bacterial growth at that specific time compared to the control group. The (<<) signal indicates that there was just a few bacterial growth at that specific time compared to the control group.

Table 2. Mean values and standard deviations of Log10 (CFU/mL) of Single Bond 2, Single Bond+EGCG, Single Bond Universal and Single Bond Universal+EGCG after 1, 3 and 5 days of biofilm. Equal letter represents no statistical differences between the groups in each day ($p>0.05$).

GROUPS	Day 1		Day 3		Day 5	
Single Bond 2 control	5.36	a	6.48	a	6.26	a
	(1.50)		(0.35)		(0.24)	
Single Bond 2 + EGCG	4.33	a	6.18	a	6.13	a
	(0.97)		(0.63)		(0.32)	
Single Bond Universal Control	5.45	a	6.27	a	6.24	a
	(1.39)		(0.51)		(0.43)	
Single Bond Universal + EGCG	5.28	a	5.97	a	6.16	a
	(1.17)		(0.64)		(0.38)	

Figures

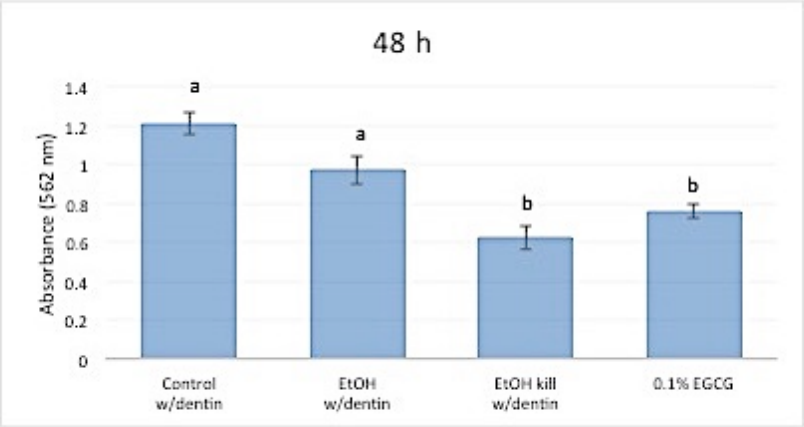

Figure 1. Pulp stems cells viability (48 h results). Student's t test and one-way ANOVA using the Tukey HSD test was applied. Equal letter represents no statistical differences between the groups ($p>0.05$).

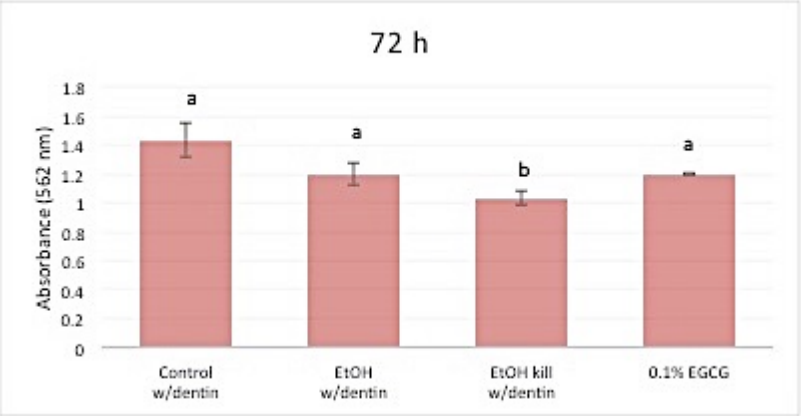

Figure 2. Pulp stems cells viability (72 h results). Student's t test or one-way ANOVA using the Tukey HSD test was applied. Equal letter represents no statistical differences between the groups ($p>0.05$).

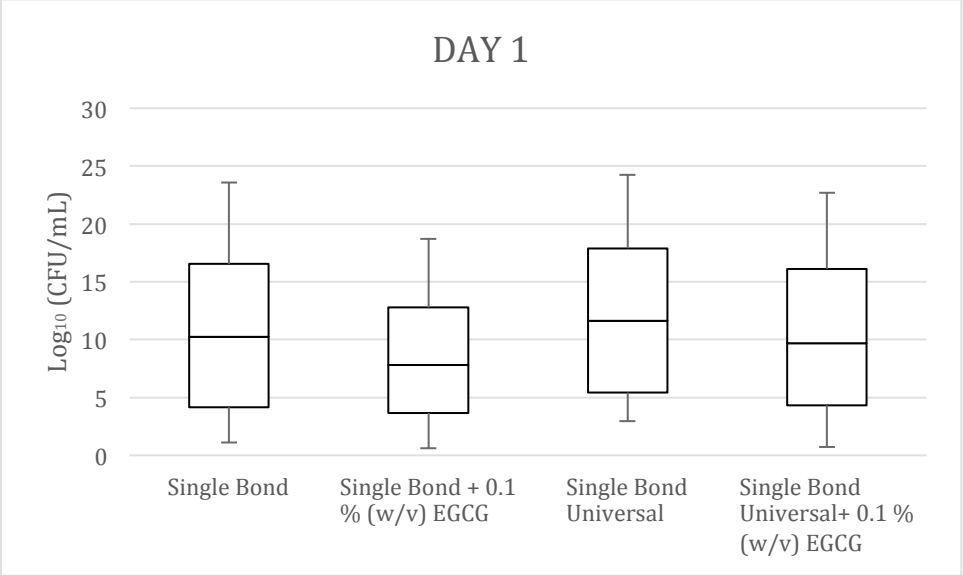

Figure 3. Box-plot of Log10 (CFU/mL) of Single Bond 2, Single Bond+EGCG, Single Bond Universal and Single Bond Universal +EGCG for Day 1. The box-plot shows the median (dash), the first and third quartiles (outer edges of box), and the highest and maximum values (error bars). A non-parametric analysis (Kruskal-Wallis) was applied.

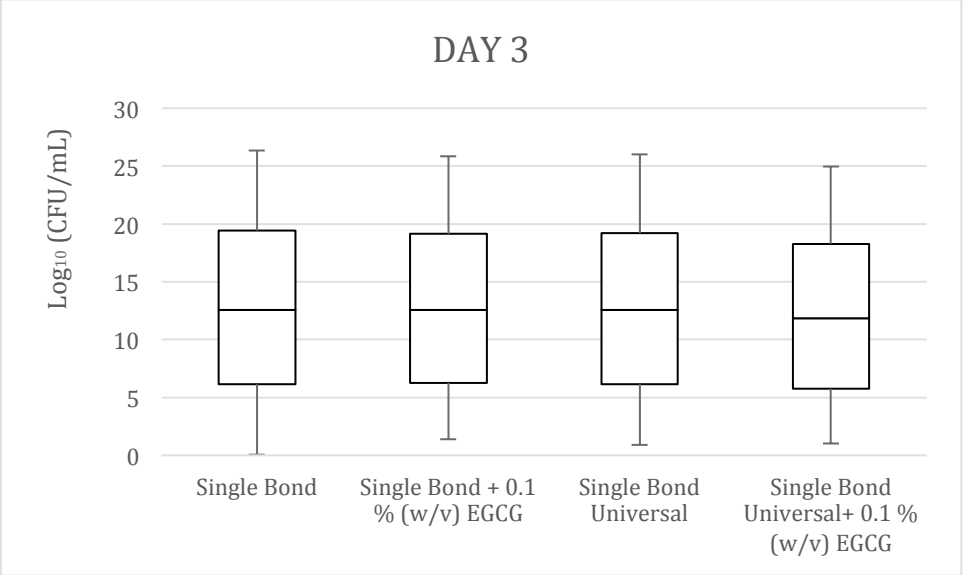

Figure 4. Box-plot of Log10 (CFU/mL) of Single Bond 2, Single Bond+EGCG, Single Bond Universal and Single Bond Universal +EGCG for Day 3. The box-plot shows the median (dash), the first and third quartiles (outer edges of box), and the highest and maximum values (error bars). A non-parametric analysis (Kruskal-Wallis) was applied.

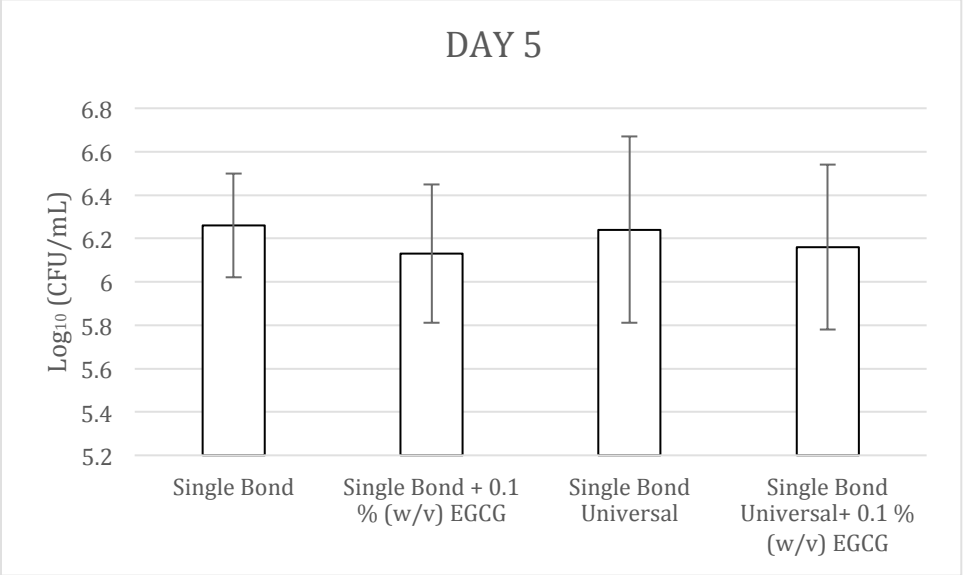

Figure 5. Mean values and standard deviations of Log10 (CFU/mL) of Single Bond 2, Single Bond+EGCG, Single Bond Universal and Single Bond Universal +EGCG for Day 5.

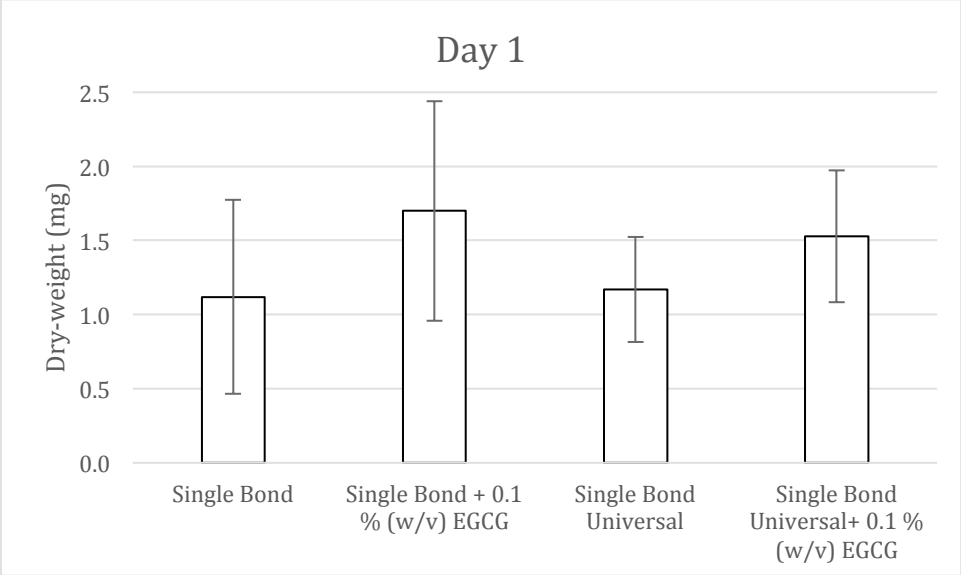

Figure 6. Mean values and standard deviations of dry-weight (mg) of Single Bond 2, Single Bond+EGCG, Single Bond Universal and Single Bond Universal +EGCG for Day 1.

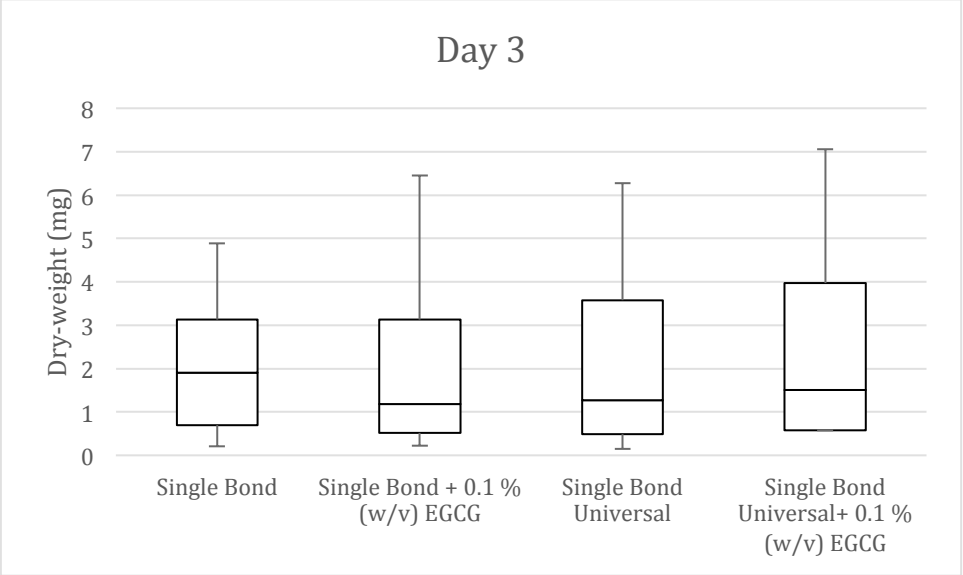

Figure 7. Box-plot of dry-weight (mg) of Single Bond 2, Single Bond+EGCG, Single Bond Universal and Single Bond Universal +EGCG for Day 3. The box-plot shows the median (dash), the first and third quartiles (outer edges of box), and the highest and maximum values (error bars). A non-parametric analysis (Kruskal-Wallis) was applied.

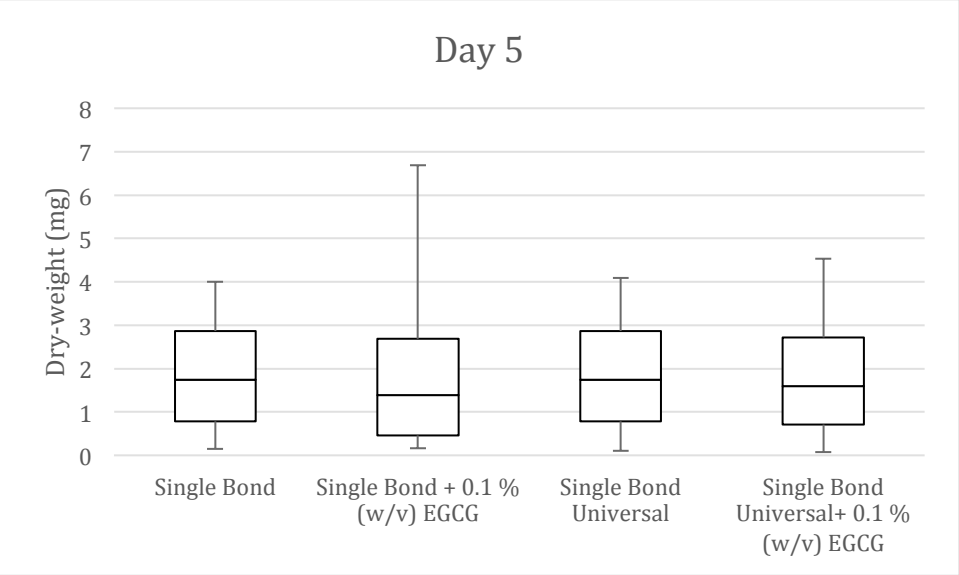

Figure 8. Box-plot of dry-weight (mg) of Single Bond 2, Single Bond+EGCG, Single Bond Universal and Single Bond Universal +EGCG for Day 5. The box-plot shows the median (dash), the first and third quartiles (outer edges of box), and the highest and maximum values (error bars). A non-parametric analysis (Kruskal-Wallis) was applied.

CAPÍTULO 2

One-year of Epigalocatechin-3-galate Dentin Pretreatment on Clinical Performance of a Universal Adhesive: A Randomized Clinical Trial.

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One-year of Epigalocatechin-3-galate Dentin Pretreatment on Clinical Performance of a Universal Adhesive: A Randomized Clinical Trial.

Abstract

Purpose: To evaluate the 1-year clinical performance of dentin pretreatment with epigallocatechin-3-gallate (EGCG) on restorations of non-carious cervical lesion (NCCLs) using a multimode adhesive Single Bond Universal, applied in etch-and-rinse and self-etch strategies. *Materials and Methods:* In this randomized clinical trial, 33 volunteers were selected and 156 NCCLs were assigned to four groups: ER: etch-and-rinse; ER-EGCG: 0.1% EGCG dentin pretreatment + etch-and-rinse; SE: self-etch; and SE-EGCG: 0.1% EGCG dentin pretreatment + self-etch. The NCCLs were incrementally restored with the nanofilled composite resin Filtek Z350XT. The restorations were evaluated at baseline, 6 and 12 months using FDI criteria for marginal staining, retention, marginal adaptation, caries and postoperative sensitivity. Impressions were taken of NCCLs and restorations to create resin replicas for observation under scanning electron microscopy. Two evaluators were blinded to the treatments performed and the Cohen's Kappa statistic measured the agreement between them. Statistical analyses were performed with Kruskal-Wallis and McNemar tests with a significance level of 5%. *Results:* Five restorations (1 from ER; 2 from SE and 2 from SE-EGCG) were lost at 12 months. Retention rates were 97.37% for ER and SE, 100% for ER-EGCG, and 94.6% for SE-EGCG, with no statistical difference among the groups ($p > 0.05$). There were no statistically differences among all other criteria at 12 months ($p > 0.05$), neither for each group when baseline and 12-month time were

compared (p>0.05). *Conclusions:* The clinical retention of the universal adhesive at 12 months does not depend on the bonding strategy and dentin pretreatment with EGCG.

Keywords: Adhesives, Catechin, Dentin bonding, Clinical trial.

Clinical significance

At 12 months, the clinical performance of the adhesive Single bond Universal may not depend on the bonding strategy employed or dentin pretreatment with epigallocatechin-3-gallate.

1. Introduction

Improve the quality of the adhesive interface and understand its degradation mechanisms have been the current research focus in adhesive Dentistry. [6, 27, 57] Degradation can weaken adhesion and lead to failures between dental structure and restorative material. Marginal staining and marginal discrepancy present it self as consequences related to degradation in composite resin restorations. [7]

The susceptibility of the adhesives systems to water and/or oral fluids sorption is directly related to this degradation [56] resulting in the resinous components [48] and silane hydrolysis. [4,58] Additionally, recent studies have emphasized role of the matrix metalloproteinases (MMPs) and cysteine cathepsins (CC) on enzymatic degradation of demineralized collagen fibrils that were not covered by resinous material or were exposed by polymer hydrolysis. [40, 55, 42] Therefore, the use of enzyme inhibitors has been accepted as effective strategies to maintain the integrity of the hybrid layer and improve the long-term bond strength to dentin. [1, 13, 36, 65]

Chlorhexidine (CHX) is a potential inhibitor of MMP and CC [18, 51] and several *in vitro* [9,30] and *in vivo* studies [5, 10, 20, 45] have concluded that, when associated with adhesive procedures (either as dentin pretreatment, [8] incorporated into phosphoric acid [54] or adhesive [47, 64]), it prevents degradation in hybrid layers. There is still no consensus regarding this benefit in the different adhesive strategies (self-etch or etch-and-rinse), and only few studies showed the clinical outcomes using CHX as MMP and CC inhibitors. [2, 17, 35]

Other natural enzymatic inhibitors have aroused the interest of the scientific community. Polyphenols found in green tea, especially epigallocatechin-3-gallate (EGCG), with proven ability to inhibit the expression and action of MMP-2 and MMP-9 [14] have recently been studied in association with adhesive procedures. Laboratory studies have demonstrated that the use of EGCG is effective in preserving adhesive bond strength when incorporated into dentin adhesive [15] or when used as dentin pretreatment. [38, 49, 63]

Despite the laboratory results demonstrated the benefits in the immediate and long-term bond strength of EGCG-associated adhesive procedures, [15, 38, 49] it is known that there is still a lack on correlation between the results from *in vitro* tests and the clinical reality to which the adhesive restorations are exposed. Regarding retention of composite resin restorations in cervical lesions, there seems to be a potential relationship between laboratory results from bond strength and clinical findings. [60] However, other aspects such as marginal adaptation, marginal staining, caries adjacent and postoperative sensitivity can only be answered in randomized clinical trials. [21]

EGCG is a very abundant natural product and a sustainable resource. The benefits already published in laboratory studies justify the application of this product in

long-term clinical evaluations, since there are no published clinical studies evaluating the benefits of EGCG application associated with adhesive restorative procedures.

The objective of the present study was to evaluate the influence of dentin pretreatment with EGCG in restorations of non-carious cervical lesions performed with a universal adhesive system in the two adhesive strategies *in vivo*. The null hypothesis tested were (1) there is no difference between the clinical performance of NCCLs restorations bonded with the etch-and-rinse strategy associated or not to dentin pretreatment with EGCG and (2) there is no difference between the clinical performance of NCCLs restorations bonded with the self-etch strategy associated or not to dentin pretreatment with EGCG.

2. Materials and methods

2.1. Experimental design and material selection

This clinical trial was a randomized with an equal allocation rate among the four groups under evaluation. The experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statement [52] and is summarized in Table 1.

A nanofilled resin and a universal adhesive system were used to restore the non-carious cervical lesions. In control groups, the adhesive system was applied in two strategies suggested by the manufacturer (i.e. self-etch and etch-and-rinse). In experimental groups, 0.1% EGCG aqueous solution was actively applied for 60 seconds, prior the adhesive application for each strategy. Table 2 summarizes the restorative materials used and applications procedures.

2.2. Sample size calculation

Considering a two-tailed test hypothesis, the sample size was calculated based on the 96% retention rate of the Single Bond Universal observed in 18-year clinical

follow up. [41] In order to determine a 25% difference between groups with a significance level of 5% and a statistical power of 80%, at least 33 restorations per group were required. Taking into consideration possible loss during the study, a 20 % increase in sample size was set.

2.3. Patient selection and ethical considerations

Study subjects were selected from Dental School according to the following conditions: (1) need at least four restorations of non-carious cervical lesions (erosion, abrasion and/or abfraction); (2) be over 18 years; (3) present adequate oral hygiene condition and absence of periodontal disease; (4) have at least 20 teeth in occlusion. The lesions should be expulsive, without retention, with no more than 50% of the margin in enamel [29] and with cervical margin in dentin. Teeth with previous cervical restorative procedure or interproximal caries were excluded.

This clinical trial protocol was approved by the Local Ethics Committee on Investigations involving human subjects (protocol 1.292.593) and registered on the Brazilian Clinical Trials Registry – ReBEC (Register Number: RBR-2hr94r). Prior to participating, all volunteers signed a written consent form, explaining the accomplishment of the study, risks and benefits to which they would be exposed.

2.4. Randomization of the sample

The NCCLs selected were randomly distributed according to each treatment following one random list generated by a computer program. For each patient, four sealed envelopes were made. The operator was aware about the treatments only at the time of the procedures, when the envelopes were opened. The sequence of the restorations was according to the sequence of the quadrants of the arches, i.e., the tooth located in the first quadrant received the treatment determined in the first envelope

opened, and so on. The patient was not aware of the location of each treatment performed.

2.5. *Clinical procedures*

Participants attended two times to perform the clinical procedures with 2 hours duration each and returned to evaluations, lasting half an hour each session.

In the first appointment, a detailed anamnesis was performed to check the patient's general and oral health. All NCCLs were classified according degree of sclerotic dentin [53] (Table 3) and according the cavity dimensions in millimeters (height, width and depth), shape of the cavity (degree of the angle labeled as <45º, 45-90º, 90-135º, >135º), presence of antagonist, presence of incisal/occlusal wear facets, presence of preoperative sensitivity and distribution among tooth types (Table 4). All lesions were photographed using a digital camera (Canon, EOS, 60D, Canon Macro EF 100mm lens) in three positions: vestibular, proximal (mesial) and incisal/occlusal. Silicone rubber impressions with heavy and light-body polyvinylsiloxane material (Adsil, Vigodent, Rio de Janeiro, RJ, Brazil) were obtained of each NCCL to construction epoxy resin replicas [43] The impressions were stored in a moist-free environment for 24 h at 37^{o}C to posterior fill with a low-viscosity epoxy resin (Epoxx Fiber MC130/FD154, Penha Circular, RJ, Brazil) and allowed to cure for 24 h.

In the second appointment, the restorations were performed. No additional retention or bevel was performed and the operatory sequence was: (1) shade selection (VITA scale, H. Rauter GmbH & Co., Bäd Sackingen, Germany); (2) local anesthesia with 1.8 ml lidocain- 0.02 g with phenylephrine- 0.0004 g (Novocol, SSWhite, Petrópolis, RJ, Brazil); (3) cotton rolls isolation with labial retractor (Arc Flex-FGM, Joinvile, SC, Brazil) and gingival retractor # 00 (Ultrapack, Ultradent, Indaiatuba, SP,

Brazil); (4) cleaning with pumice paste (SSWhite, Petrópolis, RJ, Brazil) followed by rinsing and drying; (5.1) adhesive system application in control groups (ER and SE) following the two protocols (etch-and-rinse and self-etch, respectively) suggested by the manufacturer (Table 2); (5.2) adhesive system application in experimental groups (ER-EGCG and SE-EGCG) following the same two previous protocols, plus dentin pretreatment with 0.1% aqueous solution of EGCG, actively applied for 60 seconds (Table 2) and (6) composite restoration with Filtek Z350XT resin.

The restorations were performed with up to 3 increments of composite resin, photopolymerized individually for 20 s with 600 mW / cm^2 light curing unit (BISCO Dental Products IL, USA). The initial finishing of the restorations was performed with 12 and 30 blade Multilayer Carbides (Microdont, São Paulo, SP, Brazil) and F and FF diamond tips (KG Sorensen, São Paulo, SP, Brazil). The polishing was performed in the same session with the Sof-Lex Pop-On abrasive discs (3M ESPE, St. Paul, MN, USA) according to the manufacturer's recommendations.

2.6. *Clinical evaluation*

Two experienced and calibrated dentists, unaware of the conditions and locations of the different experimental groups, performed the evaluation. The analysis was done independently and performed with a mouth mirror and an explorer. When there was disagreement among examiners, a consensus was reached before the patient dismiss. [12] The restorations were evaluated by the FDI criteria (World Dental Federation) [22,23] at the baseline (one week after the restorations) and after 6 and 12 months.

For each property of the FDI criteria, only the most relevant measurements to evaluate the clinical performance of the restorations were considered [41] (Table 5). In

aesthetic properties, only the marginal staining was analyzed. For the functional properties, the marginal adaptation and the presence of fractures or retention failure were evaluated. Finally, presence of caries and postoperative sensitivity were evaluated in the biological aspect. These variables were classified as: clinically very good and clinically good (scores 1+2); clinically sufficient/satisfactory (score 3); clinically unsatisfactory (score 4) and clinically poor (score 5).

The retention rate was calculated according to the following equation: Cumulative failure percentage = [(PF + NF) / (PF + RR)] X 100%, where PF is the number of previous failures before the current assessment, NF is the number of new failures during the current evaluation and RR is the number of restorations currently evaluated. [41]

Silicone rubber impressions were obtained from the restorations (all periods) to construction of resin replicas for observation under scanning electron microscopy. [24] Clinical photographs of the restorations were taken in two positions (vestibular and mesial).

3. Statistical analysis

The comparison of all groups in each period was performed by Kruskal-Wallis test and McNemar's test was used to compare each group with each other at different periods. The comparisons were performed for each of the criteria evaluated with a significance level of 5%. To measure agreement between examiners, the Cohen's Kappa statistic was used.

4. Results

Thirty-eight out of seventy one patients were not enrolled in the study because they did not fulfill the inclusion criteria. Thus, 33 subjects (27 patients with four

NCCLs and 6 patients with 8 NCCLs) were selected and randomly assigned in the four experimental groups. The ratio of male:female patients was 19:14 and age ranged from 22 to 66 years. Table 3 and 4 show the NCCLs features classification and distribution among tooth types, respectively. Figure 1 shows the patients flow diagram.

All patients attended to the 6- and 12-month recalls and the overall recall rate was 100% at both periods. One patient was eliminated at 12 months due orthodontic treatment.

For the primary outcome, four restorations (1 from ER; 1 from SE and 2 from SE-EGCG) were lost after 6 months and one restoration was lost (1 from SE) after 12 months. Twelve-month retention rates were 97.37% for ER and SE, 100% for ER-EGCG and 94.6% for SE-EGCG with no statistical difference among the groups ($p > 0.05$). At 12 months, four restorations (1 from SE, 1 from ER-EGCG and 2 from SE-EGCG) presented fractures that damaged the marginal quality with partial loss of restoration, but not statistically significant ($p=0.3831$).

For the marginal adaptation criteria, four restorations showed dentin exposed (1 from SE, 1 from ER-EGCG and 2 from SE-EGCG), with no statistical difference among the groups ($p= 0.5635$). Moderate marginal staining (score 3) was only observed in three restorations (one from each group, except from SE), with no statistical difference among the groups ($p=0.8065$).

Three restorations showed scores 3 or 4 for postoperative sensitivity (2 for ER group and 1 for SE group) at baseline, with no statistical difference among the groups ($p=0.2961$). At 12 months, one restoration remained the postoperative sensitivity from the ER group (score 3). For the ER-EGCG and SE groups, 1 and 2 restorations became sensitive, with no statistical difference among the groups ($p=0.5462$). Finally, caries

were not observed after 12-month of clinical service and no tooth became non-vital as a result of the cervical restoration.

Data for the clinical parameters evaluated are summarized in Table 6.

Figure 2 exemplifies one clinically satisfactory restoration from each group in all evaluated periods.

5. Discussion

The endogenous proteolysis activity in dentin by matrix metalloproteinases (MMPs) and cysteine cathepsins (CC) result in the degradation of collagen within the hybrid layer and influence the durability of dentin bond in both etch-and-rinse and self-etch adhesives. [33, 55] Universal adhesives are a multimode one-bottle adhesives that have been recently developed [19] and can be used as self-etch or etch-and rinse strategies. In theory, regardless of the strategy, the hybrid layer formed by this mild adhesive (pH 2.7) will be susceptible to the same mechanisms of enzymatic degradation of the past adhesive generations. [32] *In vitro* studies popped-up to evaluate the immediate [37] and long-term [32] bond strength of the universal adhesives to enamel and dentin, but there are few clinical studies [25,26,31,34,41,46] and no longer than 36-months [28] reporting its clinical performance.

Aiming to inhibit the enzymatic degradation, some authors [38] have suggested the use of enzymatic inhibitors as chlorhexidine digluconate (CHX) and epigallocatechin-3-gallate (EGCG) to improve long-term bond to dentin, but there is a lack of data about its effect on the bond durability of universal adhesives. This randomized clinical trial is the first report evaluating a universal adhesive, on different adhesive strategies, assessing the influence of the dentin pretreatment with EGCG as an enzymatic inhibitor. The authors failed to reject the null hypotheses because, after 12

months, there was no difference between the clinical performances of NCCLs restorations bonded with the etch-and-rinse or self-etch strategy, associated or not to dentin pretreatment with EGCG.

In the present study, the comparison of bonding techniques was performed with noncarious cervical lesions (NCCLs) restorations because these lesions are the ideal substrate for assessing the clinical behavior of adhesive systems. [59] The adhesion require a bond mainly to dentin and the lack of macro-mechanical retention indicates that the restoration loss is due to ineffective bonding, which is an objective and clinically important outcome for adhesive efficacy. [11] Besides, they are a common clinical condition, [3] and are usually found in anterior and premolar teeth, with easy access to visual inspection. [44, 59]

The present research used the clinical criteria approved by the FDI World Dental as "standard criteria" to be applied when restorative materials and/or operative techniques are under clinical investigation. The evaluation of the restorations was categorized into three groups: esthetic, functional and biological criteria. FDI criteria were used because it is a more sensitive analyses to small variations in the clinical outcomes when evaluating restorations of NCCLs. [34, 41] Besides, it is well structured and flexible, which can be selected and adjusted according to the needs of the investigator. A simplified clinical evaluation was chosen pooling scores 1 and 2 (equivalent to score A from United States Public Health Service – USPHS criteria), [12] resulting in four different scores: two acceptable: (1;2 and 3) and two unacceptable (4 and 5), one for reparable and one for replacement, respectively. [22,39]

In addition, the present investigation reproduced details from NCCL for SEM analysis. [43] The replica could be an important research tool to associate NCCL features

with clinical behavior for the composite resin restoration and, could analyze the entire interface restoration in SEM micrographs at the low magnification of 40x and 90x (Figure 3). This non-invasive procedure supplemented the findings of the clinical investigation and enhanced its accuracy. [24]

In accordance with previous in vitro studies [62] and randomized clinical trials, [25, 26, 28, 31,34, 41,46] the clinical retention for the control groups (ER and SE) presented no statistically significant difference, which shows that the bond effectiveness of universal adhesive did not depend on the bonding strategy.

For the primary outcome, for all tested groups, only five restorations failed after 1 year of clinical service, and no difference was detected between groups. This result may be explained by the Single Bond Universal (SU) composition. It is a multimode adhesive that contains 10-methacryloxydecyl dihydrogen phosphate (10-MDP), an acidic phosphate monomer capable of bonding to hydroxyapatite, producing adhesive interfaces with different chemical and morphological characteristics. This process involves micromechanical and chemical bonding to the enamel and dentin, which may have a direct impact on the retention rate and clinical behavior of SU in the current study, after 12 months. [41] Probably, differences in the effectiveness of the tested groups in this randomized clinical trial may be present only after longer observation periods. [39] Although there was no statistical difference among the groups, the higher failure rate for the restorations occurred in the first 6-months of clinical service. Other factors could be involved in these results, over challenging the adhesion, as the dentin degree of sclerosis and excessive compressive stress. [59]

At date, there is no clinical trial evaluating natural enzymatic inhibitors as EGCG pretreatment. There are few clinical trials [16, 50] reporting the clinical effect of use

of CHX before adhesive procedures in non-carious cervical lesions and, the most relevant *in vivo* results were available after the tooth extraction to *in vitro* evaluation. [5,10, 45] The present outcome may be useful for validation of the clinical effectiveness of MMP inhibitors and highlighting that EGCG is a natural product.

EGCG has proved ability to inhibit MMP-2 and -9 and also has an additional property, since it also has positive effects on mechanical properties and on the stabilizing collagen against proteolytic degradation. It has been shown [61] that galoyl radical monomeric catechins, such as EGCG, are more effective at increasing the modulus of elasticity and reducing the rates of collagen biodegradation. Vidal et al., 2014 [61] showed the effect of catechins on the biomodification of the dentin matrix, with the formation of cross-links in an already highly crosslinked structure. They concluded that EGCG is the most potent monomeric catechin with crosslinking ability in dentin. We can speculate that the effects of EGCG were not relevant in this randomized clinical trial because the relative short-term period of evaluation.

There were also no statistically differences among groups in all other evaluated criteria at 12 months, neither for each group when baseline and 12-month results were compared. When analyzing the marginal staining, caries and postoperative sensitivity, all the restorations were classified with scores from 1 to 3 that can represent a satisfactory clinical behavior at 12 months (Figure 2). [22, 39] For marginal adaptation, four restorations (1 from SE, 1 from ER-EGCG and 2 from SE-EGCG) were considered clinically unsatisfactory, but reparable (score 4) with no statistical significance. However, the not using the SQUACE method (SemiQUAntitative Clinical Evaluation) to assess the exact location of the defect especially for the criteria “marginal adaptation” and “marginal staining” may have been one of the limitations of this study.

The main target of the dentin pretreatment with EGCG is that it could be available on dentin in the moment of adhesive application and could be incorporated to the hybrid layer. As the two different strategies were tested (i.e. self-etch and etch-and-rinse), the substantivity of EGCC in both sound and demineralized dentin by phosphoric acid etching, should be subject for further studies.

Longer observation periods may be required to justify the use of EGCG as dentin pretreatment. This additional clinical step could be in confront the main idea about universal adhesives that is simplify the adhesive application, but this procedure may show future benefits on clinical performance of NCCL restorations.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Tables and Figures

Table 1. Experimental design

Procedure / Groups	33 patients /156 NCCLs			
	ER	ER-EGCG	SE	SE-EGCG
Enamel (30 s) and dentin (15 s) total etching with 37% phosphoric acid	Yes	Yes	No	No
Dentin pretreatment (60s)	No	0.1% EGCG	No	0.1% EGCG
Adhesive application	Single Bond Universal	Single Bond Universal	Single Bond Universal	Single Bond Universal
Restorative procedure	Filtek Z350-XT			
Clinical evaluation (FDI criteria)	Baseline, 6, and 12 months			

NCCL: non-carious cervical lesions; ER: etch-and-rinse strategy; SE: self-etch strategy; EGCG: epigallocateachin-3-galate.

Table 2. Restorative materials and application procedures

Material	Manufacturer	Composition	Lot number	Application procedure *
Epigallocatechin -3-gallate	Sigma- Aldrich, St Louis, MO, USA	≥ 80 % (HPLC), from green tea	#SLBL 1959V	0.1% aqueous solution actively applied for 60s with a microbrush before adhesive application.
Filtek Z350 XT	3M ESPE, St. Paul, MN, USA	Treated silanized ceramics; Silane treated silica; (UDMA); Bisphenol A polyethylene glycol diether dimethacrylate; (BisGMA); Ceramics of zirconia; Polyethylene glycol; dimethacrylate; Triethylene glycol and dimethacrylate	#190224 # 147907	Up to 3 increments of composite resin, individually photopolymerized.
Single Bond Universal	3M ESPE, St. Paul, MN, USA	Methacryloyloxydecyl dihydrogen phosphate; phosphate monomer; dimethacrylate resins; hydroxyethyl methacrylate; methacrylate-modified polyalkenoic acid copolymer; filler; ethanol; water; initiators; silane.	#582958	Etch-and-rinse strategy: Total etching with 37% phosphoric acid (Enamel- 30 and dentin-15 s) follow by rising with water and drying with air free of moisture and oil, without drying out. Adhesive application to tooth surface by scrubbing action (20 Seconds) Dry the adhesive (5 Seconds) and light cure (10 Seconds).
				Self-Etch strategy: Adhesive application to tooth surface by scrubbing action (20 Seconds) Dry the adhesive (5 Seconds) and light cure (10 Seconds).

UDMA: Urethane Dimethacrylate; BisGMA: bisphenol A-glycidyl methacrylate.

**According to the manufacturer's instructions.*

Table 3. Dentin sclerosis scale [53]

Category	Criteria
1	No sclerosis present; dentin is light yellowish or whitish, with little discoloration; dentin is opaque, with little translucency or transparency.
2	More sclerosis than in category I but less than halfway between categories 1 an 4.
3	Less sclerosis than in category 4 but more than halfway between categories 1 and 4.
4	Significant sclerosis present; dentin is dark yellow or even discolored (brownish); glassy appearance, with significant translucency or transparence evident.

Table 4. Non-carious cervical lesions classification according to shape, cervico-incisal height, degree of sclerotic dentin, presence of antagonist, presence of incisal/occlusal wear facets, presence of preoperative sensitivity and distribution among tooth types

Characteristics of NCCL	Number of lesions			
	ER	ER-EGCG	SE	SE-EGCG
Shape (degree of angle)				
<45	1	2	1	3
45-90	7	10	5	4
90-135	20	20	24	19
>135	11	7	9	13
Cervicoincisal height (mm)				
<1.5	5	4	5	4
1.5-2.5	22	18	22	19
2.5-4.0	10	14	10	13
>4.0	2	3	2	3
Degree of sclerotic dentin				
1	17	20	23	22
2	19	14	12	12
3	3	5	4	5
4	0	0	0	0
Presence of antagonist				
Yes	36	37	37	37
No	3	2	2	2
Presence of incisal/occlusal wear facets				
Yes	32	31	26	33
No	7	8	13	6
Preoperative sensitivity (air dry)				
Yes	12	15	15	17
No	27	24	24	22
Preoperative sensitivity (spontaneous)				
Yes	7	7	6	6
No	32	32	33	33
Tooth Distribution				
Anterior				
Incisores	10	9	10	8
Canines	6	3	7	2
Posterior				
Premolar	21	22	19	28
Molar	2	5	3	1
Arc distribution				
Maxilary	20	23	25	15
Mandibular	19	16	14	24

ER= etch and rinse; ER-EGCG: etch and rinse + 0.1% EGCG pretreatment; SE= self-etch; SE-EGCG: self-etch + 0.1% EGCG pretreatment.

Table 5. World Federation (FDI) criteria used for clinical evaluation [22]

	Esthetic property	**Functional properties**		**Biological properties**	
	1. Marginal staining	**2. Fracture of material and retention**	**3. Marginal adaptation**	**4. Postoperative (hypersensitivity)**	**5. Recurrence of caries**
1. Clinically very good	1.1 No marginal staining	2.1 No fractures / cracks	3.1 Harmonious outline, no gaps, no discoloration	4.1 No hypersensitivity	5.1 No caries
2. Clinically good (after correction, very good)	1.2 Minor marginal staining, easily removable by polishing	2.2 Small hairline crack	3.2.1 Marginal gap (<150µm) 3.2.2 Small marginal fracture removable by polishing 3.2.3 Minor irregularities	4.2 Minor hypersensitivity for a limited period of time	5.2 Very small and localized demineralization.
3. Clinically sufficient /satisfactory (minor short comings with no adverse effects, but not adjustable without damage to the tooth)	1.3 Moderate marginal staining, not esthetically unacceptable	2.3 Two or more or larger hairline cracks and/or material chip fracture not affecting the marginal integrity	3.3.1 Gap (<250µm) not removable 3.3.2 Several small marginal fractures 3.3.3 Major irregularities.	4.3.1 Moderate hypersensitivity 4.3.2 Delayed/mild sensitivity, no subjective complaints, no treatment needed	5.3 Larger areas of demineralization but only preventive measures necessary (dentin not exposed)
4. Clinically unsatisfactory (repair for prophylactic reasons)	1.4 Pronounced marginal staining, major intervention necessary for improvement	2.4.1 Material chip fractures which damage marginal quality. 2.4.1 Bulk fractures with partial loss (less than half of restoration)	3.4.1 Gap (>250µm) or dentin/ base exposed 3.4.2 Severe material fractures 3.4.3 Larger irregularities (repair is necessary)	4.4.1 Intense hypersensitivity 4.4.2 Delayed with minor subjective symptoms	5.4 Caries with cavitation (localized and accessible and can be repaired)
5. Clinically poor (replacement necessary)	1.5 Deep marginal staining not accessible for intervention	2.5 Partial or complete loss of restoration	3.5.1 Restoration (complete or partial) is loose but in situ. 3.5.2 Generalized major gaps or irregularities	4.5 Very intense acute pulpits or no vital; Endodontic treatment is necessary and restoration has to be replaced	5.5 Deep caries or exposed dentin that is not accessible for repair of restoration.
Overall scores	• Acceptable esthetically (n and %): • Not acceptable (n, % and reasons)	• Acceptable function (n and %): • Not acceptable (n, % and reasons)		• Acceptable biologically (n and %): • Not acceptable (n, % and reasons)	

Table 6. Clinical performance at baseline, 6 and 12 months.

Criteria	Group	Baseline				6m				*McNemar Basel. vs 6m*	12m				*McNemar Basel. vs 12m*
		Scores				Scores					Scores				
		1+2	3	4	5	1+2	3	4	5		1+2	3	4	5	
Marginal staining	ER	39	-	-	-	37	1	-	-	*p= 1.000*	36	1	-	-	*p= 1.000*
	SE	39	-	-	-	38	-	-	-	*p= 1.000*	36	-	-	-	*p= 1.000*
	ER-EGCG	39	-	-	-	39	-	-	-	*p= 1.000*	37	1	-	-	*p= 1.000*
	SE-EGCG	39	-	-	-	37	-	-	-	*p= 1.000*	36	1	-	-	*p= 1.000*
	Kruskal-Wallis	*p= 1.000*				*p= 0.3916*				----------	*p= 0.8065*				----------
Retention	ER	39	-	-	-	38	-	-	1	*p= 1.000*	37	-	-	1	*p= 1.000*
	SE	39	-	-	-	38	-	-	1	*p= 1.000*	35	-	1	2	*p= 0.2500*
	ER-EGCG	39	-	-	-	39	-	-	-	*p= 1.000*	37	-	1	-	*p= 1.000*
	SE-EGCG	39	-	-	-	35	-	2	2	*p= 0.1250*	35	-	2	2	*p= 0.1250*
	Kruskal-Wallis	*p= 1.000*				*p= 0.1072*				----------	*p= 0.3831*				----------
Marginal adaptation	ER	39	-	-	-	38	-	-	-	*p= 1.000*	37	-	-	-	*p= 1.000*
	SE	39	-	-	-	37	-	1	-	*p= 0.500*	35	-	1	-	*p= 1.000*
	ER-EGCG	39	-	-	-	39	-	-	-	*p= 1.000*	37	-	1	-	*p= 1.000*
	SE-EGCG	39	-	-	-	36	-	1	-	*p= 1.000*	35	-	2	-	*p= 0.5000*
	Kruskal-Wallis	*p= 1.000*				*p= 0.5585*				----------	*p= 0.5635*				----------
Caries	ER	39	-	-	-	38	-	-	-	*p= 1.000*	37	-	-	-	*p= 1.000*
	SE	39	-	-	-	38	-	-	-	*p= 1.000*	36	-	-	-	*p= 1.000*
	ER-EGCG	39	-	-	-	39	-	-	-	*p= 1.000*	38	-	-	-	*p= 1.000*
	SE-EGCG	39	-	-	-	37	-	-	-	*p= 1.000*	37	-	-	-	*p= 1.000*
	Kruskal-Wallis	*p= 1.000*				*p= 1.000*				----------	*p= 1.000*				----------
Postoperative sensitivity	ER	37	1	1	-	38	-	-	-	*p= 1.000*	36	1	-	-	*p= 1.000*
	SE	38	-	1	-	38	-	-	-	*p= 1.000*	34	2	-	-	*p= 1.000*
	ER-EGCG	39	-	-	-	38	1	-	-	*p= 1.000*	37	1	-	-	*p= 1.000*
	SE-EGCG	39	-	-	-	36	1	-	-	*p= 1.000*	37	-	-	-	*p= 1.000*
	Kruskal-Wallis	*p= 0.2961*				*p= 0.5691*				----------	*p= 0.5462*				----------

Scores 1+2: clinically very good + clinically good; score 3: clinically sufficient/satisfactory. score 4: clinically unsatisfactory and score 5: clinically poor.

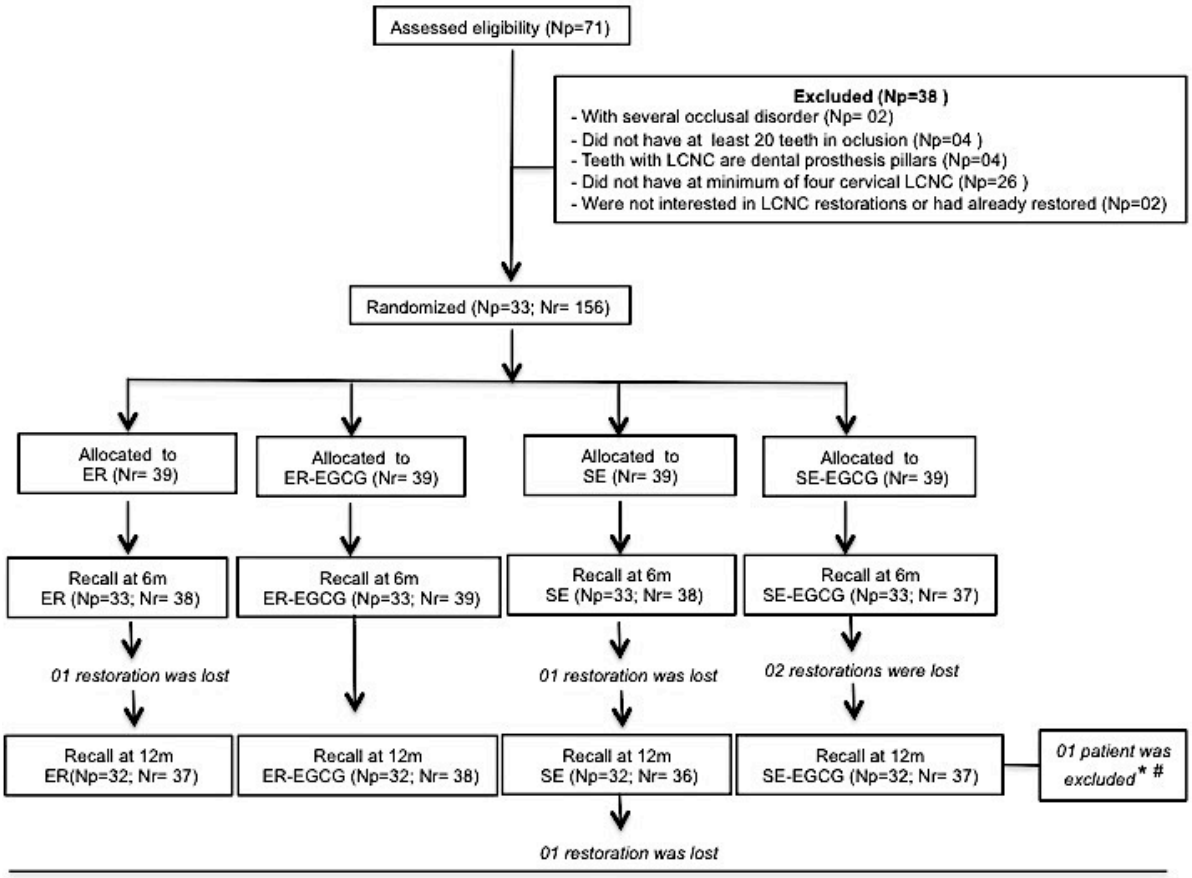

Figure 1. Patients flow diagram Np: number of patients; Nr: Number of restorations; ER: etch-and-rinse strategy; SE: self-etch strategy; EGCG: Epigallocatechin-3-gallate; (*) the patient was excluded due orthodontic treatment; (#) the patient excluded already had lost the restoration from SE-EGCG group in the 6 month recall.

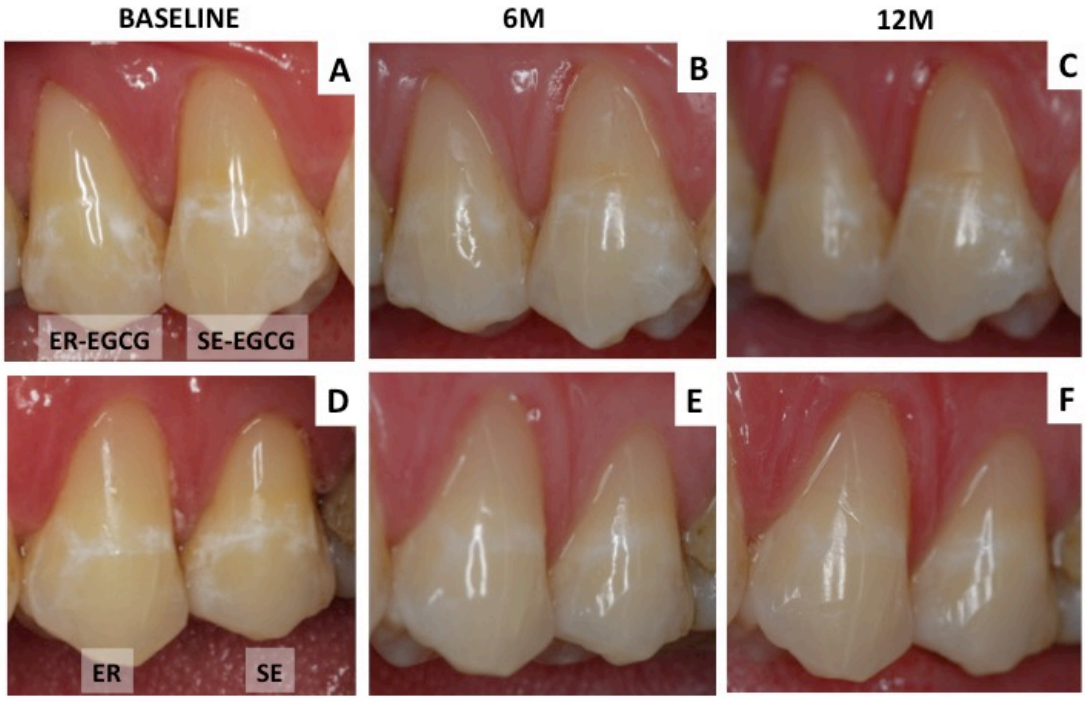

Figure 2. Cervical lesions on right and left upper premolars were restored and are exemplary of clinically satisfactory restorations. A, B and C present the baseline, 6-months and 12-months restorations from ER-EGCG (tooth 15) and SE-EGCG (tooth 14) respectively. D, E and F present the baseline, 6-months and 12-months restorations from ER (tooth 24) and SE (tooth 25) respectively.

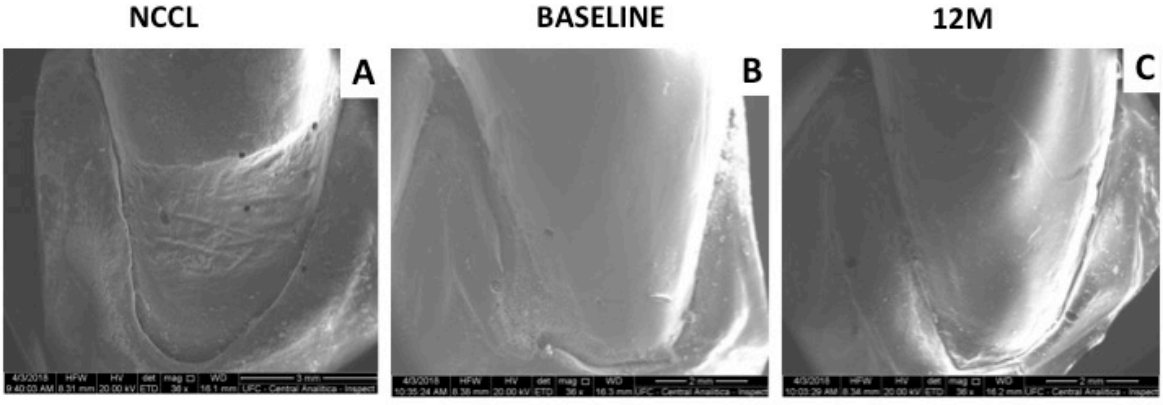

Figure 3. SEM photomicrograph of the Non-carious cervical lesion, the baseline and the 12-months restorations epoxy replicas. (A) Noncarious cervical lesion before restoration (B) Immediate aspect of the restoration at baseline showing perfect margin adaptation at the interface and (C) 12-month aspect of the restoration showing no gap, but some minor marginal imperfection.

CAPÍTULO 3

Dentin Biomodification with Epigallocatechin-3-gallate on Clinical Performance of a Universal Adhesive: a Two-year randomized clinical trial.

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Dentin Biomodification with Epigallocatechin-3-gallate on Clinical Performance of a Universal Adhesive: a Two-year randomized clinical trial.

Dentin Bimodification with EGCG: Two-year Clinical Evaluation

Clinical Relevance

At 24 months, the dentin biomodification with Epigallocatechin-3-gallate did not impair the clinical performance of the adhesive Single bond Universal regardless on the bonding strategy used.

Summary

Purpose: To evaluate the 2-year effect of dentin biomodification with epigallocatechin-3-gallate (EGCG) on clinical performance on restorations of non-carious cervical lesion (NCCLs) with Single Bond Universal, applied in two different modes (i.e. self-etch and etch-and-rinse). Materials and Methods: In this randomized clinical trial, 33 volunteers were selected and 156 NCCLs were assigned to four groups: ER: etch-and-rinse; ER-EGCG: 0.1% EGCG dentin pretreatment + etch-and-rinse; SE: self-etch; and SE-EGCG: 0.1% EGCG dentin pretreatment + self-etch. The total NCCLs were restored with the nanofilled composite resin Filtek Z350XT and evaluated at baseline, 6, 12, 18 and 24 months using FDI criteria for retention, marginal staining, marginal adaptation, caries and postoperative sensitivity. Two evaluators were blinded to the treatments performed and impressions were taken for the construction of replicas to allow indirect observations. Statistical analyses were performed with Kruskal-Wallis and McNemar tests with a significance level of 5%. Results: Six restorations (1 from ER; 2 from SE, 1 from ER-EGCG and 2 from SE-EGCG) were lost at 24-months with no significant

differences (p>0.05). The retention rates were 97.0% (ER and ER-EGCG), 94,11% (SE) and 94.21% (SE-EGCG). For marginal adaptation, a significant difference was detected on baseline and 24-month comparison for SE group (p =0.0313). There were no statistically differences among all other evaluated criteria at 24 months, neither for each group for baseline and 24-month comparisons (p>0.05). Conclusion: the clinical retention of the universal adhesive at 24 months does not depend on the bonding strategy and dentin biomodification with epigallocatechin-3-gallate.

Keywords: Adhesives, Biomodification, Catechin, Dentin bonding, Clinical trial.

1. Introduction

Randomized Clinical Trials (RCTs) are the "gold standard" to evaluate healthcare interventions [1] considered to be the ideal design for comparing different procedures [2] and the most reliable of evidence-based to dental practice in adhesive dentistry. [3] In vitro laboratory studies are widely used providing faster results but they do not take into account the complex oral environment. [4, 5] The current research in adhesive dentistry is aimed at improving the quality of the adhesive interface formation and understanding its degradation mechanisms. [6-8] Many strategies have been proposed to provide a greater longevity of the union of the restorative materials to the dental structure but few of them were evaluated under clinical conditions. [9-12]

Over the years, improvements in dental adhesives systems and new clinical strategies have been proposed to inhibit what was believed to be the only e/or main mechanism of adhesive bond degradation: the hydrolytic degradation of resin polymers by water sorption. [13] Since proteolytic degradation of the hybrid layer has been

described in the literature, [14] and associated to the host-enzymes, matrix metalloproteinases (MMPs) and cystein cathepisins (CC), numerous investigations have been conducted with potential enzymatic inhibitors. Promising laboratory results have been reached on the hybrid layer preservation and long-term durability. [15, 16] These enzymes are present in human dentin and saliva with the ability to degrade most extracellular matrix proteins, such as collagen type I, the organic component of the hybrid layer. [17, 18] Within the hybrid layer, denuded collagen fibers become vulnerable degradation by theses host-derived proteases with collagenolytic activity. [19, 20] Thus, the use of bioactive substances that inhibit the enzymes may avoid the degradation of exposed collagen fibrils at the adhesive interface. [7, 12]

Chlorhexidine (CHX) is the "gold standard" antimicrobial agent for oral health and has demonstrated inhibition of MMP-2, MMP-8, MMP-9 and CC. [21, 22] Laboratory results have proved the benefits in the immediate and long-term bond effectiviness of CHX-associated to adhesive systems. The use of chlorhexidine was the only MMP inhibitor strategy tested under clinical trials (either as dentin pretreatment or incorporated in adhesive). [23-29] The results (up to 3-year follow up) [30] showed there was no improvement on the clinical durability of adhesive restorations highlighting the lack of correlation between the results of the *in vitro* tests and the clinical reality to which the adhesive restorations are exposed. [4]

Another enzymatic inhibitors, mainly natural products, have aroused the interest of the scientific community. Epigallocatechin-3-gallate (EGCG) is the most abundant polyphenol found in green tea with a proven ability to inhibit expression and action of MMP-2 and MMP-9 [31] and CC [32,33] The use of EGCG has been recently associated with adhesive procedures, and was effective in preserving resin–dentin bond

strength up to 6 months when incorporated into the dentin adhesive [34] and up to 6 and 12 months when used as a dentin pretreatment with etch-and-rinse and self-etch adhesive systems, respectively [35,36] on *in vitro* studies. Unlike CHX, EGCG also has an additional cross-link property with positive effects on mechanical properties of collagen and its stabilization against proteolytic degradation. [37] It has been shown that galoyl radical monomeric catechins, such as EGCG, are more effective in increasing the modulus of elasticity and reducing collagen biodegradation rates.

In this field EGCG seems to be a promising bioactive substance that may maintain the integrity and stability of dentin collagen by synergic effects, with protease inhibition and crosslinking ability in dentin. To our knowledge, there are no studies evaluating the effect of EGCG as a MMP inhibitor and biomodification agent (cross-linking treatment) in a randomized clinical trial.

Thus, the objective of the present study was to evaluate the influence of dentin pretreatment with EGCG in restorations of non-carious cervical lesions performed with a universal adhesive system in the two adhesive strategies in vivo. The null hypothesis tested were (1) there is no difference between the clinical performance of NCCLs restorations bonded with the etch-and-rinse strategy associated or not to dentin biomodification with EGCG and (2) there is no difference between the clinical performance of NCCLs restorations bonded with the self-etch strategy associated or not to dentin biomodification with EGCG.

2. Materials and methods

2.1. Study design

The study design followed the Consolidated Standards of reporting Trials (CONSORT) statement [1] and is summarized in Table 1. Four adhesive procedures were

under evaluation. In the control groups, the multi-mode adhesive was applied in etch-and-rinse (ER) and self-etch (SE) strategies. In the experimental groups (ER-EGCG and SE-EGCG), dentin pretreatment with 0.1% EGCG aqueous solution was actively applied for 60 seconds and the adhesive system was used in the same etch-and-rinse and self-etch strategy, respectively. Table 2 summarizes the restorative materials used and application procedures.

2.2. Patient Selection

This trial was submitted and approved by the Local Ethics Committee on Investigations involving human subjects (protocol number: 1.292.593) and registered on the Public Clinical Registry: Brazilian Clinical Trials Registry –ReBEC (RBR-2hr94r).

Thirty-three (33) volunteers (22 to 66 years old) signed a written consent form, were informed about the accomplishment of the study, risks and benefits to which they would be exposed and authorized to participate. Inclusion criteria were: age over 18 years; good oral hygiene; no periodontal disease or deleterious habits; presence of at least 4 non-carious cervical lesions and at least 20 teeth in occlusion. Exclusion criteria were: previous cervical restorative procedure or caries lesions. All types of Class V noncarious lesions (abrasion, erosion, and abfraction) were included and all of them were expulsive, without retention, with no more than 50% of the margin in enamel and with cervical margin in dentin. [38]

2.3. Sample size calculation and sample randomization

Considering a two-tailed test hypothesis, the sample size was calculated based on the 96% retention rate of the Single Bond Universal observed in 18-year clinical follow up. [39] In order to determine a 25% difference between groups with a significance level of 5% and a statistical power of 80%, at least 33 restorations per group were

required. Taking into consideration possible loss during the study, a 20 % increase in sample size was set.

The random assignment was done using sealed envelop, which indicate either "controls" or "experimental", following one random list created by a computer program.

2.4. Restorative procedures

The sequence of the restorations was randomly distributed according to each treatment through a computer program without the patient's awareness. Each patient received at least 1 restoration per group, for a total of 156 restorations.

All NCCLs were classified according cavity dimensions in millimeters (height, width and depth), shape of the cavity (degree of the angle labeled as <45º, 45-90º, 90-135º, >135º), degree of sclerotic dentin (Table 3), presence of antagonist, presence of incisal/occlusal wear facets, presence of preoperative sensitivity and distribution among tooth types (Table 4).

Prior restorations, clinical photographs of the teeth were taken with a digital camera (Canon, EOS, 60D, Canon Macro EF 100mm lens) in three positions: vestibular, proximal (mesial) and incisal / occlusal. Silicone rubber impressions with heavy and light-body polyvinylsiloxane material (Adsil, Vigodent, Rio de Janeiro, RJ, Brazil) were obtained of each NCCL to make epoxy resin replicas (Epoxx Fiber MC130/FD154, Penha Circular, RJ, Brazil). [40]

All restorations (39 for each adhesive procedure) were performed by the same operator. Twenty-seven patients received 4 restorations and six patients received 8 restorations. No cavity preparation was carried out. Enamel margins were not beveled and no mechanical retention was placed. Local anesthesia with 1.8 ml lidocain-0.02 g with phenylephrine-0.0004 g (Novocol, SSWhite, Petrópolis, RJ, Brazil) and labial

retractor (Arc Flex-FGM, Joinvile, SC, Brazil), cotton rolls and gingival retractor # 00 (Ultrapack, Ultradent, Indaiatuba, SP, Brazil) were used for all cases. The NCCL were cleaned with pumice (SSWhite, Petrópolis, RJ, Brazil) and water in a rubber cup (KG Sorensen, Barueri, SP, Brazil) prior to restorative intervention followed by rinsing and drying.

The adhesive system application in control groups (ER and SE) followed the etch-and-rinse and self-etch protocols, respectively suggested by the manufacturer (Table 2). For experimental groups (ER-EGCG and SE-EGCG) followed the same protocols, but with dentin pretreatment with 0.1% EGCG aqueous solution, actively applied for 60 seconds (Table 2). Resin composite Filtek Z350XT (3M ESPE, St. Paul, MN, USA) increments (up to 3) were inserted and light-cured for 20 seconds using a calibrated light-curing unit (BISCO Dental Products IL, USA) at 600 mW/cm^2.

The initial finishing of the restorations was performed with 12- and 30-fluted tungsten carbide burs (Microdont, São Paulo, SP, Brazil) and F and FF diamond tips (KG Sorensen, São Paulo, SP, Brazil). The polishing was performed in the same session with the Sof-Lex Pop-On abrasive discs (3M ESPE, St. Paul, MN, USA) according to the manufacturer's recommendations and the initial Baseline evaluation were performed one week later. Another impression was taken of each restoration and resin replicas from the restoration interface were now obtained for observation under scanning electron microscopy (SEM).

2.5. Clinical Evaluation

The study was blinded for the two independent and calibrated examiners, with were responsible for the clinical evaluations. FDI criteria (World Dental Federation) [41, 42] were used to evaluate retention, presence of fractures, marginal discoloration,

marginal adaptation, caries and postoperative sensitivity at baseline and after 6, 12, 18 and 24 months. These variables were classified as: clinically very good; clinically good (scores 1+2); clinically sufficient/satisfactory (score 3); clinically unsatisfactory (score 4) and clinically poor (score 5) (Table 5). The analysis was done independently and performed with a clinical mirror and exploratory probe. In case of disagreement between the investigators, consensus was reached by reexamination and discussion, before the patient dismiss. [43]

The retention rates was calculated according to the following equation: Cumulative failure percentage = [(PF + NF) / (PF + RR)] X 100%, where PF is the number of previous failures before the current assessment, NF is the number of new failures during the current evaluation and RR is the number of restorations currently evaluated. [39]

At all evaluation periods (baseline, 6, 12, 18 and 24 months) impressions were taken from representative restorations considering each group tested and each level score from marginal adaptation criteria. [40, 44] All restorations were also photographed in two positions (vestibular and mesial).

2.6. Statistical Methods

McNemar's test was used for intragroup comparisons between baseline and others periods. Kruskal-Wallis test was used to inter-group comparing each group in each period. The comparisons were performed for all criteria evaluated with a significance level of 5%. To measure agreement between examiners, the Cohen's Kappa statistic was used.

3. Results

The Figure 1 shows the patients flow diagram. Thirty-eight out of seventy one patients were not enrolled in the study because they did not fulfill the inclusion criteria. Thus, 33 subjects were selected and randomly assigned in the four experimental groups. The ratio of male:female patients was 19:14. Recall rates were 100% for baseline, 6, 12 and 18 months. For 24 months, recall rate was 96.9%, with one patient that could not return due to health problems. One patient was eliminated at each 12 and 18 months due orthodontic treatment.

Table 6 presents data for retention/fractures, marginal staining, marginal adaptation, caries and postoperative sensitivity at baseline and for all evaluation periods. The polled scores (1+2) and score 3 present the total number of restorations classified as clinically acceptable. The scores 4 and 5 shows the number of restorations considered clinically unsatisfactory.

Representative images of clinically satisfactory restorations from each group at baseline and 24 months are presented in Figure 2.

Four restorations were lost after 6 months, one restoration was lost at 12-month recall and one restoration was lost at 18-month recall. Thus, a total of six restorations were lost at twenty-four months (one for ER, two for SE, 1 for ER-EGCG and one for SE-ECGC). The 24-month retention rates were 97.14% for ER, 94.44% for SE, 97.14% for ER-EGCG, and 94.59% for SE-EGCG, with no statistical difference between any pair of groups at 24-month recall and for each group when baseline and 24-month results were compared ($p > 0.05$; Table 6).

Two restorations had acceptable material chip fracture (1 for ER and 1 for SE) but not affecting the marginal integrity–score 3. Six restorations had bulk fractures with partial loss- score 4 (2 for each SE, ER-ECGC and SE-EGCG groups) (Figure 3).

Ten restorations were considered to have discrepancies in marginal adaptation at the 24-month recall. Half of then (1 for ER and 4 for SE) was considered as minor acceptable discrepancies, but another half (2 for SE, 1 for ER-EGCG and 2 for SE-EGCG) was considered as unacceptable discrepancies, with severe material fracture (Figure 4). No significant difference was detected between any pair of groups at the 24-month recall ($p > 0.05$) but a significant difference was detected when baseline and 24-month data were compared within SE group *($p = 0.0313$)* (Table 6).

Marginal staining was observed in seven restorations (1 for SE, 4 for ER-ECGC and 2 for SE-EGCG). Pronounced marginal staining was only observed in one restoration for the SE-EGCG group. However, no significant difference was found between groups at 24 months and within each group when baseline and 24-month findings were compared ($p>0.05$; Table 6).

Three restorations had postoperative sensitivity one week after the restorative procedures (2 for ER and 1 for SE). Two of them showed very intense sensitivity (score 4), but the sensitivity remained clinically acceptable for 6, 12 and 18-months the periods and none restoration presented postoperative sensitivity at 24 months.

Finally, caries were not observed after 24-month of clinical service and no tooth became non-vital as a result of the cervical restoration.

There was no statistically significance difference among the groups, in all periods ($p>0.05$). There was also no statistically significant difference for each group when baseline and 24-month were compared ($p > 0.05$; Table 6).

4. Discussion

Epigallocatechin-3-gallate (EGCG) is the most abundant catechin and active compound found in green tea. It has recently been introduced into the dental field because of several promising benefits such as its antibacterial effect, [45] protective effect for dental erosion [46, 47] and promising effects in preserving resin–dentin bond strength up to 6 months and 12 months associated with etch-and-rinse and self-etch adhesive systems in *in vitro* studies [34-36.]

It has been proven that EGCG might have superior clinical application due to its positive effects on both mechanical properties and stabilization of collagen against proteolytic degradation. [37] Although all these benefits proven by laboratorial studies, to our knowledge, there were no clinical reports regarding the clinical performance of adhesive systems associated with EGCG. Thus, this randomized clinical trial evaluated a universal adhesive, on different adhesive strategies, assessing the influence of the dentin biomodification with epigallocatechin-3-gallate (EGCG) prior the adhesive application.

In the current study, dentin priming with 0.1% EGCG aqueous solution was used since it was the concentration with better *in vitro* results, preserving the bond strength over time. [35,36] The 1 minute application significantly improved the bond strength on research conducted by Zheng & Chen, 2017 [16] with MMP inhibitors. In the present clinical study, this time of application represented a feasible step to be reproduced in the clinical practice.

The clinically efficacy of the four bonding procedures was evaluated by the retention of the restorations as a primary outcome. [13] Retention in non-carious cervical lesions cannot be associated to any other macromechanical retentions; which can

directly reflect the quality of the adhesive bonding. [3] Besides, the hypermineralized dentin in NCCLs makes the bonding to this substrate very unpredictable and challenging for adhesive systems. [48] After 24-month, there was no difference on retention of NCCLs restorations bonded with the etch-and-rinse or self-etch strategy, associated or not to dentin pretreatment with EGCG, which leads to fail in rejecting the null hypothesis.

The high retention rates for all tested groups after 24 months showed that the bond effectiveness of universal adhesive did not depend on the bonding strategy, in accordance with previous *in vitro* studies [49, 50] and randomized clinical trials. [39, 51-56]

This result may be explained by the Single Bond Universal (SU) chemical bond mechanism. SU contains 10-methacryloxydecyl dihydrogen phosphate (10-MDP), an acidic phosphate monomer capable of bonding to hydroxyapatite, producing adhesive interfaces with different chemical and morphological characteristics. It also contains a polyalkenoic acid copolymer.[39] This copolymer was first used in the composition of Vitrebond (3M ESPE), also known as Vitrebond copolymer or VCP. These two processes involves micromechanical and chemical bonding to the enamel and dentin, which may probably have a direct impact on the retention rate and clinical behavior of SU in the current study, after 24 months.

The retention results also showed that the EGCG priming solution did not impair the bond effectiveness, in accordance with *in vitro* studies [35,36, 57] and can be associated with both adhesive strategies. Unlike chlorhexidine, that showed a negative influence when incorporated into self-etching adhesives or used as a dentin pretreatment [58,59] This result highlight the versatile clinical application of EGCG as MMP inhibitor.

The scoring system used for the evaluation of the NCCL restorations was the FDI criteria. It is more sensitive analyses to small variations in the clinical outcomes when evaluating restorations of NCCLs. [39, 51, 56] It is a well-structured and flexible criteria which can be selected and adjusted according to the needs of the investigator. [42] For this purpose, four specific secondary outcomes were selected from the total sixteen original parameters. In this study, the inclusion of "marginal staining" and "marginal adaptation was a important measure, since they are still mainly shortcomings of cervical restorations and it has been indicated that they may be predictors of future failures.[60] Also a simplified clinical evaluation was chosen pooling scores 1 and 2 (equivalent to score A from United States Public Health Service – USPHS criteria), [43] and the FDI criteria was expressed with only four scores, two for acceptable: 1/2 and 3, and two for non-acceptable: 4 and 5 for reparable and replacement, respectively. [2, 42]

For marginal adaptation, a significant difference was detected when baseline and 24-month data were compared within SE group *(p =0.0313)*. SU is considered an ultra-mild self-etch adhesive (pH around 3.0) and, although that is no consensus that pre-etching enamel improves the bond strength of universal adhesives. [61,62] This present result may be related to the limited micromechanical retention of the enamel surface conditioned by self-etch mode. Surprisingly, the association of EGCG with the self-etch strategy did not jeopardize the marginal adaptation, and future evaluations could confirm one possible beneficial effect of EGCG priming on enamel.

After 24 months, five restorations were rating as score 4 (2 for SE, 1 for ER-EGCG and 2 for SE-EGCG) with unacceptable discrepancies, and severe material fracture. A prophylactic repair was indicated to recover the restoration integrity, avoiding premature replacement of the restoration. [42] These restorations can be scored

as “relative failure” and this result may be associated with heavy occlusal or parafunctional forces, since all teeth presented signs of occlusal wear (data not shown). A retrospective clinical study [44] showed that Class V restorations of teeth with occlusal wear facets had a 6.65-fold increased risk of failure than those of teeth without wear facets. Clinical co-variables showed on Table 5 could determining bonding effectiveness, such as shape and size of the lesion, degree of sclerosis, presence of wear facets, patient age, and tooth type. [13]

The restorations, which were rated unsatisfactory, were additionally investigated using digital photographs and epoxy resin replicas under SEM evaluation.[44] The replicas enabled indirect observation, and were useful to analyze changes on discrepancies and/or material fracture restorations on different periods. This procedure was helpful in determining the need for timely repair or replacement.

Pronounced marginal staining was only observed in one restoration for the SE-EGCG group and it was associated to color change along the whole of the restoration margin associated to smoking habits and not to the marginal discrepancy.

One reason for *in vitro* maintaining the bond strength over time by epigallocatechin-3-gallate may be associated to the dentin biomodification property of ECGC. This catechin was capable to maintain the integrity and stability of dentin collagen by synergic effects, with protease inhibition and crosslinking ability in dentin, leading to preservation of the long-term dentin bond effectiveness. [37] This effect could not be proved by the current results, probably by the relative short-term evaluation. Since the best way to evaluate the effectiveness of a new biomaterial or a bonding system, is *in vivo* experimentation in randomized controlled trials, more-longer follow-

up is required to validate the experimental strategies aiming to enhance the adhesive interface.

Conclusion

Despite the limitation of the study, the clinical retention of the universal adhesive at 24 months does not depend on the bonding strategy and dentin biomodification with epigallocatechin-3-gallate.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Tables and Figures

Table 1. Experimental design

Procedure / Groups	33 patients /156 NCCLs			
	ER	ER-EGCG	SE	SE-EGCG
Enamel (30 s) and dentin (15 s) total etching with 37% phosphoric acid	Yes	Yes	No	No
Dentin pretreatment (60s)	No	0.1% EGCG	No	0.1% EGCG
Adhesive application	Single Bond Universal	Single Bond Universal	Single Bond Universal	Single Bond Universal
Restorative procedure	Filtek Z350-XT			
Clinical evaluation (FDI criteria)	Baseline, 6, and 12, 18 and 24months			

NCCL: non-carious cervical lesions; ER: etch-and-rinse strategy; SE: self-etch strategy; EGCG: epigallocateachin-3-galate.

Table 2. Restorative materials and application procedures

Material	Manufacturer	Composition	Lot number	Application procedure *
Epigallocatechin -3-gallate	Sigma- Aldrich, St Louis, MO, USA	≥ 80 % (HPLC), from green tea	#SLBL 1959V	0.1% aqueous solution actively applied for 60s with a microbrush before adhesive application.
Filtek Z350 XT	3M ESPE, St. Paul, MN, USA	Treated silanized ceramics; Silane treated silica; (UDMA); Bisphenol A polyethylene glycol diether dimethacrylate; (BisGMA); Ceramics of zirconia; Polyethylene glycol; dimethacrylate; Triethylene glycol and dimethacrylate	#190224 # 147907	Up to 3 increments of composite resin, individually photopolymerized.
Single Bond Universal	3M ESPE, St. Paul, MN, USA	Methacryloyloxydecyl dihydrogen phosphate; phosphate monomer; dimethacrylate resins; hydroxyethyl methacrylate; methacrylate-modified polyalkenoic acid copolymer; filler; ethanol; water; initiators; silane.	#582958	Etch-and-rinse strategy: Total etching with 37% phosphoric acid (Enamel- 30 and dentin-15 s) follow by rising with water and drying with air free of moisture and oil, without drying out. Adhesive application to tooth surface by scrubbing action (20 Seconds) Dry the adhesive (5 Seconds) and light cure (10 Seconds).
				Self-Etch strategy: Adhesive application to tooth surface by scrubbing action (20 Seconds) Dry the adhesive (5 Seconds) and light cure (10 Seconds).

UDMA: Urethane Dimethacrylate; BisGMA: bisphenol A-glycidyl methacrylate.

**According to the manufacturer's instructions.*

Table 3. Dentin sclerosis scale [63]

Category	Criteria
1	No sclerosis present; dentin is light yellowish or whitish, with little discoloration; dentin is opaque, with little translucency or transparency.
2	More sclerosis than in category I but less than halfway between categories 1 an 4.
3	Less sclerosis than in category 4 but more than halfway between categories 1 and 4.
4	Significant sclerosis present; dentin is dark yellow or even discolored (brownish); glassy appearance, with significant translucency or transparence evident.

Table 4. Non-carious cervical lesions classification according to shape, cervico-incisal height, degree of sclerotic dentin, presence of antagonist, presence of incisal/occlusal wear facets, presence of preoperative sensitivity and distribution among tooth types

Characteristics of NCCL	Number of lesions			
	ER	ER-EGCG	SE	SE-EGCG
Shape (degree of angle)				
<45	1	2	1	3
45-90	7	10	5	4
90-135	20	20	24	19
>135	11	7	9	13
Cervicoincisal height (mm)				
<1.5	5	4	5	4
1.5-2.5	22	18	22	19
2.5-4.0	10	14	10	13
>4.0	2	3	2	3
Degree of sclerotic dentin				
1	17	20	23	22
2	19	14	12	12
3	3	5	4	5
4	0	0	0	0
Presence of antagonist				
Yes	36	37	37	37
No	3	2	2	2
Presence of incisal/occlusal wear facets				
Yes	32	31	26	33
No	7	8	13	6
Preoperative sensitivity (air dry)				
Yes	12	15	15	17
No	27	24	24	22
Preoperative sensitivity (spontaneous)				
Yes	7	7	6	6
No	32	32	33	33
Tooth Distribution				
Anterior				
Incisores	10	9	10	8
Canines	6	3	7	2
Posterior				
Premolar	21	22	19	28
Molar	2	5	3	1
Arc distribution				
Maxilary	20	23	25	15
Mandibular	19	16	14	24

ER= etch and rinse; ER-EGCG: etch and rinse + 0.1% EGCG pretreatment; SE= self-etch; SE-EGCG: self-etch + 0.1% EGCG pretreatment.

Table 5. World Federation (FDI) criteria used for clinical evaluation [42]

	Esthetic property	**Functional properties**		**Biological properties**	
	1. Marginal staining	**2. Fracture of material and retention**	**3. Marginal adaptation**	**4. Postoperative (hypersensitivity)**	**5. Recurrence of caries**
1. Clinically very good	1.1 No marginal staining	2.1 No fractures / cracks	3.1 Harmonious outline, no gaps, no discoloration	4.1 No hypersensitivity	5.1 No caries
2. Clinically good (after correction, very good)	1.2 Minor marginal staining, easily removable by polishing	2.2 Small hairline crack	3.2.1 Marginal gap (<150µm) 3.2.2 Small marginal fracture removable by polishing 3.2.3 Minor irregularities	4.2 Minor hypersensitivity for a limited period of time	5.2 Very small and localized demineralization.
3. Clinically sufficient /satisfactory (minor short comings with no adverse effects, but not adjustable without damage to the tooth)	1.3 Moderate marginal staining, not esthetically unacceptable	2.3 Two or more or larger hairline cracks and/or material chip fracture not affecting the marginal integrity	3.3.1 Gap (<250µm) not removable 3.3.2 Several small marginal fractures 3.3.3 Major irregularities.	4.3.1 Moderate hypersensitivity 4.3.2 Delayed/mild sensitivity, no subjective complaints, no treatment needed	5.3 Larger areas of demineralization but only preventive measures necessary (dentin not exposed)
4. Clinically unsatisfactory (repair for prophylactic reasons)	1.4 Pronounced marginal staining, major intervention necessary for improvement	2.4.1 Material chip fractures which damage marginal quality. 2.4.1 Bulk fractures with partial loss (less than half of restoration)	3.4.1 Gap (>250µm) or dentin/ base exposed 3.4.2 Severe material fractures 3.4.3 Larger irregularities (repair is necessary)	4.4.1 Intense hypersensitivity 4.4.2 Delayed with minor subjective symptoms	5.4 Caries with cavitation (localized and accessible and can be repaired)
5. Clinically poor (replacement necessary)	1.5 Deep marginal staining not accessible for intervention	2.5 Partial or complete loss of restoration	3.5.1 Restoration (complete or partial) is loose but in situ. 3.5.2 Generalized major gaps or irregularities	4.5 Very intense acute pulpits or no vital; Endodontic treatment is necessary and restoration has to be replaced	5.5 Deep caries or exposed dentin that is not accessible for repair of restoration.
Overall scores	• Acceptable esthetically (n and %): • Not acceptable (n, % and reasons)	• Acceptable function (n and %): • Not acceptable (n, % and reasons)		• Acceptable biologically (n and %): • Not acceptable (n, % and reasons)	

Table 6. Number of evaluated restorations for baseline and 6, 12, 18 and 24 months recalls according to the experimental groups. ER= etch and rinse; ER-EGCG: etch and rinse + 0.1% EGCG pretreatment; SE= self-etch; SE-EGCG: self-etch + 0.1% EGCG pretreatment.

Criteria	Group	Baseline				6m				12m				18m				24m			
		Scores				Scores				Scores				Scores				Scores			
		1+2	3	4	5	1+2	3	4	5	1+2	3	4	5	1+2	3	4	5	1+2	3	4	5
Marginal staining	ER	39	-	-	-	37	1	-	-	36	1	-	-	35	-	-	-	34	-	-	-
	SE	39	-	-	-	38	-	-	-	36	-	-	-	35	-	-	-	33	1	-	-
	ER-EGCG	39	-	-	-	39	-	-	-	37	1	-	-	32	3	-	-	30	4	-	-
	SE-EGCG	39	-	-	-	37	-	-	-	36	1	-	-	35	1	-	-	33	1	1	-
Retention/ Fracture	ER	39	-	-	-	38	-	-	1	37	-	-	1	35	-	-	1	33	1	-	1
	SE	39	-	-	-	38	-	-	1	35	-	1	2	34	-	1	2	31	1	2	2
	ER-EGCG	39	-	-	-	39	-	-	-	37	-	1	-	34	-	1	1	32	-	2	1
	SE-EGCG	39	-	-	-	35	-	2	2	35	-	2	2	34	-	2	2	33	-	2	2
Marginal adaptation	ER	39	-	-	-	38	-	-	-	37	-	-	-	35	-	-	-	33	1	-	-
	SE	39	-	-	-	37	-	1	-	35	-	1	-	33	1	1	-	28	4	2	-
	ER-EGCG	39	-	-	-	39	-	-	-	37	-	1	-	34	-	1	-	33	-	1	-
	SE-EGCG	39	-	-	-	36	-	1	-	35	-	2	-	34	-	2	-	33	-	2	-
Caries	ER	39	-	-	-	38	-	-	-	37	-	-	-	35	-	-	-	34	-	-	-
	SE	39	-	-	-	38	-	-	-	36	-	-	-	35	-	-	-	34	-	-	-
	ER-EGCG	39	-	-	-	39	-	-	-	38	-	-	-	35	-	-	-	34	-	-	-
	SE-EGCG	39	-	-	-	37	-	-	-	37	-	-	-	36	-	-	-	35	-	-	-
Postoperative sensitivity	ER	37	1	1	-	38	-	-	-	36	1	-	-	34	1	-	-	34	-	-	-
	SE	38	-	1	-	38	-	-	-	34	2	-	-	35	-	-	-	34	-	-	-
	ER-EGCG	39	-	-	-	38	1	-	-	37	1	-	-	35	-	-	-	34	-	-	-
	SE-EGCG	39	-	-	-	36	1	-	-	37	-	-	-	36	-	-	-	35	-	-	-

Scores 1+2: clinically very good + clinically good; score 3: clinically sufficient/satisfactory. score 4: clinically unsatisfactory and score 5: clinically poor.

(*) Significant difference was detected when baseline and 24-month data were compared within SE group *(p =0.0313).*

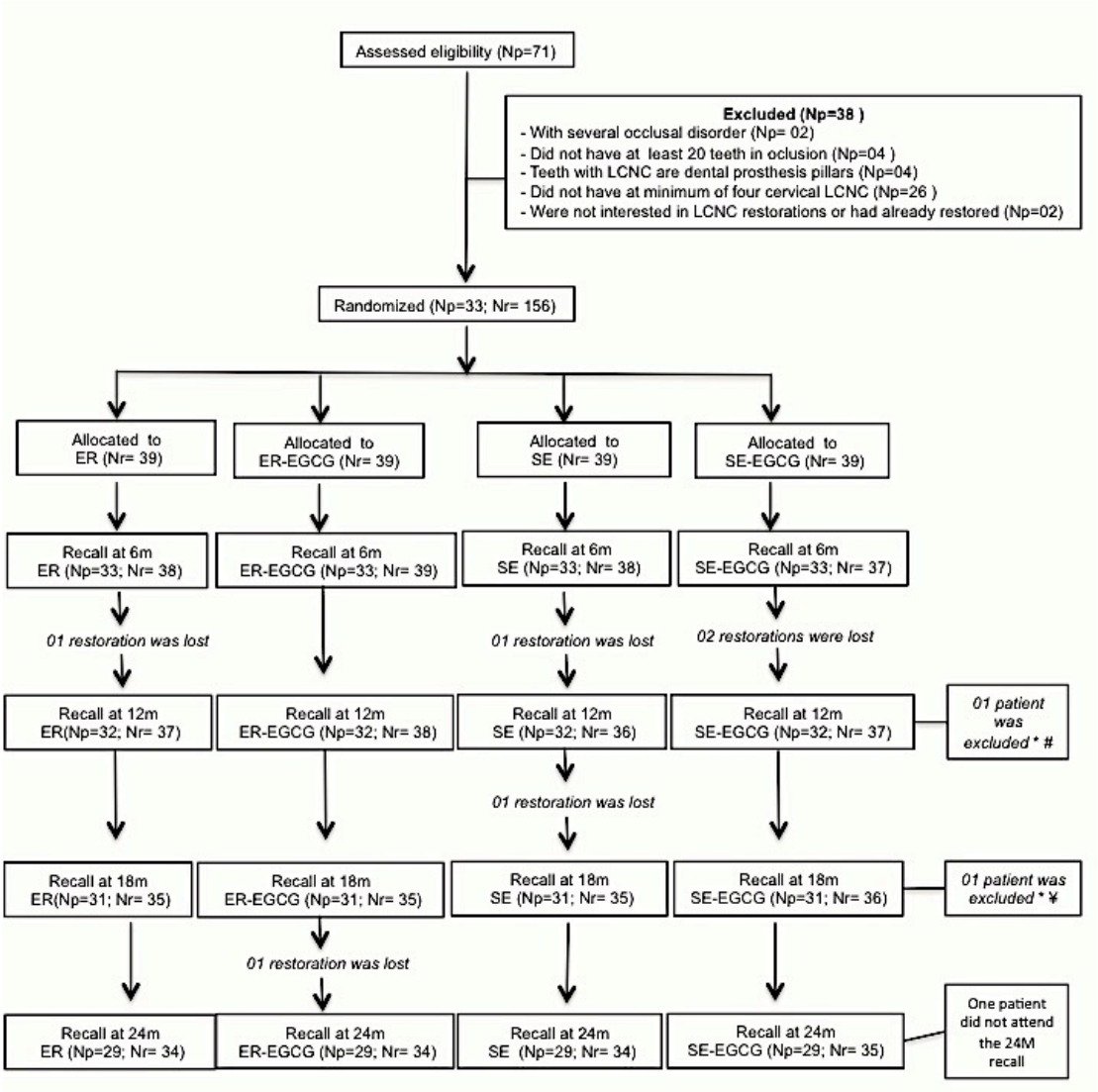

Figure 1. Patients flow diagram Np: number of patients; Nr: Number of restorations; ER: etch-and-rinse strategy; SE: self-etch strategy; EGCG: Epigallocatechin-3-gallate; (*) the patient was excluded due orthodontic treatment; (#) the excluded patient already had lost the restoration from SE-EGCG group in the 6-month recall. ¥ The excluded patient had 8 NCCLs restored and already had lost the restoration from SE and SE-EGCG groups at 12-month recall.

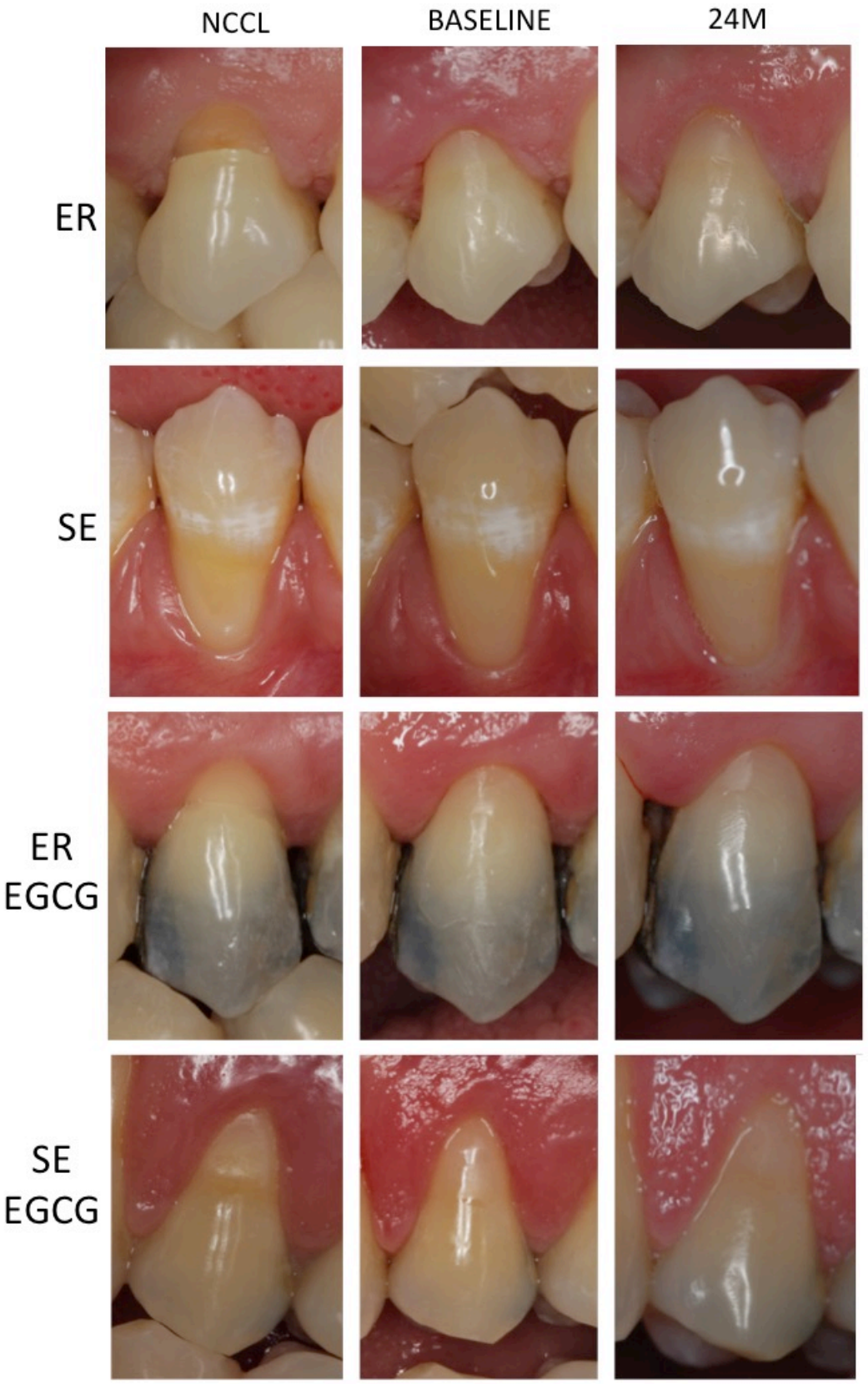

Figure 2. Cervical lesions exemplary of NCCLs from each groups and clinically satisfactory restorations at baseline and 24 months

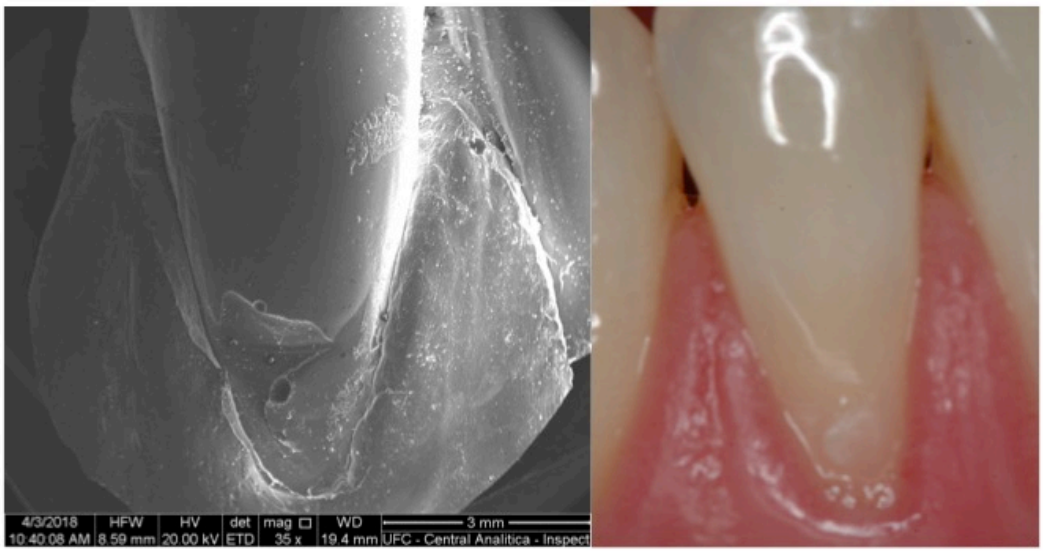

Figure 3. On left, SEM photomicrograph of resin epoxy replicas from the resin fracture. On right, clinical photograph from the same resin restoration rated as clinically unsatisfactory, but reparable (material fracture - score 4) at 24-month recall.

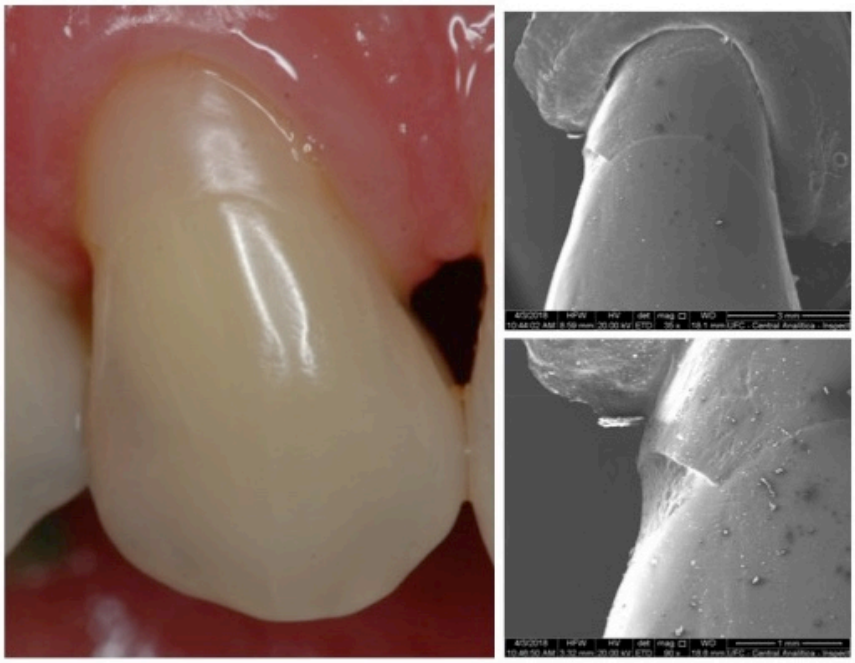

Figure 4. On left, clinical photograph of resin restoration (SE group) rated as clinically unsatisfactory, but reparable (marginal adaptation - score 4) at 24-month recall. On right, SEM photomicrographs of resin epoxy replicas from the same restoration (superior right) and an approximated view (inferior right) from the resin fracture.

CONCLUSÃO GERAL

4 CONCLUSÃO GERAL

- O pré-tratamento dentinário com epigalocatequina-3-galato a 0.1% demonstrou ser biocompatível para células tronco de polpa dentária humana por não reduzir a viabilidade celular. A incorporação direta de epigalocatequina-3-galato a 0,1% (p/v) em adesivos dentários comerciais não mostrou atividade antibacteriana por teste de contato direto, nem na formação e desenvolvimento do biofilme de *Streptococcus mutans*.
- O pré-tratamento dentinário com solução aquosa de EGCG a 0.1% não prejudicou a retenção clínica do adesivo universal no períodp imediato e após 6, 12 18 e 24 meses em restaurações de lesões cervicais não cariosas, realizadas com o sistema adesivo *Single Bond Universal* utilizando-se duas estratégias adesivas (condicionamento total e autocondicionante).

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ANEXOS

ANEXO A – PARECER DO COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DA EPIGALOCATEQUINA-3-GALATO NO DESEMPENHO CLÍNICO DE UM SISTEMA ADESIVO UNIVERSAL: ESTUDO CLÍNICO RANDOMIZADO

Pesquisador: CECILIA ATEM GONÇALVES DE ARAÚJO COSTA

Área Temática:

Versão: 1

CAAE: 49671515.6.0000.5054

Instituição Proponente: Departamento de Odontologia Restauradora

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.292.593

Apresentação do Projeto:

Projeto de pesquisa da doutoranda Cecília Atem Gonçalves de Araújo Costa sobre o efeito de substâncias naturais como a epigalocatequina-3-galato(EGCG) na adesão dentinária das restaurações cervicais. Tem por objetivo avaliar "in vivo", a influência do pré-tratamento dentinário com EGCG em restaurações de lesões cervicais não cariosas (LCNC), utilizando-se um adesivo universal, aplicado nas duas estratégias adesivas, condicionamento total e autocondicionante. Nesse estudo clínico randomizado, 40 voluntários serão selecionados e 160 restaurações de LCNC serão realizadas, aleatoriamente, distribuídas de acordo com o pré-tratamento e o tipo de estratégia adesiva. As restaurações serão avaliadas pelo método proposto pela FDI (World Dental Federation) nos períodos imediato e após 6, 12, 18 e 24 meses. Os critérios adotados para as avaliações do desempenho clínico serão: retenção, adaptação marginal, cárie, sensibilidade pós-operatória e descoloração marginal. A comparação dos tratamentos entre si, em cada tempo, será feita pelo teste de McNemar e a de cada tratamento em cada período será realizada pelo teste exato de Fisher, ambos com nível de significância de 5%.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar o efeito da epigalocatequina-3-galato no desempenho clínico de um sistema adesivo

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

universal através de um ensaio clínico randomizado.

Objetivo Secundário:
Avaliar “in vivo” (estudo clínico randomizado) a influência do pré-tratamento dentinário com EGCG a 0.1% em restaurações de lesões cervicais não cariosas, realizadas com o sistema adesivo universal Single Bond Universal® (3M ESPE) utilizando-se de duas estratégias adesivas (condicionamento total e autocondicionante), através de avaliação clínica de restaurações nos períodos imediato e após 6, 12, 18 e 24 meses.

Avaliação dos Riscos e Benefícios:
A pesquisa apresenta baixo risco a não ser pelo desconforto do procedimento odontológico. As restaurações destas lesões cervicais já apresentam protocolo bem estabelecido e tanto as resinas compostas como os sistemas de condicionamento e de adesão já são largamente utilizados na clínica de dentisteria.
Quanto aos benefícios, os pacientes terão seus dentes restaurados, serão acompanhados por 24 meses e a sintomatologia com frio e doce, se existir, desaparecerá.

Comentários e Considerações sobre a Pesquisa:
Trata-se de um estudo clínico randomizado, com uma taxa de alocação igual entre os quatro grupos em avaliação e o delineamento experimental seguiu a declaração CONSORT.

Considerações sobre os Termos de apresentação obrigatória:
A pesquisadora apresentou a este comitê: projeto, folha de rosto devidamente preenchida e assinada pelo chefe do Departamento de Odontologia Restauradora, orçamento, cronograma, TCLE, currículo lattes, carta de encaminhamento, declaração institucional e de infra-estrutura, anuência dos participantes da pesquisa.

Recomendações:
Recomenda-se a inclusão no cronograma a avaliação de 24 meses após o procedimento odontológica como está previsto no projeto e no TCLE.

Conclusões ou Pendências e Lista de Inadequações:
Não há pendências éticas nem documental.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Continuação do Parecer: 1.292.593

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_582630.pdf	29/09/2015 19:05:57		Aceito
Projeto Detalhado / Brochura Investigador	Projeto_final_5.docx	29/09/2015 13:43:02	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Cronograma	CRONOGRAMA_5.docx	29/09/2015 13:41:59	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_final.docx	27/09/2015 21:47:38	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Outros	Curriculo_lattes.pdf	11/09/2015 20:35:39	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Outros	encaminhamento_corrigido.pdf	11/09/2015 20:33:43	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Outros	declaracaocusteio.pdf	04/09/2015 10:46:48	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Declaração de Pesquisadores	concordancia.pdf	04/09/2015 10:42:49	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Declaração de Instituição e Infraestrutura	carta_de_autorizacao.pdf	04/09/2015 10:42:19	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Folha de Rosto	folhaderosto.pdf	04/09/2015 10:38:02	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito
Orçamento	orcamento.pdf	04/09/2015 10:36:30	CECILIA ATEM GONÇALVES DE ARAÚJO COSTA	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Continuação do Parecer: 1.292.593

FORTALEZA, 22 de Outubro de 2015

Assinado por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador)

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

ANEXO B – REGISTRO BRASILEIRO DE ENSAIOS CLÍNICOS

APÊNDICES

APÊNDICE A - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

PROJETO: "EFEITO DA EPIGALOCATEQUINA-3-GALATO NAS PROPRIEDADES FÍSICO-QUÍMICAS E NO DESEMPENHO CLÍNICO DE UM SISTEMA ADESIVO UNIVERSAL: ESTUDOS IN VITRO E IN VIVO"

Responsáveis: Prof. Dr. Sérgio Lima Santiago / Doutoranda: Cecília Atem Gonçalves de Araújo Costa

Você está sendo convidado(a) a participar de um projeto de pesquisa clínica odontológica. As informações seguintes tem por objetivo dar condições para que você tome a decisão de participar deste estudo conscientemente.

1. Nesta pesquisa serão realizadas restaurações com uma resina composta (da cor do dente), tendo como tratamento prévio a aplicação de uma substância: a Epigalocatequina-3-galato (EGCG), um produto natural, derivado do chá verde, sem contra-indicações e liberado para uso em odontologia.

2. O estudo irá avaliar a durabilidade dessas restaurações após uma semana, 6, 12,18 e 24 meses. Trata-se de procedimentos do dia-a-dia em clínicas odontológicas. Os materiais utilizados encontram-se disponíveis no mercado e foram previamente estudados, de modo que não causam nenhum risco ao ser humano.

3. As consultas para as restaurações demorarão aproximadamente de 1 a 2 horas (cada 3 dentes) e o tempo total dependerá do número de restaurações que necessitem ser realizadas. Após uma semana, 6, 12,18 e 24 meses da colocação das restaurações, você será chamado(a) para um novo exame. Cada consulta para reavaliação demorará aproximadamente 15 a 30 minutos. Entretanto, em qualquer tempo e diante de

qualquer situação de dor ou desconforto referente às áreas restauradas o paciente poderá entrar em contato com os responsáveis da pesquisa para receber orientações e providências clínicas cabíveis.

4. Não haverá nenhum custo para você por estas consultas.

5. Você será diretamente beneficiado(a) por participar deste estudo pelo fato de ter as restaurações realizadas. O tratamento terá como objetivo recuperar a estética dos dentes com lesões cervicais , bem como acabar ou minimizar os sintomas de dor causados por fatores como: água gelada, alimentos doces etc.

6. Todas as anotações relativas as seu(s) dente(s) e à sua pessoa serão mantidas confidenciais. Você não será identificado em nenhum relatório ou publicação.

7. Você poderá sair deste projeto de pesquisa a qualquer hora. A saída não afetará sua oportunidade de obter tratamento no Curso de Odontologiada Universidade Federal do Ceará.

8. Questões sobre o projeto e sua participação serão respondidas pelos responsáveis pela pesquisa:

Dra. Cecília Atem Gonçalves de Araújo Costa- Universidade Federal do Ceará- Rua: Monsenhor Furtado, s/n –Rodolfo Teófilo 60430-355 - Fortaleza – Ceara – Brasil / **Telefone: (85) 8891-1887**

Prof. Dr. Sérgio Lima Santiago - Universidade Federal do Ceará - Rua: Monsenhor Furtado, s/n –Rodolfo Teófilo 60430-355 - Fortaleza – Ceara – Brasil - **Telefone: (85) 3366-8232**

Este projeto está em processo de aprovação pelo comitê de Ética em Pesquisa da UFC. Se você tiver qualquer dúvida ou precisar informar ocorrências irregulares ou

danosas durante a sua participação no estudo, dirija-se ao: **Comitê de Ética em Pesquisa da Universidade Federal do Ceará Rua Coronel Nunes de Melo, 1127, Rodolfo Teófilo Telefone: (85) 3366-8338**

Tendo lido esta declaração, eu concordo em participar deste projeto de clínica.

Fortaleza, _____/_____/_____

_____

Assinatura do paciente

_____

Assinatura do pesquisador

APÊNDICE B - FICHA DE DADOS PESSOAIS E ANAMNESE

Identificação: **Prontuário : _____**

Nome: _____

End. Residencial: _____

Bairro: _____ Cidade: _____ UF: _____ CEP: _____

Fone(s): fixo/celular_____

Data de nascimento: ____ /____ /____ Estado Civil: _____

Profissão_____

End. Comercial: _____

Bairro: _____ Cidade: _____ UF: _____ CEP: _____

Fone(s) comercial _____

História Médica:

1. ( ) Está ou esteve recentemente sob tratamento médico?_____
2. ( ) Está tomando algum medicamento? Qual(is)? _____
3. ( ) Tem ou teve alguma dessas doenças: febre reumática, hipertensão, hipotensão, prolapso da válvula mitral, angina, diabetes, doença renal, hepatite, tuberculose, pneumonia, asma, anemia, câncer, aids? Qual(is)? _____
4. ( ) Usa marcapasso ou válvula cardíaca artificial?_____
5. ( ) Já teve convulsões? _____
6. ( ) Tem alergia a medicamento, alimento, anestésico ou outro? Qual(is)? _____
7. ( ) Ao se ferir, sangra muito ou demora a cicatrizar? _____
8. ( ) Costuma desmaiar ou sentir tonturas com frequência? _____
9. ( ) Costuma ter formigamento ou inchaço nos pés ou pernas? _____
10. ( ) Tem tosses persistentes ou já provocou sangue? _____
11. ( ) Tem dores de cabeça frequentemente? _____
12. ( ) É fumante? Quantos cigarros fuma por dia? _____
13. ( ) Já se submeteu a alguma cirurgia? _____
14. ( ) Tem alguma doença ou condição que mereça cuidados e que não foi mencionado aqui? Qual(is)? _____
15. Para mulheres: ( ) Está Grávida?

História Bucal:

2. Quanto à sua higiene bucal: Qual a frequência da sua escovação dental? _____

Utiliza fio dental? ____Faz uso de algum bochecho ? Qual? Com que frequência? _____

3. Já se submeteu a anestesia dentária ? Houve algum problema? _____

4. Sua gengiva é dolorida, inchada ou sangra com frequência? _____

5. Tem dificuldades ou ouve barulho ao abrir ou fechar a boca? _____

6. Tem hábitos com roer unhas, morder objetos ou ranger os dentes?_____

7. Consome frequentemente alimentos com açúcar entre as refeições? _____

8. Com que frequência vai ao dentista? _____

Declaro verdadeiros todos os dados aqui informados.

Fortaleza, _____ / _____/ _____

_____

Assinatura do paciente

APÊNDICE C - ALEATORIZAÇÃO GERADA ATRAVÉS DO SITE https://sealedenvelope.com

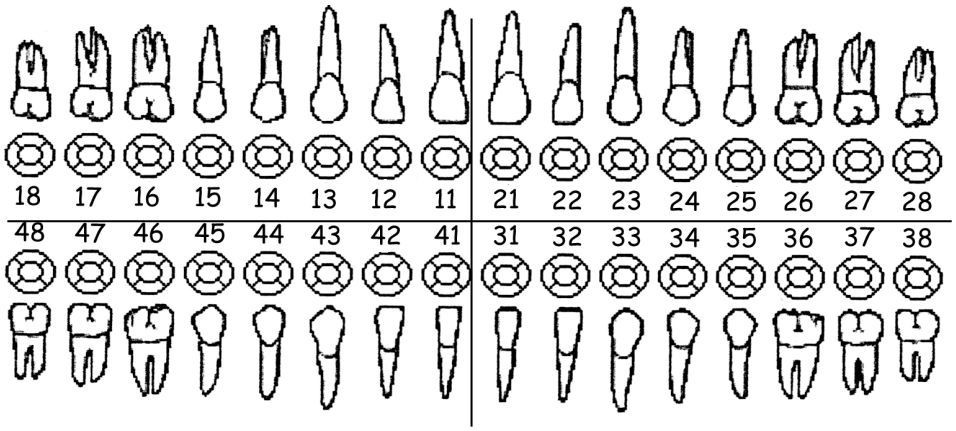
Paciente	nº Dentes	Sequencia	Tratamento	Paciente	nº Dentes	Sequencia	Tratamento
1	4	1	AUTO	11	4	1	CT-EGCG
1	4	2	CT	11	4	2	CT
1	4	3	CT-EGCG	11	4	3	AUTO
1	4	4	AUTO-EGCG	11	4	4	AUTO-EGCG
2	4	1	AUTO	12	4	1	CT-EGCG
2	4	2	CT-EGCG	12	4	2	AUTO-EGCG
2	4	3	AUTO-EGCG	12	4	3	AUTO
2	4	4	CT	12	4	4	CT
3	4	1	CT	13	4	1	AUTO-EGCG
3	4	2	CT-EGCG	13	4	2	CT-EGCG
3	4	3	AUTO-EGCG	13	4	3	AUTO
3	4	4	AUTO	13	4	4	CT
4	4	1	CT-EGCG	14	4	1	AUTO-EGCG
4	4	2	AUTO-EGCG	14	4	2	CT-EGCG
4	4	3	CT	14	4	3	CT
4	4	4	AUTO	14	4	4	AUTO
5	4	1	CT-EGCG	15	4	1	CT
5	4	2	AUTO	15	4	2	AUTO-EGCG
5	4	3	AUTO-EGCG	15	4	3	CT-EGCG
5	4	4	CT	15	4	4	AUTO
6	4	1	AUTO-EGCG	16	4	1	CT-EGCG
6	4	2	AUTO	16	4	2	AUTO-EGCG
6	4	3	CT	16	4	3	AUTO
6	4	4	CT-EGCG	16	4	4	CT
7	4	1	CT	17	4	1	CT
7	4	2	AUTO-EGCG	17	4	2	AUTO
7	4	3	AUTO	17	4	3	CT-EGCG
7	4	4	CT-EGCG	17	4	4	AUTO-EGCG
8	4	1	AUTO-EGCG	18	4	1	AUTO
8	4	2	AUTO	18	4	2	AUTO-EGCG
8	4	3	CT	18	4	3	CT-EGCG
8	4	4	CT-EGCG	18	4	4	CT
9	4	1	AUTO-EGCG	19	4	1	AUTO
9	4	2	CT	19	4	2	CT
9	4	3	CT-EGCG	19	4	3	AUTO-EGCG
9	4	4	AUTO	19	4	4	CT-EGCG
10	4	1	CT-EGCG	20	4	1	CT
10	4	2	CT	20	4	2	CT-EGCG
10	4	3	AUTO	20	4	3	AUTO-EGCG
10	4	4	AUTO-EGCG	20	4	4	AUTO

Paciente	nº Dentes	Sequencia	Tratamento	Paciente	nº Dentes	Sequencia	Tratamento
21	4	1	CT-EGCG	31	4	1	AUTO
21	4	2	CT	31	4	2	CT
21	4	3	AUTO	31	4	3	CT-EGCG
21	4	4	AUTO-EGCG	31	4	4	AUTO-EGCG
22	4	1	AUTO-EGCG	32	4	1	AUTO
22	4	2	AUTO	32	4	2	CT
22	4	3	CT-EGCG	32	4	3	CT-EGCG
22	4	4	CT	32	4	4	AUTO-EGCG
23	4	1	AUTO	33	4	1	CT-EGCG
23	4	2	CT-EGCG	33	4	2	AUTO
23	4	3	CT	33	4	3	CT
23	4	4	AUTO-EGCG	33	4	4	AUTO-EGCG
24	4	1	CT-EGCG	34	4	1	CT-EGCG
24	4	2	CT	34	4	2	CT
24	4	3	AUTO	34	4	3	AUTO-EGCG
24	4	4	AUTO-EGCG	34	4	4	AUTO
25	4	1	AUTO	35	4	1	CT-EGCG
25	4	2	CT	35	4	2	AUTO-EGCG
25	4	3	CT-EGCG	35	4	3	AUTO
25	4	4	AUTO-EGCG	35	4	4	CT
26	4	1	AUTO	36	4	1	CT
26	4	2	AUTO-EGCG	36	4	2	AUTO-EGCG
26	4	3	CT-EGCG	36	4	3	CT-EGCG
26	4	4	CT	36	4	4	AUTO
27	4	1	CT-EGCG	37	4	1	AUTO-EGCG
27	4	2	AUTO-EGCG	37	4	2	CT
27	4	3	CT	37	4	3	AUTO
27	4	4	AUTO	37	4	4	CT-EGCG
28	4	1	AUTO	38	4	1	CT-EGCG
28	4	2	AUTO-EGCG	38	4	2	CT
28	4	3	CT-EGCG	38	4	3	AUTO-EGCG
28	4	4	CT	38	4	4	AUTO
29	4	1	CT-EGCG	39	4	1	CT-EGCG
29	4	2	CT	39	4	2	AUTO
29	4	3	AUTO	39	4	3	CT
29	4	4	AUTO-EGCG	39	4	4	AUTO-EGCG
30	4	1	CT-EGCG				
30	4	2	AUTO				
30	4	3	CT				
30	4	4	AUTO-EGCG				

APÊNDICE D - FICHA DE ACOMPANHAMENTO DOS PROCEDIMENTOS REALIZADOS E RETORNOS

PRONTUÁRIO nº: _____ DATA: ___ /___ /___

NOME : _____



Solicitar radiografias:
( ) Dentes: _____
( ) panorâmica ( ) interproximais

CLASSIFICAÇÃO / RESTAURAÇÃO DAS LESÕES SELECIONADAS

Dente	Sensibilidade		Angulação	Esmalte margens	Geometria (mm)			Faceta desgaste	Grau de Esclerose	Presença Antagonista	Rest. DATA	Grupo
	Esp	Ar			Alt	Larg	Prof					

Grau de esclerose: 1 a 4 (fundamental com a foto)
Faceta de desgaste: sim ou não (fotos podem auxiliar)
Geometria: anotar em milímetros: (fotos podem auxiliar)
Borda em esmalte: avaliar percentualmente a quantidade de esmalte na borda
Sensibilidade espontânea e a jato de ar: (10s a 2cm): sim ou não
Angulação da lesão: < 45º, entre 45-90º, entre 90-145º e >135º
Presença de antagonista: sim ou não

TRATAMENTOS REALIZADOS

( ) Avaliação Inicial/ Triagem (____ /____/ ____)

( ) Anamnese / TCLE (____ /____/ ____)

( ) Exame completo / seleção das LCNC (___ /___/ ____)

( ) Moldagens iniciais (____ /____/ ____)

( ) Classificação das lesões selecionadas (___ /___/ ____)

( ) Fotografias iniciais (____ /____/ ____)

( ) Restaurações (____ /____/ ____)

( ) Moldagens finais (____ /____/ ____)

( ) Fotografias finais (____ /____/ ____)

( ) Avaliação Baseline (____ /____/ ____)

( ) Avaliação 6M (____ /____/ ____)

( ) Avaliação 12M (____ /____/ ____)

( ) Avaliação 18M (____ /____/ ____)

( ) Avaliação 24M (____ /____/ ____)

APÊNDICE E - FICHA DE AVALIAÇÃO CLÍNICA DAS RESTAURAÇÕES

PACIENTE (nº): _____

DATA DA RESTAURAÇÃO: ____ / ____ / _____ DENTES: | | | | | | | | |

DATA DA AVALIAÇÃO: ____ / ____ / ____

( ) BASELINE
( ) 6M
( ) 12M
( ) 18M
( ) 24M

AVALIADOR 1: _____

AVALIADOR 2: _____

MOLDAGEM: ( ) SIM ( ) NÃO / DENTES: _____

FOTOGRAFIAS: ( ) SIM ( ) NÃO / DENTES: _____

Critérios Avaliados	Propriedade Estética				Propriedades Funcionais								Propriedades Biológicas							
	Pigmentação marginal				Fraturas e retenção				Adaptação marginal				Sensibilidade pós-operatória				Cáries			
DENTES																				
GRUPOS																				
1. Clinicamente excelente / muito boa																				
2. Clinicamente boa (após o polimento provavelmente muito boa)																				
3. Clinicamente suficiente /satisfatório (pequenas deficiências, não há defeitos inaceitáveis, mas não ajustável sem dano para o dente)																				
4. Clinicamente insatisfatória (mas reparável)																				
5. Clinicamente pobres (substituição necessária)																				

APÊNDICE F - CARTÃO DE RETORNO ENTREGUE AO PACIENTE APÓS A AVALIAÇÃO BASELINE

UNIVERSIDADE FEDERAL DO CEARÁ
PROGRAMA DE PÓS GRADUAÇÃO DE ODONTOLOGIA
DOUTORADO EM ODONTOLOGIA
PESQUISA CLÍNICA

PARTICIPANTE: _____

RETORNOS:

6 MESES: _____ / _____ / _____

1 ANO: _____ / _____ / _____

2 ANOS: _____ / _____ / _____

3 ANOS: _____ / _____ / _____

4 ANOS: _____ / _____ / _____

OBRIGADA POR PARTICIPAR DA PESQUISA.
AGUARDAR O CONTATO PARA AGENDAMENTO DO RETORNO
DRA CECÍLIA: 988911887
ceciliaatem@hotmail.com

APÊNDICE G: CARTA DE CONTRA-REFERÊNCIA A SER APRESENTADA EM CASO DE ATENDIMENTO ODONTOLÓGICO REALIZADO POR PROFISSIONAIS EXTERNOS AO ESTUDO.

UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM
DOUTORADO EM ODONTOLOGIA

APRESENTAR EM EVENTUAIS ATENDIMENTOS ODONTOLÓGICOS

Caro colega,

O paciente: _____

está participando de uma pesquisa clínica na Universidade Federal do Ceará e teve lesões cervicais não cariosas restauradas nos seguinte dentes: _____

Solicitamos encarecidamente não realizar nenhum procedimento nos dentes supracitados e solicitar que o paciente entre em contato com a pesquisadora para a mesma realizar o tratamento adequado.

Atenciosamente,

Dra. Cecília Atem Gonçalves de Araújo Costa

Doutoranda em Odontologia

CRO-CE: 3448