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INCORPORAÇÃO DE AGENTES DE LIGAÇÃO CRUZADA EM SISTEMAS ADESIVOS APLICADOS EM DENTINA AFETADA POR CÁRIE

FORTALEZA

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Área de concentração: Clínica Odontológica.

Orientador: Prof. Dr. Vicente de Paulo Aragão Saboia.

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Aprovada em ___/___/___.

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Aos meus pais, Sérgio e Káthia;

Ao meu marido, Ronaldo;

E à minha filha, Alice

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RESUMO

A dentina cariada (DC) é um substrato usualmente encontrado na prática clínica, em que há maior dificuldade para adesão e maior taxa de degradação. Agentes de ligação cruzada naturais como a proantocianidina (PA) e epigalocatequina-3-galato (EGCG), têm mostrado efeitos positivos nas propriedades mecânicas e estabilidade estrutural da dentina sadia (DS) em longo prazo. No primeiro capítulo da presente tese, foi realizada uma revisão sistemática de estudos in vitro acerca do efeito de agentes de ligação cruzada aplicados em dentina cariada nas propriedades mecânicas desse tecido e na manutenção da integridade da interface adesiva. As estratégias de buscas foram utilizadas em cinco bases de dados, obtendo 594 artigos, dos quais 11 foram incluídos na revisão sistemática. Todos os artigos apresentaram risco médio de viés. Controvérsias foram encontradas quanto ao efeito em longo prazo dos agentes devido a não haver uma padronização nos estudos em relação ao modo/tempo de aplicação e concentração empregada. No segundo capítulo, um estudo laboratorial, foram selecionados 96 terceiros molares humanos hígidos, distribuídos em grupos definidos de acordo com os seguintes critérios: dentina hígida ou dentina afetada por cárie induzida por modelo de biofilme microcosmo; adesivo Ambar Universal ou adesivo Clearfil SE Bond; aplicação de adesivo sem incorporação de agentes de ligação cruzada ou incorporados com PA 1% ou EGCG 1% (n=8). Após a aplicação do adesivo, foram construídos platôs de resina composta, e os dentes foram seccionados para o teste de microtração (μTBS). Os palitos de cada grupo foram subdivididos em dois subgrupos: teste após 24 horas ou teste após 12 meses de armazenamento em água destilada. Foram observados, então, a resistência de união, o padrão de fratura e o número de fraturas prematuras dos espécimes. Três palitos aleatórios por grupo foram selecionados para análise de nanoinfiltração (NL). Duas fatias dos grupos cariados – 24 horas, e duas dos grupos controle dos dois adesivos utilizados foram escolhidas para o teste de zimografia *in situ* da interface resina-dentina. Os dados foram submetidos ao teste estatístico ANOVA um fator e *post-hoc* de Bonferroni ($p < 0,05$). Nenhum grupo apresentou diferença estatística em diferentes momentos (24 h x 12 M), independentemente do tipo de dentina. Quando aplicada à DC, a incorporação de PA ou EGCG não mostrou efeito na resistência de união, independentemente do tempo de avaliação e do adesivo ($p> 0,05$). O grupo Ambar - PA foi o único que apresentou aparente diminuição da atividade enzimática e manteve os valores de resistência de união quando a comparação entre DC x DS foi feita após 12 M ($p = 0,451$). O padrão de fratura

mostrou predominância de falhas adesivas / mistas. Todos os grupos exibiram depósitos de nitrato de prata na interface de união. O efeito da incorporação de agentes de ligação cruzada depende do adesivo utilizado e do tipo de substrato. A incorporação de PA no adesivo Ambar Universal manteve os valores de resistência de união e também mostrou uma diminuição da atividade enzimática na DC, o que não foi observado no EGCG.

Palavras-chave: Colágeno Dentinário. Inibidores de Metaloproteinases de Matriz. Proantocianidinas. Cárie Dentária. Adesão Dentinária.

ABSTRACT

Caries-affected dentin (CAD) is a substrate widely found in clinical practice and in which there is a greater difficulty in adherence and higher rate of degradation. Natural crosslinking agents, such as proanthocyanidin (PA) and epigallocatechin-3-gallate (EGCG), have been researched for positive effects on mechanical properties and structural stability of sound dentin (SD) overtime. In the first chapter, a systematic review of *in vitro* studies was conducted about the effect of crosslinking agents applied on carious dentin in the mechanical properties of this tissue and the maintenance of the resin-dentin interface integrity. The search strategies were used in 5 databases, obtaining 594 articles, of which 11 were included in the systematic review. All articles had a medium risk of bias. Controversies have been found in the literature regarding the long-term effect of the agents because there is no standardization in the studies regarding the mode/time of application nor the concentration employed. In the second chapter, an *in vitro* study, 96 selected healthy human third molars were divided into groups defined according to the following criteria: sound dentin or caries-affected dentin induced by microcosm biofilm model; Ambar Universal adhesive or Clearfil SE Bond adhesive; adhesive application with no incorporation of crosslinking agents or application with 1% PA or 1% EGCG (n = 8). After adhesive application, composite resin restoration was constructed incrementally and the teeth were sectioned for the microtensile test (μTBS). The sticks in each group were subdivided into two subgroups: 24-hours test and test after 12 months of storage in distilled water. The bond strength, fracture pattern and the number of premature fractures of the specimens were then observed. Three random sticks per group were selected for nanoinfiltration (NL) analysis. Two slices of the 24-hours CAD groups, as well as two slices of the sound control groups of the two adhesives used, were chosen for the in situ zymography test of the resin-dentin interface. Data were evaluated according to the statistical one-way ANOVA test and Bonferroni post-hoc test ($p < 0.05$). No group showed a statistical difference in the comparison at different times (24 h x 12 M) regardless of the type of dentin. When applied to CAD, the incorporation of PA or EGCG showed no effect on bond strength, regardless of the evaluation time and the adhesive used ($p > 0.05$). The Ambar - PA group was the only group that showed a decrease in enzymatic activation and showed maintenance of the bond strength when the comparison between CAD x SD was made after 12 M ($p = 0.451$). The fracture pattern showed a high predominance of adhesive/mixed failures. All groups exhibit silver nitrate

deposits along with the bonding interface. The effect of incorporating crosslinking agents depends on the adhesive used and the substrate type. The incorporation of PA in Ambar Universal adhesive maintained the bond strength values and also showed a decrease in enzymatic activity in CAD, which was not observed in EGCG.

Keywords: Dentin collagen. Matrix MMP inhibitors. Proanthocyanidins. Dental caries. Dentin bonding.

SUMÁRIO

INTRODUÇÃO GERAL

1. INTRODUÇÃO

A manutenção da estabilidade da união entre os sistemas adesivos e a dentina ainda constitui alvo de diversos estudos, pois sabe-se que a interface resina-dentina degrada com o tempo (ALBUQUERQUE *et al*., 2019; BRESCHI et al., 2008; DE MACEDO *et al*., 2018; LIU et al., 2011; PERDIGÃO et al., 2013). A principal estrutura responsável pela retenção micromecânica dos materiais adesivos à estrutura dentinária é conhecida como camada híbrida, composta por monômeros resinosos, colágeno, água residual e cristais de hidroxiapatita (NAKABAYASHI, KOJIMA, MATSUHARA, 1982). Para sua formação, é necessária a desmineralização superficial da dentina. Porém, o que ocorre em ambos os sistemas adesivos, convencionais e autocondicionantes é uma desmineralização que excede a infiltração dos monômeros resinosos, o que gera uma camada de fibrilas colágenas expostas sem recobrimento e, portanto, susceptíveis à degradação hidrolítica e enzimática (MAZZONI *et al*., 2006). Existem duas formas principais de degradação da matriz dentinária, que, por sua vez, levam a uma deteriorização da interface de união resina-dentina, ocasionando uma diminuição da longevidade da restauração: a degradação promovida pela hidrólise e a degradação que decorre da ativação de enzimas colagenolíticas (BRESCHI *et al*., 2008; PERDIGÃO *et al*., 2013). Uma vez que o substrato dentinário possui, inerentemente, na sua composição, tanto água quanto essas enzimas, essas degradações são agrupadas e chamadas de intrínsecas da matrix dentinária.

A matriz dentinária trata-se de um complexo conjunto de fibrilas que constituem a parte orgânica da dentina (MARSHALL *et al*., 1997). Apesar de grande parte de seu conteúdo ser composto por colágeno tipo I, há também proteínas da matriz dentinária que desempenham um papel fundamental como mediadoras de interações químicas, na maturação e na mineralização da dentina (PINNA *et al*., 2015). As MMPs (metaloproteinases da matriz) constituem um grupo de mais de 20 enzimas proteolíticas, uma classe de endopeptidases zinco/cálcio dependentes que, na dentina mineralizada, estão cobertas com nanocristais de apatita, tornando-as imóveis e não funcionais (NISHITANI *et al*., 2006). Porém, em sua forma ativa, são capazes de degradar as proteínas da matriz dentinária e membrana basal (BRESCHI *et al*., 2008; VISSE, NAGASE, 2003).

As MMPs são secretadas como pró-enzimas e podem ser ativadas quando a matriz dentinária for solubilizada por cáries (TJÄDERHANE *et al*., 1998), por proteinases, por agentes químicos, por calor excessivo (CHAUSSAIN-MILLER et al., 2006), estresse mecânico (WANG et al., 2012) e durante a diminuição do pH do meio onde se encontram, o que ocorre durante a desmineralização promovida pelos procedimentos adesivos (MAZZONI *et al*., 2006; ZHOU et al., 2019). A degradação intrínseca da matriz dentinária é considerada o fator decisivo na diminuição da resistência de união entre compósito restaurador e dentina (FANG *et al*., 2012; MAZZONI et al., 2015).

Algumas estratégias foram pensadas para prevenir efetivamente a degradação promovida pelas MMPs, a fim de melhorar a longevidade da união resina-dentina. Uma dessas alternativas é o uso de substâncias sintéticas que imitam a ação de inibidores endógenos da matriz dentinária (LIU *et al*., 2011; TJÄDERHANE *et al*., 2013b) e outra consiste na inativação dessas enzimas pelo uso de inibidores de MMPs exógenos, que agem na estabilidade funcional da união adesivo-dentina por intermédio de ligações cruzadas, chamadas em inglês de *crosslinkers* (MAZZONI *et al*., 2014).

As ligações cruzadas intermoleculares e interfibrilares são a base para a estabilidade, resistência e viscoelasticidade da matriz dentinária (KIM *et al*., 2017). A quantidade e o tipo de ligações cruzadas – que influenciam na qualidade da ligação – também são fatores decisivos na manutenção do colágeno e na sua capacidade de resistir à biodegradação (BEDRAN-RUSSO et al., 2014). Portanto, o aumento das ligações cruzadas do colágeno da dentina exposta tem como objetivo melhorar a estabilidade, principalmente da base da camada híbrida, considerada o ponto crítico e inicial da degradação endógena da interface de união, uma vez que, dificilmente, as fibrilas de colágeno dessa região são encapsuladas pelos monômeros resinosos do adesivo e tornam-se susceptíveis a hidrólise (MAZZONI *et al*., 2013;TJÄDERHANE *et al*., 2013a;TJADERHANE *et al*., 2013).

Nos últimos anos, vem crescendo entre os pesquisadores um interesse sobre os agentes de ligação cruzada por se tratarem de uma estratégia promissora no que tange ao aumento da resistência de união resina-dentina e à manutenção da estabilidade dessa união (COSTA *et al*., 2019; FONSECA *et al*., 2019; NERI *et al*., 2016). Nesse sentido, há uma predileção por alguns agentes químicos naturais, tais como os flavonoides e polifenóis, devido sua à biocompatibilidade, à baixa toxicidade aos tecidos dentinários e

à sua presença abundante na natureza, nas frutas, nos vegetais, no fruto, nas sementes e flores (BEDRAN-RUSSO *et al*., 2008; SANTIAGO *et al*., 2013; ISLAM *et al*., 2012; ISLAM *et al*., 2014).

As proantocianidinas (PAs), frequentemente extraídas da semente de uva, são um exemplo de agente de ligação cruzada natural que se mostrou capaz de melhorar as propriedades mecânicas da dentina através da formação de ligações cruzadas nas fibras colágenas (BALALAIE et al., 2018; CASTELLAN, 2010; FANG et al., 2012). Devido, também, a seu potencial efeito inibidor de MMPs, torna-se importante a tentativa de incorporá-las aos adesivos atuais a fim de buscar uma melhoria na resistência à degradação do colágeno da matriz dentinária (BEDRAN-RUSSO *et al*., 2008; EPASINGHE *et al*., 2012; HASS *et al*., 2016).

A epigalocatequina-3-galato (EGCG) é o principal flavonoide extraído do chá verde (*Camelia sinensis*) que tem demonstrado ser um efetivo inibidor de MMPs (CARVALHO *et al*., 2016; DE MACEDO *et al*., 2019; TJADERHANE *et al*., 2013). Estudos recentes demonstraram efeitos benéficos do EGCG incorporado no adesivo dentinário na manutenção da resistência de união dentina-resina ao longo do tempo (DU *et al*., 2012; KHAMVERDI; REZAEI-SOUFI; ROSTAMZADEH, 2015; SANTIAGO, *et al*., 2013).

Buscando tornar as pesquisas laboratoriais mais relevantes e compatíveis com as condições clínicas encontradas na prática odontológica, pesquisas têm sido realizadas visando ao aumento da resistência de união à dentina afetada por cárie, substrato encontrado com maior frequência durante os procedimentos restauradores (DA SILVA *et al*., 2015; FIALHO *et al*., 2019; PEIXOTO *et al*., 2015). Porém, sabe-se que a resistência de união diminui significativamente, quando sistemas adesivos são aplicados em dentina cariada em comparação à aplicação em dentina hígida (FIALHO *et al*., 2019; ISOLAN *et al*., 2018; JOVES *et al*., 2013; PINNA *et al*., 2015).

A heterogeneidade de uma lesão de cárie natural bem como o envolvimento de diversas profundidades de dentina, são fatores que poderiam comprometer os resultados dos materiais e das condições a serem testadas em estudos *in vitro*. Visando sobrepor essa dificuldade, modelos microbianos *in vitro* simplificados ou complexos têm sido amplamente utilizados para produzir lesões artificiais de cárie (MASKE et al, 2017). Os biofilmes artificiais são cultivados a partir de monoculturas, consórcios de várias espécies

definidas ou de microcosmos microbianos complexos usando diferentes abordagens de cultura (MASKE *et al*., 2017), em prol de simular, de forma controlada e fidedigna, o crescimento e a proliferação de microorganismos que compõem o biofilme dentário.

É sabido que um bom modelo *in vitro* de geração de biofilme deve incluir oscilações de pH e diversidade microbiológica compatíveis com as condições encontradas na cavidade oral para o desenvolvimento de lesões de cárie semelhantes às clinicamente vistas. Nesse sentido, um método microbiológico de produção artificial de tecido dentário afetado por cárie foi desenvolvido (VAN DE SANDE *et al*., 2011). Trata-se de um modelo microcosmo que abrange diferentes espécies bacterianas associadas ao processo carioso, que possui variações diárias de pH do meio de cultura, que conta com tempo relativamente curto de desenvolvimento da cárie e que se têm atingido resultados satisfatórios na reprodução *in vitro* de lesões naturais de cárie em dentina (MASKE *et al*., 2015) e esmalte (SIGNORI *et al*., 2016).

Com base em investigações prévias indicando que as MMPs-2, 3, 8, 9 e 20 estão presentes na matriz dentinária humana (MAZZONI *et al*., 2007), que a expressão das MMPs-2, 8 e 9 foi identificada nas formas ativa e inativa em tecido cariado (TJÄDERHANE *et al*., 1998), que o uso das PAs e do EGCG já demonstrou diminuir a degradação endógena da dentina e aumentar a longevidade da camada híbrida (SANON; SANCHAVANAKIT; SRISAWASDI, 2019; SANTIAGO *et al*., 2013), faz-se necessário averiguar se a utilização desses agentes de ligação cruzada atuam na estabilização e preservação da integridade da interface resina-dentina afetada por cárie ao longo do tempo.

No cotidiano da prática odontológica é interessante, tanto para pacientes quanto para dentistas, que os sistemas adesivos sejam os mais simplificados possíveis, diminuindo, dessa forma, o tempo clínico operatório e a sensibilidade da técnica. Dessa forma, clínicos tendem a rejeitar a adição de passos extras nos procedimentos restauradores, como o que ocorre com pré-tratamentos da dentina, o que torna a incorporação dos agentes de ligação cruzada nos adesivos dentinários uma alternativa atraente em face do pré-tratamento, que é preconizado na maioria dos estudos laboratoriais (EPASINGHE *et al*., 2012; ISLAM *et al*., 2014).

Portanto, a realização de uma revisão sistemática a fim de verificar o estado atual da arte das pesquisas laboratoriais que utilizam inibidores de MMP em dentina cariada,

incorporados ou não aos adesivos dentinários contemporâneos, e o efeito dessa aplicação na resistência de união resina-dentina em curto e longo prazo seria relevante, no intuito de guiar pesquisas clínicas futuras. Ainda, um estudo que avaliasse o efeito dos agentes de ligação cruzada proantocianidina 1% e epigalocatequina-3-galato 1% na estabilização das interfaces resina-dentina afetada por cárie, por meio da verificação do comportamento de restaurações de resina composta, seria de fundamental importância para a comprovação da eficácia desses compostos na técnica adesiva.

PROPOSIÇÕES

2. PROPOSIÇÕES

2.1 Objetivo geral:

Realizar uma revisão sistemática da literatura sobre o efeito da utilização de inibidores de MMP em dentina cariada, incorporados ou não aos adesivos dentinários contemporâneos, na resistência de união resina-dentina em curto e longo prazo, bem como avaliar *in vitro* o efeito da incorporação de agentes de ligação cruzada em dois sistemas adesivos autocondicionantes na resistência de união da interface formada por esses adesivos e a dentina hígida ou afetada por cárie, gerada por modelo microcosmo, 24 horas após a restauração ou após armazenamento em água por 12 meses.

2.2. Objetivos específicos:

1. Verificar, por meio da realização da revisão sistemática, se há na literatura associação positiva entre a utilização de inibidores de MMP e aumento da resistência de união, em curto e longo prazo, em dentina afetada por cárie.
2. Comparar a resistência de união entre resina e dentina afetada por cárie com a resistência de união entre resina e dentina hígida, quando aplicados os adesivos Ambar Universal ou Clearfil SE Bond, incorporados ou não por agentes de ligação cruzada, após 24 horas de armazenamento em água destilada.
3. Comparar a resistência de união entre resina e dentina afetada por cárie com a resistência de união entre resina e dentina hígida, quando aplicados os adesivos Ambar Universal ou Clearfil SE Bond, incorporados ou não por agentes de ligação cruzada, pós 12 meses de armazenamento em água destilada.
4. Comparar a resistência de união entre resina e dentina afetada por cárie na qual foi aplicado adesivo autocondicionante incorporado com agentes de ligação cruzada com a resistência de união entre resina e dentina afetada por cárie na qual foi aplicado adesivo autocondicionante sem incorporação, após 24 horas e após 12 meses de armazenamento em água destilada.
5. Comparar a resistência de união entre resina e dentina hígida na qual foi aplicado adesivo autocondicionante incorporado com agentes de ligação cruzada com a resistência de união entre resina e dentina hígida na qual foi aplicado adesivo autocondicionante sem incorporação, após 24 horas e após 12 meses de armazenamento em água destilada.

6. Avaliar o padrão de fratura dos espécimes após o teste de resistência à microtração.

7. Descrever a expressão da atividade proteolítica das MMPs por meio de zimografia *in situ* da interface de união nos grupos em que os adesivos incorporados com agentes de ligação cruzada foram aplicados em dentina afetada por cárie, no período de 24 horas de armazenamento em água.

8. Descrever a expressão da nanoinfiltração dos grupos estudados, por meio de Microscopia Eletrônica de Varredura, após 24 horas e após 12 meses de armazenamento em água destilada.

CAPÍTULOS

3. CAPÍTULOS

REGIMENTO INTERNO

Esta tese está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para teses de doutorado, permitindo a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de pesquisa envolvendo seres humanos ou parte deles, o projeto de pesquisa deste trabalho 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 – 3.048.195 (Anexo A). Desse modo, a presente tese de doutorado é composta pelos seguintes capítulos:

- Capítulo 1: **Effects of MMP inhibitors on caries-affected dentin – a systematic review**

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Periódico: Brazilian Oral Research *

- Capítulo 2: **Effect of Proanthocyanidin and Epigallocatechin-3-gallate incorporation into a universal and a self-etch dental adhesive applied on sound and caries-affected dentin.**

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CAPÍTULO 1

Effects of MMP inhibitors on caries-affected dentin – a systematic review

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Effects of MMP inhibitors on adhesion to caries-affected dentin – a systematic review

ABSTRACT

The aim of this study was to systematically review the current literature for *in vitro* studies that evaluated the effect of metalloproteinase (MMPs) inhibitors on immediate and long-term resin-dentin bond strength to caries-affected dentin. A search of publications was carried out in Pubmed (Medline), Web of Science, Embase, Cochrane Library and BVS databases, with no language or publication year limits, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) statement. Only in vitro studies that assessed the use of metalloproteinase inhibitors in adhesive bond strength (BS) to caries-affected dentin (CAD) were included. From 594 potentially eligible articles, 23 were selected for full-text reading and 11 were included in the systematic review once they met all the eligibility criteria. Two independent reviewers selected the studies, extracted the data and assessed the risk of bias. The most commonly used synthetic MMP inhibitor was chlorexidine (CHX), present in 9 of 11 studies, and this agent was usually applied as dentin pretreatment prior to etch-and-rinse adhesives. From CHX results, 4 articles concluded that it did not prevent decrease of BS over time, 2 studies of them that observed only immediate BS stated that no difference was noticed between CHX and control and 3 researches reported that CHX maintain BS to CAD overtime. Naturally delivered crosslinking agent as epigallocatechin-3-gallate (EGCG), proanthocyanidin (PA) and riboflavin (R) were also achieved, but in a very low frequency compared to CHX. All of included studies had medium risk of bias. Controversies have been found in the literature regarding the long-term effect of the agents because there is no standardization in the studies regarding the mode/time of application nor the concentration employed. Further studies are needed to be conducted to determine the optimal percentage for *in vitro* and clinical use and as explore mechanisms of action and long-term effectiveness.

Keywords: Dental caries. Matrix Metalloproteinase Inhibitors. Dentin. Tensile strength.

1. Introduction

Even today, adhesion to the dentin substrate remains a challenge for researchers and clinicians. Dentin is an organic substrate with high water content, which makes it difficult to infiltrate hydrophobic monomers by adhesive systems [1]. Furthermore, this tissue functions as a semipermeable membrane, allowing water to accumulate near the adhesive layer, thereby facilitating the degradation of this layer by hydrolysis.

This challenge of dentin adhesion becomes even more difficult when the dentin involved in the restorative procedure is caries-affected dentin (CAD). The current Odontology´s philosophy defends the minimally invasive dentistry, which proposes the selective removal of caries and the maintenance of CAD, that can be remineralized [2]. However, this tissue is damaged, disorganized, with collagen fibrils exposed by acids released during caries progression, with micro porosities and even higher water content than sound dentin (SD), which makes this substrate even more sensitive to degradation over time [3].

Besides hydrolytic, there is also another degradation pathway which is promoted by the activation of endogenous enzymes known as matrix metalloproteinases (MMPs). These enzymes, produced and secreted by odontoblasts, assist in the dentin formation phase[2,4]. After playing their role, they remain inactive in the dentin matrix, surrounded by hydroxyapatite crystals [5]. However, during the development of caries process or after hybridization promoted by adhesive systems, processes involving acidic products that solubilize the inorganic part of dentin and lower the pH, these enzymes are released and reactivated [6,7]. Nevertheless, at this time, they are able to degrade the entire dentin matrix, promoting tissue destruction at a molecular level but which may lead to a gradual decrease in the longevity of an adhesive restoration [8].

Some attempts to reduce this degradation have been researched during the last years, among them, a promising possibility comes from the use of MMP inhibitors directly on the demineralized or sound dentin [9]. Chlorhexidine, glutaraldehyde, riboflavin and a class of natural polyphenols from teas, seeds, fruits or leaves called proanthocyanidins are popular among these inhibitors [10-15]. However, although this approach is frequent in the literature, it still needs further studies regarding the inactivation of MMPs in carious lesions. In fact, until now, there is no systematic review that embraced *in vitro* studies

that evaluated the effect of matrix metalloproteinase (MMP) inhibitors during the adhesive procedure on the immediate and long-term resin-CAD bond strength.

Based on that, this study aimed to conduct a systematic review of in vitro studies which evaluated the bond strength between adhesive systems and CAD when different crosslinker agents are applied directly to the dentin or inserted into the adhesive system.

2. Material and methods

This systematic review was carried out according to the guidelines of the PRISMA strategy (Preferred Reporting Items for Systematic Reviews And Meta-Analyze) 2015 [16] and followed the flow diagram based on PRISMA Statement. PICO structure was adapted as follows: P(patient) – caries-affected dentin; I (intervention) – use of MMP inhibitors; C (control) – without MMP inhibitors; O (outcome) – bond strength by mechanical test. The research question guiding this review was: **do crosslinker agents applied in caries affected dentin before or inside adhesives systems improve the immediate or long-term bond strength?**

2.1 Literature search and study selection

An electronic search in five distinct electronic databases - Pubmed (Medline), Web of Science, Embase, Cochrane Library and BVS – was performed systematically by two independent reviewers up to December 2019 with no limited language and date restrictions. The keywords and search strategy used in Pubmed are listed in Table 1 and were adapted for the other databases. The reviewers hand-searched the reference lists of included articles for additional manuscripts. After the screening of articles, all of the selected studies were imported into Mendeley Desktop 1.17.11 software to remove duplicates. The full-text articles were independently assessed in duplicate by two review authors (DCMR and JSR). Discrepancies were solved by a discussion with a third reviewer (VPS).

2.2 Eligibility criteria and data extraction

The eligibility criteria consisted of selecting studies that evaluated the use of crosslinking agents in dentin bond strength of adhesives systems applied in caries-affected dentin (CAD). Only laboratory studies that evaluated bond strength from

adhesive to dentin were considered. Reviews, conference abstracts, case reports, case series, pilot studies, clinical trials, studies of intrinsic cross-linking of dentin, studies of crosslinker applications in other collagenous tissues or tooth-like structures, studies that bond strength was not the main outcome and irrelevant studies were excluded. In addition, studies that used only sound, clarified or eroded dentin was excluded.

A standardized protocol for data collection was developed by authors using a Microsoft Excel spreadsheet. The following data were extracted from all selected articles: author/year of publication, dental substrate, adhesive/manufacturer, adhesive system approach, crosslinker agent, pretreatment or incorporation, bond strength test, storage/aging, results, and conclusion. The data extraction was also performed independently by two reviewers (DCMR and JSR), and the third reviewer was involved only when a consensus was not possible (VPS).

2.2 Risk of bias assessment

Risk of bias was evaluated according to the articles' description of the following parameters for study quality assessment based on Cochrane Collaboration tool for intervention studies [17]: Random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. If the authors reported the parameter, the article had a ''Y'' (yes) on that specific parameter; if it was not possible to find the information, the article received an ''N'' (no). Articles that reported one or two items were classified as having high risk of bias, three or four items as medium risk, and five to seven items as low risk of bias (Fig.2).

3. Results

After research in databases, 1117 articles were found. After the removal of duplicates, 594 articles remained for the title and abstract screening. Then, 23 papers were selected for full-text reading, when 12 studies were excluded for not comply with the eligibility criteria. Finally, 11 studies composed this systematic review. Details of article selection and reasons for exclusions are shown in Fig 1. The characteristics of the studies included are summarized in Table 2.

All of the 11 studies included presented medium risk of bias. The results are described in Fig.2, according to the parameters considered in the analysis.

Between the 11 studies included in this review, all used human teeth, 5 tested adhesive systems to natural caries lesion [18–22] while 6 tested *in vitro* induced caries [15,23–27]. All of them used the pretreatment approach of crosslinker application, only one study compared pretreatment with incorporating strategy [23]. Most studies (91%) used an etch-and-rinse adhesive and chlorhexidine (CHX) was the preferred MMP inhibitor among the researchers (82%). The prevailing mechanical test was microtensile bond strength.

4. Discussion

Despite all the advances in research over the past decade, the dentin substrate remains a problematic issue of restorative procedures. If adhesion to this tissue in its healthy form is already considered a challenge, the application of current adhesive systems on this substrate in its carious way becomes even more challenging [28]. In this sense, studies have been conducted in order to biomodify dentin prior to restorative procedures[29,30]. A visionary alternative is the use of MMP inhibitors which can also crosslink dentin molecules, transforming this substrate into a more resistant tissue to intrinsic degradation of the collagen matrix and making dentin restoration last longer[31].

Several studies have been conducted *in vitro* regarding the search for the best performing crosslinking agent [15], or its ideal concentration [13,32–36] and application form [23], or even the comparison between several inhibitors of MMPs and their long-term effect on bond strength [14,37–39]. However, the vast majority of articles in the literature perform these experiments on healthy dentin, which does not reflect the clinical reality of restorative procedures or the maintenance of caries-affected dentin currently considered in minimally invasive dentistry.

In the present systematic review, according to our inclusion and exclusion criteria, 11 articles composed this research. All of them were performed in vitro and used caries-affected dentin as the main substrate for adhesive restorations. 55% percent of the articles used naturally carious dentin, which makes comparing this factor with other articles, which induced caries in the laboratory, a difficult task. This mainly happens because natural caries is a heterogeneous process that involves different levels and depths of dentin [40] so that even within a single tooth there will be specimens that will have different degrees of tissue disorganization. In addition, because it is a chronic and gradual process, it involves the individual's response, such as the presence of reaction dentin, which could

influence the results obtained in these studies. However, according to Joves et al., 2013[28], which evaluated mineral density, morphology and bond strength of natural versus artificial caries-affected dentin, the artificial-caries affected dentin showed similar mineral content and bond strength yet lower variability compared to the natural caries-affected substrate. But, the same author affirmed that with the lack of mineral casts in dentinal tubules of artificial caries-affected dentin, their morphologies are different [28]. The development of laboratory caries models is an effort to simulate clinical conditions under well-controlled environment [41].

Of all the selected studies, only 1 used a 3-step experimental adhesive [42] while the others used commercially available adhesives. Comparison between the 10 commercial adhesives showed a preference for using Adper Single Bond 2 (3M ESPE), considered in the literature as a gold standard among the two-step etch-and-rinse adhesive systems [43].

From the 11 articles, 9 used conventional adhesives. Of the 2 studies that opted for self-etching adhesives, one compared self-etching with etch-and-rinse approaches of adhesives of the same trademark [18] while the other used only a self-etching adhesive – Clearfil SE Bond [44]. For the first one[18], favorable results were observed when etch-and-rinse adhesive was used, whereas self-etching adhesive was not benefited by MMP inhibitor approach. On the second study[44], optimal results were achieved once 5% CHX was able to maintain bond strength of Clearfil SE Bond to CAD over 2 years of evaluation.

All selected articles had chlorhexidine as at least one of the MMP inhibitors applied to caries-affected dentin, except Ahn et al., 2018[18] who tested riboflavin or riboflavin with UVA activation and Macedo et al., 2009[45] who evaluated the application of 5% Glutaraldehyde or 6.5% Proanthocyanidin. Only Czech et al., 2019[23] tested the incorporation of crosslinker agents – 0.02% EGCG or 2% CHX - into Adper Single Bond 2 and compared this strategy with pretreatment. All other studies used crosslinker agents as pretreatment. All studies that used pretreatment applying the crosslinker agent for 60 seconds, except for Macedo et al., 2009[45] who used 1h, an unfeasable clinical time, and Erhardt et al., 2008[46] who did pretreatment for 120 s. Mobarak, 2011[44] and Ahn et al., 2018[18] used the mechanical shear test, while the other studies evaluated the bond strength by the microtensile test. Three studies evaluated bond strength only after 24h [18,45,46], while the other studies aimed at long-term verification of the effect of crosslinker application.

Among the results of the chosen studies, when the MMP inhibitor evaluated was CHX, some studies stated that this agent did not prevent the decrease in bond strength [19,25] ,while others concluded that CHX was able to maintain bond strength for 6 months [22,26] and even for 2 years [44]. Still, a study showed that the application of CHX did not affect the bond strength when compared to the control [20].

Among the studies that evaluated the use of naturally delivered MMP inhibitors, only one evaluated the performance of riboflavin and compared it with riboflavin + UVA applied as a pretreatment of a etch-and-rinse adhesive or a self-etching adhesive [18]. This study concluded that the combination of riboflavin + UVA improved the bond strength of etch-and-rinse adhesives to CAD, but had no effect when using self-etching adhesives. Also, the application time of riboflavin in this study is quite high for the standards of clinical pretreatment (4 min), which could make the choice of this approach unfeasible. Still, there is only one study among the 11 chosen that deals with the use of grape seed extract, from which the main substance extracted is proanthocyanidin. In this study [27], both proanthocyanidin and glutaraldehyde significantly increased the bond strength of two different etch-and-rinse adhesives to sound and CAD. However, there was no long-term evaluation to verify that this increase in bond strength was stable and the time of application of these MMP inhibitors on dentin was 1 hour, which makes this alternative unattractive clinically. Another natural crosslinker agent researched was EGCG, derived from green tea extract. Among the chosen studies, 3 of them researched this MMP inhibitor. Of these surveys, one [23] concluded that 0.02% of EGCG as pretreatment or incorporated was unable to maintain bond strength in the 6 and 12 month assessment, according to another study [36] who stated that EGCG at 0.02%, 0.05% or 0.2% was unable to maintain bond strength in the 12-month assessment. However, the third study [15] concluded that 2% of green tea extract was able to maintain the bond strength in the 6-month evaluation. As we can see, the percentage of EGCG used seems to define the long-term effectiveness of this approach in CAD.

This review found that all studies showed a medium risk of bias based on the method used for the risk of bias assessment, as described in Fig.2. Most of studies reported random sequence generation but 4 of them did not mentioned it. This finding is not related to performing or not of the randomization but to how the experiments were performed and, mainly, reported. The absence of information in this studies may not imply the authors did not carry out this procedure, but this requires the reader to deduce by giving

insufficient methodological detail. In this sense, there is a need for reporting guidelines for *in vitro* research to improve the quality and transparency of studies [47].

A methodological limitation was observed in our systematic review once it was not possible to perform a meta-analysis since the methodologies for choosing crosslinkers, their concentrations, the adhesive used, the time of application and the measurement of outcomes were heterogeneous.

5. *Conclusion*

Within the limitations of this review, it is possible to conclude that chlorexidine is the most commonly used synthetic MMP inhibitor and this agent is usually applied as dentin pretreatment prior to etch-and-rinse adhesives. Among naturally agents, EGCG is the most frequent researched, but there is still few studies about it. Mainly, in the present published literature so far, there is no consensus about the benefits of the application of crosslinker agents on CAD before adhesive systems. Once that different agents, concentrations and times of pretreatment application have been found, this can lead to a lack of a standardized methodology that could provide a better comparison between these studies. In addition, this systematic review also reinforces the weakness and limitation of the available evidence related to the topic searched. Therefore, this systematic review was able to infer that more *in vitro* studies should be conducted in caries-affected dentin to establish a protocol of using crosslinking agents on this substrate.

6. *References*

1. Ito S., Saito T., Tay FR., Carvalho RM., Yoshiyama M., Pashley DH. Water content and apparent stiffness of non-caries versus caries-affected human dentin. J Biomed Mater Res - Part B Appl Biomater. 2005;72(1):109–16. https://doi.org/10.1002/jbm.b.30130.

2. Mazzoni A., Tjäderhane L., Checchi V., Di Lenarda R., Salo T., Tay FR., et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res. 2015;94(2):241–51. https://doi.org/10.1177/0022034514562833.

3. Tjäderhane L., Nascimento FD., Breschi L., Mazzoni A., Tersariol ILS., Geraldeli S., et al. Strategies to prevent hydrolytic degradation of the hybrid layer - A review. Dent Mater. 2013;29(10):999–1011. https://doi.org/10.1016/j.dental.2013.07.016.

4. Palosaari H., Pennington CJ., Larmas M., Edwards DR., Tjäderhane L., Salo T.

Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue. Eur J Oral Sci. 2003;111(2):117–27. https://doi.org/10.1034/j.1600-0722.2003.00026.x.

5. Hannas AR., Pereira JC., Granjeiro JM., Tjäderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand. 2007;65(1):1–13. https://doi.org/10.1080/00016350600963640.

6. Tjäderhane L., Larjava H., Sorsa T., Uitto VJ., Larmas M., Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res. 1998;77(8):1622–9. https://doi.org/10.1177/00220345980770081001.

7. Sulkala M., Wahlgren J., Larmas M., Sorsa T., Teronen O., Salo T., et al. The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. J Dent Res. 2001;80(6):1545–9. https://doi.org/10.1177/00220345010800061301.

8. Carrilho MRO., Carvalho RM., De Goes MF., Di Hipólito V., Geraldeli S., Tay FR., et al. Chlorhexidine preserves dentin bond in vitro. J Dent Res. 2007;86(1):90–4. https://doi.org/10.1177/154405910708600115.

9. Montagner AF., Sarkis-Onofre R., Pereira-Cenci T., Cenci MS. MMP inhibitors on dentin stability: A systematic review and meta-analysis. J Dent Res. 2014;93(8):733–43. https://doi.org/10.1177/0022034514538046.

10. Loguercio AD., Stanislawczuk R., Polli LG., Costa JA., Michel MD., Reis A. Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. Eur J Oral Sci. 2009;117(5):587–96. https://doi.org/10.1111/j.1600-0722.2009.00663.x.

11. Santiago SL., Neri JR., Osorio R., Toledano M., de Carvalho RM. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. J Adhes Dent. 2013;15(6):535–40. https://doi.org/10.3290/j.jad.a29532.

12. Bedran-Russo AKB., Pashley DH., Agee K., Drummond JL., Miescke KJ. Changes in stiffness of demineralized dentin following application of collagen crosslinkers. J Biomed Mater Res - Part B Appl Biomater. 2008;86(2):330–4. https://doi.org/10.1002/jbm.b.31022.

13. Epasinghe DJ., Yiu CKY., Burrow MF. Mechanical properties, water sorption characteristics, and compound release of grape seed extract-incorporated resins. J Appl Oral Sci. 2017;25(4):412–9. https://doi.org/10.1590/1678-7757-2016-0448.

14. Hass V., Luque-Martinez IV., Gutierrez MF., Moreira CG., Gotti VB., Feitosa VP., et al. Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition. Dent Mater. 2016 Jun;32(6):732–41. https://doi.org/10.1016/j.dental.2016.03.008.

15. Carvalho C., Fernandes FP., Freitas V da P., Franca FMG., Basting RT., Turssi CP., et al. Effect of green tea extract on bonding durability of an etch-and-rinse adhesive system to caries-affected dentin. J Appl Oral Sci. 2016;24(3):211–7.

https://doi.org/10.1590/1678-775720150518.

16. Moher D., Shamseer L., Clarke M., Ghersi D., Liberati A., Petticrew M., et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015 Jan;4:1. https://doi.org/10.1186/2046-4053-4-1.

17. Higgins JPT, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011 Oct 18;343(oct18 2):d5928–d5928.

18. Ahn Y., Lamy R., Darling CL., Stewart JM., Pinzon LM. Photochemical crosslinking of caries-affected dentin combined with total- or self-etch systems. Am J Transl Res. 2018;10(9):2990–5.

19. Ekambaram M., Yiu CKY., Matinlinna JP., King NM., Tay FR. Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine. J Dent. 2014 Jun;42(6):709–19. https://doi.org/10.1016/j.jdent.2014.04.001.

20. Erhardt MCG., Osorio R., Toledano M., M.C.G. E., R. O., M. T. Dentin treatment with MMPs inhibitors does not alter bond strengths to caries-affected dentin. J Dent. 2008 Dec;36(12):1068–73. https://doi.org/10.1016/j.jdent.2008.09.002 LK

21. Mobarak EH. Effect of Chlorhexidine Pretreatment on Bond Strength Durability of Caries-affected Dentin Over 2-Year Aging in Artificial Saliva and Under Simulated Intrapulpal Pressure. Oper Dent. 2011;36(6):649–60. https://doi.org/10.2341/11-018-L.

22. Komori PCP., Pashley DH., Tjaderhane L., Breschi L., Mazzoni A., de Goes MF., et al. Effect of 2% Chlorhexidine Digluconate on the Bond Strength to Normal Versus Caries-affected Dentin. Oper Dent. 2009;34(2):157–65. https://doi.org/10.2341/08-55.

23. Czech R., Oliveira CAR., Franca FMG., Basting RT., Turssi CP., Amaral FLB. Incorporation of EGCG into an etch-and-rinse adhesive system: mechanical properties and bond strength to caries affected dentin. J Adhes Sci Technol. 2019 Nov;33(22):2430–42. https://doi.org/10.1080/01694243.2019.1642978.

24. Fialho MPN., Hass V., Nogueira RP., Franca FMG., Turssi CP., Basting RT., et al. Effect of epigallocatechin-3- gallate solutions on bond durability at the adhesive interface in caries-affected dentin. J Mech Behav Biomed Mater. 2019 Mar;91:398–405. https://doi.org/10.1016/j.jmbbm.2018.11.022.

25. Giacomini MC., Scaffa P., Chaves LP., Vidal C., Machado TN., Honorio HM., et al. Role of Proteolytic Enzyme Inhibitors on Carious and Eroded Dentin Associated With a Universal Bonding System. Oper Dent. 2017;42(6):E188–96. https://doi.org/10.2341/16-178-L.

26. Lenzi TL., Tedesco TK., Maxnuck Soares FZ., Loguercio AD., Rocha R de O. Chlorhexidine application for bond strength preservation in artificially-created caries-affected primary dentin. Int J Adhes Adhes. 2014 Oct;54:51–6. https://doi.org/10.1016/j.ijadhadh.2014.04.007.

27. Macedo G V., Yamauchi M., Bedran-Russo AK., G.V. M., M. Y., A.K. B-R. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res. 2009 Dec;88(12):1096–100. https://doi.org/10.1177/0022034509351001 LK -

28. Joves GJ., Inoue G., Nakashima S., Sadr A., Nikaido T., Tagami J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. Dent Mater J. 2013;32(1):138–43. https://doi.org/10.4012/dmj.2012-243.

29. Fang M., Liu R., Xiao Y., Li F., Wang D., Hou R., et al. Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds. J Dent. 2012 Jun;40(6):458–66. https://doi.org/10.1016/j.jdent.2012.02.008.

30. Neri JR., Yamauti M., Silveira FD d., Mendonça JS., Carvalho RM d., Santiago SL. Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: Twelve-month results. Int J Adhes Adhes. 2016;71:81–6. https://doi.org/10.1016/j.ijadhadh.2016.08.007.

31. Bedran-Russo AK., Pauli GF., Chen SN., McAlpine J., Castellan CS., Phansalkar RS., et al. Dentin biomodification: Strategies, renewable resources and clinical applications. Dent Mater. 2014;30(1):62–76. https://doi.org/10.1016/j.dental.2013.10.012.

32. Epasinghe DJ., Yiu CKY., Burrow MF., Tay FR., King NM. Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength. J Dent. 2012 Mar;40(3):173–80. https://doi.org/10.1016/j.jdent.2011.11.013.

33. Du X., Huang X., Huang C., Wang Y., Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent. 2012 Jun;40(6):485–92. https://doi.org/10.1016/j.jdent.2012.02.013.

34. Neri JR., Neri JR., Yamauti M., Feitosa VP., Pires APM., Dos Santos Araújo R., et al. Physicochemical properties of a methacrylate -based dental adhesive incorporated with epigallocatechin -3 -gallate. Braz Dent J. 2014;25(6):528–31. https://doi.org/10.1590/0103-6440201300096.

35. de Macedo FAA., Souza NO., Lemos MVS., De-Paula DM., Santiago SL., Feitosa VP. Dentin bonding and physicochemical properties of adhesives incorporated with epigallocatechin-3-gallate. Odontology. 2019;107(1):23–8. https://doi.org/10.1007/s10266-018-0367-0.

36. Fialho MPN., Hass V., Nogueira RP., França FMG., Turssi CP., Basting RT., et al. Effect of epigallocatechin-3- gallate solutions on bond durability at the adhesive interface in caries-affected dentin. J Mech Behav Biomed Mater. 2019;91(November 2018):398–405. https://doi.org/10.1016/j.jmbbm.2018.11.022.

37. Castellan CS., Bedran-Russo AK., Antunes A., Pereira PNR. Effect of dentin biomodification using naturally derived collagen cross-linkers: one-year bond strength study. Int J Dent. 2013;2013:918010. https://doi.org/10.1155/2013/918010.

38. Costa CAG de., Passos VF., Neri JR., Mendonça JS., Santiago SL. Effect of

Metalloproteinase Inhibitors on Bond Strength of a Self-etching Adhesive on Erosively Demineralized Dentin. J Adhes Dent. 2019;21(4):337–44. https://doi.org/10.3290/j.jad.a42930.

39. Fonseca BM., Barcellos DC., Silva TM da., Borges ALS., Cavalcanti B das N., Prakki A., et al. Mechanical-physicochemical properties and biocompatibility of catechin-incorporated adhesive resins. J Appl Oral Sci. 2019;27:1–11. https://doi.org/10.1590/1678-7757-2018-0111.

40. Marquezan M., Correa FNP., Sanabe ME., Rodrigues Filho LE., Hebling J., Guedes-Pinto AC., et al. Artificial methods of dentine caries induction: A hardness and morphological comparative study. Arch Oral Biol. 2009 Dec;54(12):1111–7. https://doi.org/10.1016/j.archoralbio.2009.09.007.

41. Maske TT, van de Sande FH, Arthur RA, Huysmans MCDNJM, Cenci MS. In vitro biofilm models to study dental caries: a systematic review. Biofouling. 2017 Sep 14;33(8):661–75.

42. Ekambaram M., Yiu CKY., Matinlinna JP., King NM., Tay FR. Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine. J Dent. 2014;42(6):709–19. https://doi.org/10.1016/j.jdent.2014.04.001.

43. Zanatta RF., Silva TM., Esper MALR., Bresciani E., Gonçalves SEP., Caneppele TMF. Bonding performance of simplified adhesive systems in noncarious cervical lesions at 2-year followup: A double-blind randomized clinical trial. Oper Dent. 2019;44(5):476–87. https://doi.org/10.2341/18-049-C.

44. Mobarak EH. Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. Oper Dent. 2011;36(6):649–60. https://doi.org/10.2341/11-018-L.

45. MacEdo G V., Yamauchi M., Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res. 2009;88(12):1096–100. https://doi.org/10.1177/0022034509351001.

46. Erhardt MCG., Osorio R., Toledano M. Dentin treatment with MMPs inhibitors does not alter bond strengths to caries-affected dentin. J Dent. 2008 Dec;36(12):1068–73. https://doi.org/10.1016/j.jdent.2008.09.002.

47. Sarkis-Onofre R, Cenci MS, Demarco FF, Lynch CD, Fleming PS, Pereira-Cenci T, et al. Use of guidelines to improve the quality and transparency of reporting oral health research. J Dent. 2015 Apr;43(4):397–404.

TABLES AND FIGURES

Table 1. Search strategy used in Pubmed / MEDLINE

#1	(("Dental Caries"[Mesh] OR (dental decay) OR (caries, dental) OR (decay, dental) OR (carious dentin) OR OR (dentin, carious) OR (dentin, carious) OR (dental) OR (caries-affected dentin) OR (Caries-affected dentine) OR (caries-like lesion) OR (CAD) OR (Dental Decay) OR (Decay, Dental) OR (Carious Dentin) OR (Carious Dentins) OR (Dentin, Carious) OR (Dentins, Carious) OR (Caries) OR (carious-affected hybrid layer) OR (caries-affected hybrid layer)) AND ((“Matrix Metalloproteinase Inhibitors”[Mesh] OR (Inhibitors, Matrix Metalloproteinase) OR (Metalloproteinase Inhibitors, Matrix) OR (MMP Inhibitors) OR (Inhibitors, MMP) OR (Stromelysin Inhibitors) OR (Inhibitors, Stromelysin) OR (Gelatinase Inhibitors) OR (Inhibitors, Gelatinase) OR (Collagenase Inhibitors) OR (Inhibitors, Collagenase) OR (Crosslinking reagentes) OR (Bioflavonoids) OR (MMP inhibitor) OR (Crosslinkers) OR (Crosslink$) OR (Matrix metalloproteinases) OR (Cross-linking) OR (MMPs) OR (Collagen cross-linking) OR (Natural collagen cross-linkers) OR (collagen crosslinker) OR (Protease inhibitor) OR (Proanthocyanidins) OR (Grape seed extract) OR (Green tea) OR (epigallocatechin)) AND ((Tensile Strength [Mesh]) OR (Strength, Tensile) OR (Strengths, Tensile) OR (Tensile Strengths) OR (Microtensile bond strength) OR (Bond strength))

Table 2. Characteristics of the studies included in the Systematic Review

Author (Year)	*Dental substrate*	*Adhesive used/Manufacturer*	*Adhesive system approach*	*MMP inhibitor*	*Pretreatment or incorporation*	*Bond strength test (BS)*	*Storage/ aging*	*Results*	*Conclusion*
Ahn et al. (2018)	Natural human caries	All-Bond 3 TE (Bisco) or All-Bond SE (Bisco)	E&R or SE	0,1% Riboflavin or 0,1% Riboflavin + UVA	Pretreatment for 4 min or 4min + 3min (UVA light)	Shear bond	None	**For E&R:** R+UVA increased BS **For SE:** not affected by R or R+UVA	The combination of R+UVA improves BS on CAD when using an E&R adhesive. SE may not benefit from this strategy.
Carvalho et al. (2016)	Caries induction	Adper Single Bond 2 (3M ESPE)	E&R	2% CHX or 2% green tea extract	Pretreatment for 60 s	µTBS	24 h or 6m WS	**24h:** no difference noticed **6m:** 2% green tea increased BS	Application of 2% green tea extract was able to increase long-term BS to CAD
Czech et al. (2019)	Caries induction	Adper Single Bond 2 (3M ESPE)	E&R	0.02% EGCG or	Pretreatment (PRE) or	µTBS	24h or 6m or	**24h:** no difference noticed (CHX was the worse)	EGCG (PRE or INC) was not able to prevent decrease of BS overtime.

				2% CHX	Incorporation (INC)		12m WS	**6m and 12m:** all groups decreased BS	
Ekambaram et al. (2014)	Natural human caries	Experimental 3-step adhesive	E&R	2% CHX	Pretreatment for 60s	μTBS	24h or 12m Artificial saliva	**24h:** WWB + CHX was able to maintain BS to CAD compared to SD and also preserved BS in SD at 12m **12m:** WWB + CHX did not preserve BS in CAD	CHX with WWB preserved the bond strength of the hydrophobic adhesive to SD after 12 months; A significant drop in bond strength was observed in CAD after aging
Erhardt et al. (2008)	Natural human caries	Adper Scotchbond 1 (3M ESPE)	E&R	EDTA or 5% CHX	EDTA: application instead of H_3PO_4; CHX: 35% H_3PO_4 + Pretreatment for 120s + rinsed for 10s	μTBS	None	No difference was noticed	Neither the use of 5% CHX or conditioning with EDTA did not affect BS to SD and CAD compared to the control group.

Fialho et al. (2019)	*Caries induction*	*Adper Single Bond 2 (3M ESPE)*	*E&R*	*0.02% EGCG or* *0.2% EGCG or* *0.5% EGCG or* *2% CHX*	*Pretreatment for 60 s*	*µTBS*	*24h* *or* *12m* *WS*	***24h:** no difference noticed between groups* ***12m:** all groups decreased BS. No difference noticed between groups*	*EGCG solutions and CHX were incapable of reducing the decrease of BS to CAD over time*
Giacomini et al. (2017)	Caries induction	Adper Single Bond Universal (3M ESPE)	E&R	2% CHX	Pretreatment for 60 s	µTBS	24h or 6m Artificial saliva	**24h:** lower BS for CAD, even if CHX was applied **6m:** Lower BS for CAD, even when CHX was applied	Bond strength trended lower in CAD, even with CHX as pretreatment
Lenzi et al. (2014)	Caries induction	Adper Single Bond 2 (3M ESPE)	E&R	2% CHX	Pretreatment for 60 s	µTBS	24h or 6m WS	**24h:** Lower BS for CAD **6m:** Lower BS for CAD, but when CHX was applied it preserves dentin from time degradation	CHX prevents the decrease in BS for SD and CAD in primary dentin after 6m

Macedo et al. (2009)	Caries induction	Adper Single Bond Plus (3M ESPE) or One Step Plus (Bisco)	E&R	5% GLU or 6,5% GSE	Pretreatment for 1 h	µTBS	None	Bond to CAD was lower than SD for both adhesives. GSE and GLU increased BS for SD and CAD	BS increased with the use of biochemical crosslinkers in both CAD and SD
Mobarak (2011)	Natural human caries	Clearfil SE Bond (Kuraray)	SE	2% CHX or 5% CHX	Pretreatment for 60s	Micro shear bond	24h or 2y Artificial saliva	**24h:** no difference noticed (for both SD and CAD) **2y:** all groups decreased BS except the 5% CHX on CAD	5% CHX was able to maintain BS in CAD overtime under pulpal pressure while 2% CHX did not.
Komori (2009)	Natural human caries	Scotchbond Multi-Purpose (3M ESPE) or Adper Single Bond 2 (3M ESPE)	E&R	2% CHX	Pretreatment for 60s	µTBS	24h or 6m Artificial saliva	**24h:** no difference noticed (for both SD and CAD and adhesives testes) **6m:** decrease of BS except for CHX groups	CHX demonstrated its therapeutic action on the preservation of bond strength of E&R to normal dentin.

Abbreviations: E&R: etch-and-rinse adhesive; SE: self-etch adhesive; CAD: caries-affected dentin; SD: sound dentin; CHX: chlorexidine; R: riboflavin; BS: bond strength ; μTBS: microtensile bond strength; WS: water storage; EGCG: epigallocatechin-3-gallate; GLU: glutaraldehyde; GSE: grape seed extract ; WWB: water-wet bonding; EDTA: Ethylenediamine tetraacetic acid.

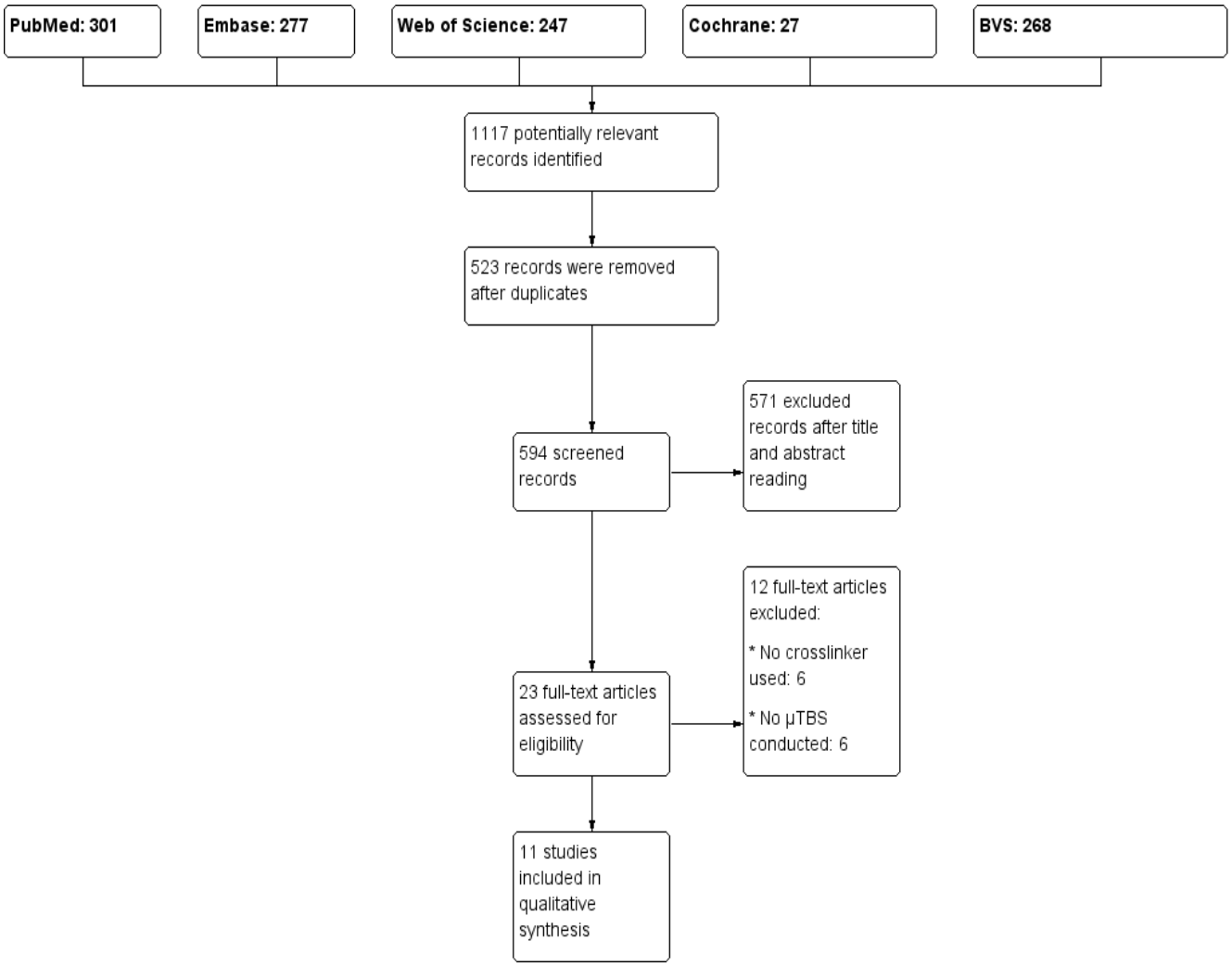

Fig. 1 Search flowchart according to the PRISMA statement.

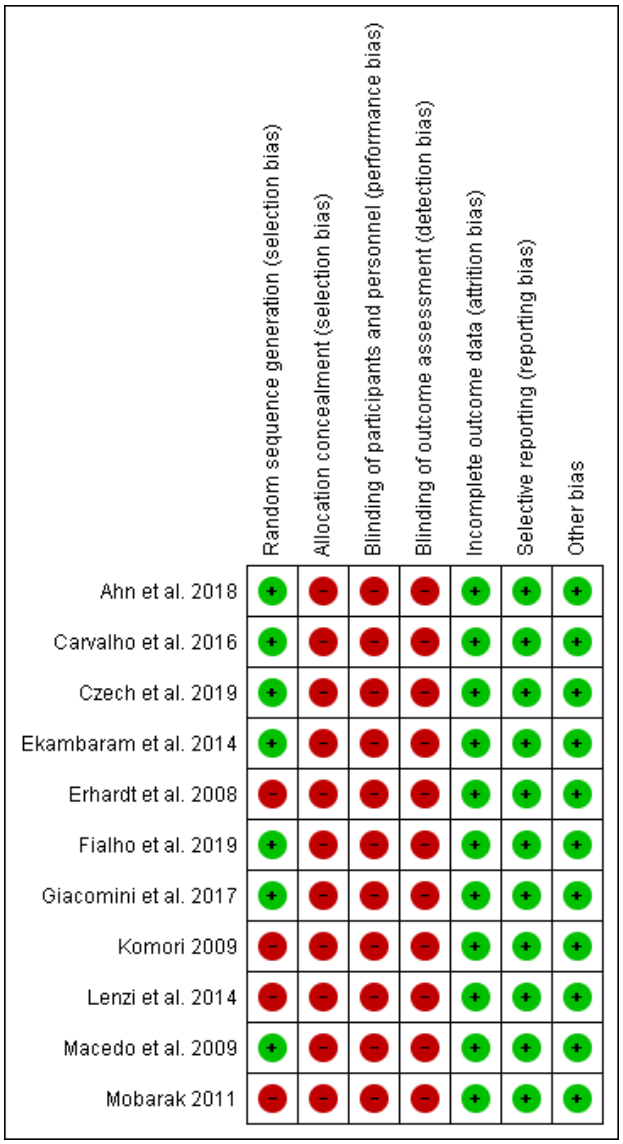

Fig. 2 Risk of bias summary author´s judgment on each item for each included study.

CAPÍTULO 2

Effect of Proanthocyanidin and Epigallocatechin-3-gallate incorporation into a universal and a self-etch dental adhesive applied on sound and caries-affected dentin.

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Effect of Proanthocyanidin and Epigallocatechin-3-gallate incorporation into a universal and a self-etch dental adhesive applied on sound and caries-affected dentin.

ABSTRACT

The aim of this *in vitro* study was to evaluate the incorporation of PA or EGCG on the resin-dentin micro tensile bond strength (μTBS), fracture pattern, nanoleakage (NL), as well as *in situ* inhibition potential of MMP´s activity, in immediate (IM) time and after 12 months of water storage (12M), when using two types of adhesive systems on sound dentin (SD) or CAD. 96 dentin middle surfaces of human molars were exposed and half of them were submitted to a microbiological cariogenic challenge – microcosm, for 14 days. Then, all specimens were randomly allocated into twelve groups (n=8) regarding 1. adhesive incorporation at three levels (1% EGCG x 1% PA x none incorporation); 2. dentin status (CAD x SD); 3. adhesive approach: one-bottle universal adhesive (Ambar Universal, FGM) and two-bottle self-etch adhesive (Clearfil SE Bond, Kuraray). After the bond procedure, specimens were cut into resin-dentin sticks for μTBS and the following tests. Half of the sticks were tested in 24 h (IM) and the other half after 12 months of water storage (12M). Three sticks per group were submitted to NL evaluation and two slices of the immediate CAD groups, as well as two slices of the sound control groups of the two adhesives used, were chosen for the in situ zymography test of the resin-dentin interface. Data were tabulated and evaluated according to one-way ANOVA, t-student´s test and Bonferroni post-hoc ($p < 0.05$). The frequency of fracture was expressed as a relative frequency and analyzed by Fisher's exact test. No group showed a statistical difference in the comparison at different times (24 h x 12 M) regardless of the type of dentin used (SD x CAD). When applied to CAD, the incorporation of PA or EGCG showed no effect on the BS, regardless of the evaluation time and adhesive used ($p>0.05$). The Ambar - PA group was the only group that showed a decrease in enzymatic activation demonstrated by in situ zymography and showed maintenance of the bond strength when the comparison between CAD x SD was made after 12 M ($p = 0.451$). The fracture pattern showed a high predominance of adhesive/mixed failures in most groups. All groups exhibit silver nitrate deposits along with the bonding interface. The effect of incorporating crosslinkers depends on the adhesive used and the substrate type. The

incorporation of PA in Ambar Universal maintained the bond strength values as well as showed a decrease in enzymatic activity in CAD, which was not observed for EGCG. **Significance:** The use of proanthocyanidin incorporated into adhesives may achieve a better and durable bonding interface, even if some caries-affected dentin was left on the restoration cavity.

Keywords: Adhesives. Dental caries. Matrix metalloproteinase inhibitors. Dentin

1. Introduction

The most ordinary substrate at the contemporary restorative technique, after the advent of minimally invasive Dentistry, is caries-affected dentin (CAD). Due to the ability of remineralization inherent of this tissue, during caries removal, it is highly recommended to let CAD on the bottom of dentin cavities. Nevertheless, CAD has a different composition of the smear layer when compared to sound dentin, mainly because of the different mineral/organic composition [1]. CAD´s smear layer is full of organic components, disorganized collagen traps minerals and appears to be thicker than unaltered dentin [2]. All that ends up with poor infiltration of demineralized collagen which leads to premature degradation of hybrid layer over time due to activation of MMPs and hydrolysis of the non-reinforced fibrils [3].

The main structure responsible for the micromechanical retention of adhesive materials to the dentin is known as the hybrid layer (HL)[4], composed of adhesive monomers, collagen mix, resin, water and hydroxyapatite crystals. For its formation, the superficial demineralization of dentin is required [5]. However, what occurs in both conventional and self-etching adhesive systems is a demineralization that exceeds the infiltration of resin monomers, which generates a layer of uncoated collagen fibrils and thus susceptible to hydrolytic and enzymatic degradation [6,7]. Some authors consider the intrinsic degradation of the dentin matrix to be the decisive factor in reducing the bond strength between restorative composite and dentin [8,9].

During the demineralization phase of dental caries, hydroxyapatite is solubilized by organic acids produced by oral bacteria [8]. The acidic environment created by bacterial acids can facilitate the activation of endogenous MMPs [10]. Also, during the application of adhesive systems, the acidic resin monomers contained either in etch-and-rinse or self-etch adhesives may activate these MMPs as well [11].

In this context, alternatives were been developed to inactivate MMPs, improving adhesion to dentin and the use of natural collagen cross-linking agents has gained space[12–14]. A great variety of bioactivities have been reported for polyphenols from plants. In dental research, a particular interest in proanthocyanidins (PA) was born. They belonging to a category known as condensed tannins, highly hydroxylated structures capable of forming an insoluble complex with carbohydrates and proteins[15]. Because

of the PA affinity for proline-rich proteins, a decrease of in vitro caries progression was shown to be promoted by PA-rich grape seed extract[15].

Epigallocatechin-3-gallate (EGCG) is another naturally derived crosslinker. It is the main flavonoid extracted from *Camellia sinensis*, has antioxidant and anti-inflammatory properties, and exhibits an effective inhibition of matrix-metalloproteinases (MMPs) and cysteine cathepsins [16]. Also, EGCG promotes collagen cross-linking through hydrogen bonding, thus improving collagen properties such as modulus of elasticity [17]. EGCG has been shown to be a promising agent in the maintenance of long-term dentin bond once that reduces dentin bond deterioration and improve the longevity of adhesive restorations[16,17].

Most studies use the strategy of crosslinker agents as a pretreatment, after acid conditioning and before primer/adhesive [16–18]. However, the improvement of current adhesive techniques and procedures leads to the use frequently of simplified systems, self-etching or universal ones, which require less clinical time and lower sensitivity of the restorative technique. Therefore, the incorporation of these MMP´s inhibitors agents in adhesives aims to simplify and facilitate their application by clinicians, since it does not increase the number of steps already existing in the restorative protocol. In addition, since the dentin smear layer is a constituent part of the hybrid layer instituted by self-etching adhesives, we believe that incorporating crosslinkers into the adhesives will achieve greater retention in dentin, slow and gradual release of these substances and thus beneficial long-term effects.

Although the use of crosslinking agents and MMP inhibitors have shown promising results [19–21], there is still limited information on the literature about the effect of their incorporation on self-etch and universal adhesives on mechanical properties and bonding durability to sound and, mainly, caries-affected dentin. In fact, only a few studies examined the use of EGCG to improve adhesion to CAD [16,18,22] and one study has examined the use of PA to the same substrate[23].

Thus, the aim of this *in vitro* study was (1) to evaluate the stability of the adhesive interfaces created by two commercial adhesives, with and without incorporation of 1% proanthocyanidin or 1% epigallocatechin-3-gallate and dentin on both sound and caries-affected dentin through by micro tensile, (2) to investigate nanoleakage expression and (3) collagenolytic activity of the adhesive interface through *in situ* zymography analyze.

The null hypothesis tested was that neither the crosslinker agents incorporation, the dentin status nor the water storage period would have any effect on bond strength to the substrate.

2. Material & Methods

2.1 Ethical aspects

This study was submitted to the Ethics Committee on Research with Human Beings, of the Federal University of Ceará. The project was approved by Protocol no 3.048.195.

2.2 Selection and preparation of teeth

Ninety-six caries-free, healthy third molars extracted for reasons unrelated to our research were obtained, cleaned with a periodontal curette and stored in an aqueous solution of 0.1% thymol at 4 ºC for no more than 30 days before the experiment. The occlusal third and the roots of the teeth were removed with a high-concentration diamond saw at a constant speed undercooling, mounted in a metallographic cutter (Struers A/S, Copenhagen, Denmark) generating a 4 mm thick disc with a flat enamel-free dentine surface. Dentin surfaces were examined under a 40x magnification stereoscopic microscope to ensure the absence of enamel remnants (Leica DM 1000 - Leica Microsystems GmbH - Wetzlar, Germany). The occlusal dentin surfaces were then roughed with 600-grit SiC paper for the 20s under running water to produce a standard smear layer.

2.3 Experimental Design

The *in vitro* study involved a microbiological model of multispecies caries induction, called microcosmos, performed according to the protocol of [24]. The factors under study were:

1. Adhesive incorporation at three levels: 1% EGCG (Epigallocatechin-3-gallate); 1% PA (Proanthocyanidins from grape seed extract) and NT (no treatment group as a negative control);
2. Dentin status: caries-affected dentin and sound dentin;
3. Adhesive approach: One-bottle Universal adhesive (Ambar Universal, FGM) and Two-bottle self-etch adhesive (Clearfil SE Bond, Kuraray);

4. Time of storage in water: 24 h and 12 months.

As a quantitative variable response, the micro tensile test (in megapascal – MPa) was obtained. The qualitative response variables were failure mode, adhesive interface analysis by scanning electron microscopy (SEM) for NL at 1200x magnification and *in situ* zymography by confocal laser scanning microscopy (CLSM).

The schematic representation of the experimental protocol is showed in Figure 1.

2.4 Caries-affected dentin (CAD) – Microcosm model

The protocol used for caries induction has been previously described and valid [24]. For caries-affected induced dentin, all discs were sterilized by gamma radiation and kept at 4 °C in a humid atmosphere until use. 20 mL of fresh saliva stimulated by paraffin film was collected from a healthy volunteer (a 23-year-old male) who had not been under antibiotic therapy for at least 6 months. No saliva volume was discarded before the collection. The volunteer abstained from oral hygiene for 24 h and from food ingestion for 2 h prior to collection. A 0.4-mL volume of saliva was inoculated onto each of the 48 dentin discs in a 24-microwell plate, and it remained at rest for 1 h at 37 °C.

After this period, the saliva was gently aspirated from the bottom of each well, 1.8 mL of defined medium enriched with mucin (DMM)[25] containing 1 % sucrose was added, and the plates were incubated at 37 °C under an anaerobic atmosphere (5–10 % CO_2, less than 1 % O_2) [24]. After 4 h, the specimens were rinsed with 2 mL of sterile saline, inserted into a new plate containing DMM without sucrose, and incubated for 20 h under the same conditions.

The biofilms were formed individually on the specimens in each well for 14 days, during which the same daily routine of alternate exposure to DMM supplemented with and without sucrose was followed. After this period, dentin discs were carefully cleaned with cotton soaked in distilled water, following adhesive and restorative procedures.

2.5 Preparation of adhesives

Two self-etching adhesive systems were used: a two-step adhesive (Clearfil SE Bond) and another universal single-step adhesive (Ambar Universal) (Table 1), which were used as controls. For the experimental groups, the same adhesives had their commercial composition modified by the addition of PA powder (*V. Vinifera*,

Meganatural Gold, Madera, CA, USA) or EGCG power (Sigma Aldrich, St. Louis, MO, USA) in order to generate a mixture with the concentration of PA 1.0 w/w or EGCG 1.0 w/w [13, 14]. For this, four drops of the adhesive primer were portioned to the proportional amount of powder, reaching the concentration desired. To obtain a homogeneous mixture, the incorporated adhesives were mixed by a shaker (QL-901; Biomixer, Sao Paulo, SP, Brazil) in the dark for 1 minute. The homogeneity of the dilution was carefully checked and only the adhesive was used without crystals and the color stabilized. The mixture was used immediately.

2.6 Restorative procedures

All adhesives were applied on the dentin surfaces according to their respective manufacturer's instructions by a single trained operator (Table 1). Then, they were photoactivated for 10s by a light source (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) with an intensity of 1200 mW / cm². After the bonding procedures, a resin composite (Filtek Z350 XT, 3M ESPE, MN, EUA) was applied to all teeth in four increments of 1 mm each. Each composite layer was individually light-cured for 20 s. All bonded specimens were stored in distilled water at 37°C for 24 h prior to micro tensile bond strength testing.

The specimens were longitudinally sectioned in both the mesiodistal and buccolingual directions across the bonded interface in a cutting machine (Struers A/S, Copenhagen, Denmark), to obtain resin–dentin sticks (1 mm²). The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan). These sticks were randomly divided into two groups, according to storage time in distilled water before the micro tensile test: 24 h or 12 months (12 M) at 37 °C. The water in which the sticks were stored was changed one a week.

2.7 Microtensile bond strength (μTBS) and failure mode

For this test, a total of 96 teeth (n=8 teeth per group) were used. Each bonded stick was attached to a jig for micro tensile testing with cyanoacrylate resin (Super Bonder Gel, Loctite, São Paulo, Brazil) and subjected to a tensile force in a universal testing machine (EMIC DL 1000, Equipment and Systems; Sao Jose dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min and a 500N load cell until fracture. The bond strength

values were expressed in MPa. The comparison was conducted using the average value of each tooth (n = 8).

The failure mode of each stick fractured was analyzed using a stereomicroscope at a magnification of 40X magnification. Failures were classified as adhesive/mixed (failure at the adhesive-dentin interface or mixed with cohesive failure of the neighboring substrates) or cohesive (failure exclusively within dentin or composite). The number of premature fractures was also recorded.

2.8 Nanoleakage Evaluation (NL)

Three resin-bonded sticks from each group, that were not tested in μTBS, were randomly selected. Firstly, the specimens were immersed in 50 wt% ammoniacal silver nitrate [Ag $(NH_3)_2$] NO_3 (aq) solution in total darkness for 24 h. Then, they were rinsed thoroughly in distilled water and immersed in the photo-developing solution for 8 h under fluorescent light (60 cm from the specimens) to reduce the silver ions into metallic silver grains along with the resin-dentin interface. The specimens were included in epoxy resin and wet-polished down until 2500-grit SiC paper and 1 μm diamond paste (Buehler Ltd., Lake Bluff, IL, USA). They were ultrasonically cleaned between each SiC paper, air-dried, dehydrated overnight, coated with carbon and observed using a scanning electron microscope (SEM), in the backscattered mode at 12 kV (Quanta FEG, FEI, Amsterdam, Netherlands). To investigate nanoleakage expression, three images were taken of each specimen: the first image was in the center of the stick, while the other two were obtained 0.3 mm left and right from the first picture, respectively.

2.9 In situ zymography

The in situ zymographic analysis was performed following the protocol of [11]. Two resin-dentin bonded slices of 0.5mm thickness from two different teeth of each CAD 24 h tested groups (Amb – Control – CAD, Amb – PA– CAD, Amb – EGCG – CAD, Clear – Control – CAD, Clear – PA – CAD, Clear – EGCG – CAD), plus 24 h sound dentin groups (Amb – Control – SD and Clear – Control – SD) were used to evaluate MMPs activity. These slices, obtained as previously described for other tests, were wet-polished down until 2500-grit SiC paper and 1 μm diamond paste (Buehler Ltd., Lake

Bluff, IL, USA). The specimens were covered with diluted self-quenched fluorescein-conjugated gelatine (D- 12060; Molecular Probes, Eugene, OR, USA), protected with a glass cover-slip and in a dark humid chamber at 37°C and incubation for 24 hours. Then, the specimens were examined using confocal laser microscopy (Leica SP5 CLSM, Heidelberg, Germany) equipped with a 63×/1.4 NA oil immersion lens using 468-nm laser illumination. The z-stack scans (one at each micrometer up to 20 μm below the surface) were compiled into single projections. To investigate the collagenolytic activity of the adhesive interface, images representing the MMP-activity along the bonded interfaces were captured.

2.10 Statistical analysis

The μTBS values from the same dentin surface at each storage period and group were averaged so that the statistical unit was the tooth, not the stick. For μTBS (MPa) data were submitted to the Shapiro-Wilk normality test, expressed as mean and standard deviation and analyzed by Student's t-test and ANOVA-1-way followed by Bonferroni post-test (parametric data). Sticks with premature were not included in the calculation of mean value for the tooth due to their low frequency in the experiment although the number of premature failures were recorded for posterior computation. The frequency of fracture was expressed as a relative frequency and analyzed by Fisher's exact test. Statistical calculations were performed with SPSS 20 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1 Microtensile bond strength (μTBS) and failure mode analysis

The results of μTBS can be observed in Table 2. For Ambar adhesive applied to SD, the incorporation of PA and EGCG did not influence the BS values after 24 hours whereas, at 12 months evaluation, the incorporation of EGCG resulted in a significant increase of the BS ($p = 0.022$) compared with the control and PA groups. When applied to CAD, the incorporation of PA or EGCG showed no effect on the BS, regardless of the evaluation time. When comparing the BS in SD x CAD, it was observed that there was no significant difference between the groups, except for the incorporation of EGCG which showed a decrease in CAD after 12 months ($p = 0.006$).

For Clearfil SE Bond adhesive, applied to SD or CAD, the incorporation of PA and EGCG showed no effect on the BS after 24 hours or after 12 months. When

comparing the BS in SD x CAD, only a statistically significant decrease for CAD was observed for the PA ($p = 0.01$) and EGCG ($p = 0.038$) groups after 12 months of water storage.

No group showed a statistical difference in the comparison at different times (24 h x 12 M) regardless of the type of dentin used (SD x CAD).

The failure mode showed a high predominance of adhesive/mixed failures for most of the groups as can be seen in Table 3.

3.2 Nanoleakage Evaluation (NL)

Representative backscattering SEM images of the adhesive interface produced in all conditions tested can be seen for Ambar (Fig. 2) and for Clearfil SE Bond (Fig. 3). Evaluation of the SEM images allowed to state that all experimental groups, in both times of evaluation, has silver nitrate deposition on the hybrid layer. When dentin was affected by caries, a pattern of superficial disorganized dentin can be seen (Fig. 2 and Fig. 3 D, E, F, J, K, L), although practically same silver nitrate deposition along the adhesive interface was observed in comparison to SD groups (Fig. 2 A, B, C, G, H, I and Fig. 3 A, B, C, G, H, I).

3.3 In situ zymography

Confocal laser scanning microscopy images of resin-dentin interfaces are shown in Fig. 4. There is a difference in gelatinolytic activity between SD and CAD, once that CAD has a stronger intensity of green fluorescence. Between CAD groups (Fig. 4 B, C, D, F, G, and H), rather a difference in MMPs activation was noticed for PA incorporated to Ambar (Fig .4C) where less MMP´s activity may be detected at hybrid layer. For all groups, gelatinolytic activity was mainly observed within dentin tubules and slightly on exposed collagen surfaces.

4. Discussion

This study aimed to assess the effect of two natural crosslinkers, incorporated into two different adhesives - one 2- step self-etch and other universal, applied at a self-etching way, when applied to SD or CAD, on 24 hours or 12 months. The null hypothesis was

partially rejected, once the results of this research have shown that adhesion to SD is superior to that in CAD after 12 M. Also, the incorporation of EGCG in Ambar Universal applied at SD exhibited an increase in bond strength even after water storage for 12 M. On the other hand, water storage period did not show any effect on BS of the adhesive interface. However, until now, there is no consensus about the minimal period of water storage that promotes expressive degradation in the hybrid layer [16]. So, maybe increasing time-lapse for 18 or 24 months a difference between control and incorporated groups could be seen.

The present study worked with caries-affected dentin. It is well known that this tissue has the ability of remineralization and should be preserved [26]. However, its configuration, which is more disorganized, wet, porous and that has different levels of collagen alteration and demineralization [1,7], make the adhesion of current adhesive systems to this surface a real challenge. This substrate is an obstacle because of the previous activation of MMPs, owing to the bacteria lactic fermentation, which leads to the liberation of acid products that lower the pH [10]. It is important to develop approaches that improve the performance of contemporary adhesives on this substrate in order to provide greater longevity to clinical adhesive restorations.

For this reason, the use of some natural delivered crosslinking agents, as proanthocyanidins (PA) and epigallocatechin-3-gallate (EGCG), has been defended and researched [15,19,27–29]. Many studies conclude that they are able to enhance the dentin mechanical properties [13,16,17], reduce the biodegradation of this tissue by the host-derived matrix metalloproteinases [30] and act as antioxidant agents, then preserving adhesive bond to dentin over time [31–33]. Concentrations of PA and EGCG chosen on this in vitro study was based to recent outcomes that up to 1% of PA can be added into a dental adhesive resin without interfering with the mechanical properties or solubility of the resins [29] and that EGCG concentrations of 1.0 wt% showed better biological and mechanical performance, preserved bond strength and adhesive interface and reduced cytotoxicity [20]. We performed incorporation by adding crosslinkers powder into commercial adhesives (into the primer, in case of Clearfil SE Bond). No water or ethanol was used to solubilize these agents in order to preserve the hydrophilicity of the adhesive systems and did not change their proportion of solvents.

A recent systematic review about bonding to CAD [34], as well as a current meta-analytic review [35], have shown that higher bond strengths may be seen at SD, irrespectively of adhesive approach. Lower immediate bond strength related to CAD is associated with lower mineral content, changes in collagen structure and irregular and deep tissue demineralization [1,35]. Besides, when hydroxyapatite is lost due to the carious process, the remaining spaces are filled by water, which alters water content from approximately 10% to approximately 14–53%, thus contributing to hydrolytic degradation [36].

However, in the present study, when SD x CAD comparison for an immediate time was made, no significant difference was noticed for both adhesive systems. We suppose that it can be due to the 10-MDP component action, present in this two adhesive approaches used in this experiment, which produces a stable chemical bonding to calcium ions of hydroxyapatite, even when the concentration of these ions is lower, and turns adhesive-dentin bonds reliable [37].

Nevertheless, after 12 months of water storage, for SD x CAD comparison, our study found out that adhesion to the carious substrate was not stable, once for almost all incorporated groups, the bond strength decreased significantly, except for Ambar – PA. Proanthocyanidins are capable of complex interactions with collagen, and it is believed that stabilization of the bond with hydrogen bridges established between protein amide carbonyl and PA phenolic hydroxyl [15]. Thus, they may support the stability of the collagen structure, increasing their resistance to enzymatic-hydrolytic degradation [27]. Also, PA can increase the resistance of collagen against collagenases *via* masking the cleavage sites of the collagen matrix what can explain the improvement of hybrid layer long-term stability [15,27].

The incorporation of PA did not show maintenance of bond strength for Clearfil SE Bond, when sound dentin was compared to caries-affected dentin, after 12 months of water storage. We believe that the environment created by the acid pH of this self-etching adhesive (pH=2.0) associated with the previously demineralized dentin by caries bacteria products may over activated MMPs, which can be caused a collagen matrix breakdown in a way that even PA, through their crosslinking mechanism, was not able to recover. Maybe, this behavior of combination of two-step self-etching adhesive with PA could not obtain a great long-term performance because a phase division, creating a hydrophobic layer that could enhance water sorption and, then, increased the hydrolytic degradation.

The use of 0.1% EGCG as a dentin pretreatment solution previously to a two-step etch-and-rinse adhesive was shown to maintain the bond strength of sound dentin after 6 months of water storage [31]. Adversely, Fialho et al 2019[18] tested different EGCG concentrations as a pretreatment of a two-step etch-and-rinse adhesive and concluded that EGCG was unable to reduce degradation of the adhesive interface to CAD over time (12 months), similarly to our study. On the other hand, in the present study, the incorporation of 1% EGCG or 1% PA, in both adhesives, was able to maintain the long-term bond strength on a level equal or superior to that of the control group, for SD. Besides, after 12 M, the incorporation of EGCG into Ambar Universal adhesive applied on SD showed higher MPa values when compared to results of control and PA incorporation on the same adhesive. The resin tags that occlude the dentinal tubules in addition to the adhesive resin coating collagen fluids and the adhesive layer overlying have been speculated to sequestrate the EGCG-soaked demineralized matrix from the interstitial fluids. This, in turn, causes prolonged retention and liberation of EGCG and thereby that MMPs are inhibited. However, this finding of greater EGCG performance incorporated in Ambar after 12 M was found in sound dentin and did not occur in CAD. This can be due to the disorganization of CAD tissue and the thicker hybrid layer, which may lead to lower bioavailability and release of EGCG.

To evaluate the activity and presence of MMPs, as well as the efficiency of inhibiting them by EGCG and PA, was performed the *in situ* zymography. Since self-etching adhesives were used, a relative enzymatic activity can be seen even in SD groups, due to the incorporation of acidic monomers present in these adhesives in the hybrid layer (Fig 4., A and E). In CAD groups the enzymatic activity was even more intense, due to the pre-activation of these enzymes by the acidic products released during the carious process, except for the Ambar incorporated with PA, where a decrease in intensity and the frequency of fluorescence can be observed. It seems that PA is compatible with the formulation of Ambar Universal. Once that PA is highly hydroxylated and belongs to the condensed tannins category, it is difficult to hydrolyze it [38]. So, rather than solubilize at the water of Ambar Universal content, PA stayed attach to CAD being released and thus inactivating MMPs.

5. Conclusion

Within the limitation of this study, we can conclude that the effect of incorporating crosslinkers depends on the adhesive used and the substrate type. The incorporation of the crosslinking agents was able to maintain the bond strength over the 12 M period compared to the 24 h interval, regardless of the dentin type and the adhesive used. The incorporation of PA in Ambar Universal maintained the bond strength values as well as showed a decrease in enzymatic activity in CAD, which was not observed for EGCG. All groups presented silver deposits at the adhesive interface.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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References

[1] Joves GJ, Inoue G, Nakashima S, Sadr A, Nikaido T, Tagami J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. Dent Mater J 2013;32:138–43. doi:10.4012/dmj.2012-243.

[2] Erhardt MCG, Osorio R, Toledano M. Dentin treatment with MMPs inhibitors does not alter bond strengths to caries-affected dentin. J Dent 2008;36:1068–73. doi:10.1016/j.jdent.2008.09.002.

[3] Pinna R, Maioli M, Eramo S, Mura I, Milia E, R. P, et al. Carious affected dentine: its behaviour in adhesive bonding. Aust Dent J 2015;60:276–93. doi:10.1111/adj.12309 LK

[4] Nakabayashi, N., Kojima, K., Matsuhara E. Promotion of adhesion by infiltration monomers into tooth substrates. J Biomed Mat Res 1982;16:265–73.

[5] Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003;28:215–35.

[6] Perdigão J, Reis A, Loguercio AD, J. P, A. R, A.D. L, et al. Dentin adhesion and MMPs: a comprehensive review. J Esthet Restor Dent 2013;25:219–41.

doi:10.1111/jerd.12016 LK

[7] Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, Cenci MS. MMP inhibitors on dentin stability: A systematic review and meta-analysis. J Dent Res 2014;93:733–43. doi:10.1177/0022034514538046.

[8] Mazzoni A, Tjäderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res 2015;94:241–51. doi:10.1177/0022034514562833.

[9] Cai J, Palamara JEA, Burrow MF. Effects of Collagen Crosslinkers on Dentine: A Literature Review. Calcif Tissue Int 2018;102:265–79. doi:10.1007/s00223-017-0343-7.

[10] Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998;77:1622–9. doi:10.1177/00220345980770081001.

[11] Mazzoni A, Apolonio FM, Saboia VPA, Santi S, Angeloni V, Checchi V, et al. Carbodiimide inactivation of MMPs and effect on dentin bonding. J Dent Res 2014;93:263–8. doi:10.1177/0022034513516465.

[12] Kalaiselvam R, Ganesh A, Rajan M, Kandaswamy D. Evaluation of bioflavonoids on the immediate and delayed microtensile bond strength of self-etch and total-etch adhesive systems to sound dentin. Indian J Dent Res 2018;29:133–6. doi:10.4103/ijdr.IJDR_284_17.

[13] Neri JR, Neri JR, Yamauti M, Feitosa VP, Pires APM, Dos Santos Araújo R, et al. Physicochemical properties of a methacrylate -based dental adhesive incorporated with epigallocatechin -3 -gallate. Braz Dent J 2014;25:528–31. doi:10.1590/0103-6440201300096.

[14] Islam MS, Hiraishi N, Nassar M, Yiu C, Otsuki M, Tagami J. Effect of hesperidin incorporation into a self-etching primer on durability of dentin bond. Dent Mater [Internet]. 2014 Nov;30(11):1205–12.

[15] Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, et al. Dentin biomodification: Strategies, renewable resources and clinical applications. Dent Mater 2014;30:62–76. doi:10.1016/j.dental.2013.10.012.

[16] Carvalho C, Fernandes FP, Freitas V da P, Franca FMG, Basting RT, Turssi CP, et al. Effect of green tea extract on bonding durability of an etch-and-rinse adhesive system to caries-affected dentin. J Appl Oral Sci 2016;24:211–7. doi:10.1590/1678-775720150518.

[17] de Macedo FAA, Souza NO, Lemos MVS, De-Paula DM, Santiago SL, Feitosa VP. Dentin bonding and physicochemical properties of adhesives incorporated with epigallocatechin-3-gallate. Odontology 2019;107:23–8. doi:10.1007/s10266-018-0367-0.

[18] Fialho MPN, Hass V, Nogueira RP, França FMG, Turssi CP, Basting RT, et al. Effect of epigallocatechin-3- gallate solutions on bond durability at the adhesive interface in caries-affected dentin. J Mech Behav Biomed Mater 2019;91:398–405. doi:10.1016/j.jmbbm.2018.11.022.

[19] Costa CAG de, Passos VF, Neri JR, Mendonça JS, Santiago SL. Effect of Metalloproteinase Inhibitors on Bond Strength of a Self-etching Adhesive on Erosively Demineralized Dentin. J Adhes Dent 2019;21:337–44. doi:10.3290/j.jad.a42930.

[20] Fonseca BM, Barcellos DC, Silva TM da, Borges ALS, Cavalcanti B das N, Prakki A, et al. Mechanical-physicochemical properties and biocompatibility of catechin-incorporated adhesive resins. J Appl Oral Sci 2019;27:1–11. doi:10.1590/1678-7757-2018-0111.

[21] Hass V, Luque-Martinez IV, Gutierrez MF, Moreira CG, Gotti VB, Feitosa VP, et al. Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition. Dent Mater 2016;32:732–41. doi:10.1016/j.dental.2016.03.008.

[22] Czech R, Oliveira CAR, Franca FMG, Basting RT, Turssi CP, Amaral FLB. Incorporation of EGCG into an etch-and-rinse adhesive system: mechanical properties and bond strength to caries affected dentin. J Adhes Sci Technol 2019;33:2430–42. doi:10.1080/01694243.2019.1642978.

[23] MacEdo G V., Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res 2009;88:1096–100. doi:10.1177/0022034509351001.

[24] Maske TT, Isolan CP, van de Sande FH, Peixoto AC, Faria-e-Silva AL, Cenci MS, et al. A biofilm cariogenic challenge model for dentin demineralization and dentin bonding analysis. Clin Oral Investig 2015;19:1047–53. doi:10.1007/s00784-014-1331-1.

[25] Wong L, Sissions CH. A comparison of human dental plaque microcosm biofilms grown in an undefined medium and a chemically defined artificial saliva. Arch Oral Biol 2001;46:477–86. doi:10.1016/S0003-9969(01)00016-4.

[26] Mazzoni A, Tjäderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res 2015;94:241–51. doi:10.1177/0022034514562833.

[27] Balalaie A, Rezvani MB, Mohammadi Basir M. Dual function of proanthocyanidins as both MMP inhibitor and crosslinker in dentin biomodification: A literature review. Dent Mater J 2018;37:173–82. doi:10.4012/dmj.2017-062.

[28] Costa AR, Garcia-Godoy F, Correr-Sobrinho L, Naves LZ, Raposo LHA, de Carvalho FG, et al. Influence of different dentin substrate (Caries-affected, caries-infected, sound) on long-term μTBS. Braz Dent J 2017;28:16–23. doi:10.1590/0103-6440201700879.

[29] Epasinghe DJ, Yiu CKY, Burrow MF. Mechanical properties, water sorption characteristics, and compound release of grape seed extract-incorporated resins. J Appl Oral Sci 2017;25:412–9. doi:10.1590/1678-7757-2016-0448.

[30] Vidal CMP, Tjäderhane L, Scaffa PM, Tersariol IL, Pashley D, Nader HB, et al. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. J Dent Res

2014;93:269–74. doi:10.1177/0022034513516979.

[31] Santiago SL, Neri JR, Osorio R, Toledano M, de Carvalho RM. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. J Adhes Dent 2013;15:535–40. doi:10.3290/j.jad.a29532.

[32] da Silva APP, Gonçalves RS, Borges AFS, Bedranrusso AK, Shinohara MS. Effectiveness of plant-derived proanthocyanidins on demineralization on enamel and dentin under artificial cariogenic challenge. J Appl Oral Sci 2015;23:295–301. doi:10.1590/1678-775720140304.

[33] Castellan CS, Bedran-Russo AK, Antunes A, Pereira PNR. Effect of dentin biomodification using naturally derived collagen cross-linkers: one-year bond strength study. Int J Dent 2013;2013:918010. doi:10.1155/2013/918010.

[34] Ekambaram M, Yiu CKY, Matinlinna JP. Bonding of resin adhesives to caries-affected dentin - A systematic review. Int J Adhes Adhes 2015;61:23–34. doi:10.1016/j.ijadhadh.2015.04.011.

[35] Isolan CP, Sarkis-Onofre R, Lima GS, Moraes RR. Bonding to sound and caries-affected dentin: A systematic review and meta-analysis. J Adhes Dent 2018;20:7–18. doi:10.3290/j.jad.a39775.

[36] Ito S, Saito T, Tay FR, Carvalho RM, Yoshiyama M, Pashley DH. Water content and apparent stiffness of non-caries versus caries-affected human dentin. J Biomed Mater Res - Part B Appl Biomater 2005;72:109–16. doi:10.1002/jbm.b.30130.

[37] Wang R, Shi Y, Li T, Pan Y, Cui Y, Xia W. Adhesive interfacial characteristics and the related bonding performance of four self-etching adhesives with different functional monomers applied to dentin. J Dent 2017;62:72–80. doi:10.1016/j.jdent.2017.05.010.

[38] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009;2:270–8. doi:10.4161/oxim.2.5.9498.

FIGURES

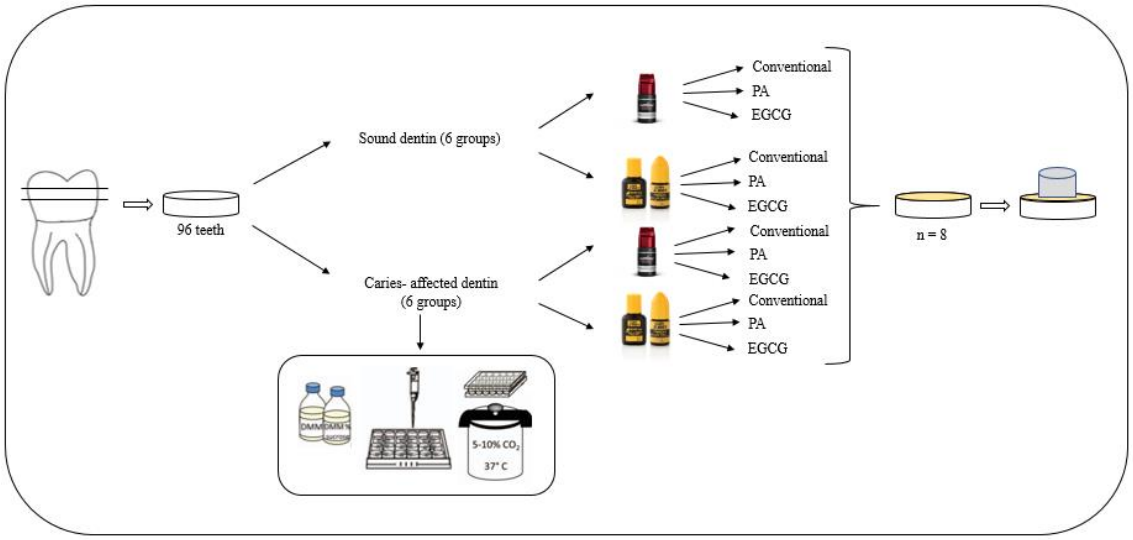

Figure 1. Squematic representation of the experimental protocol

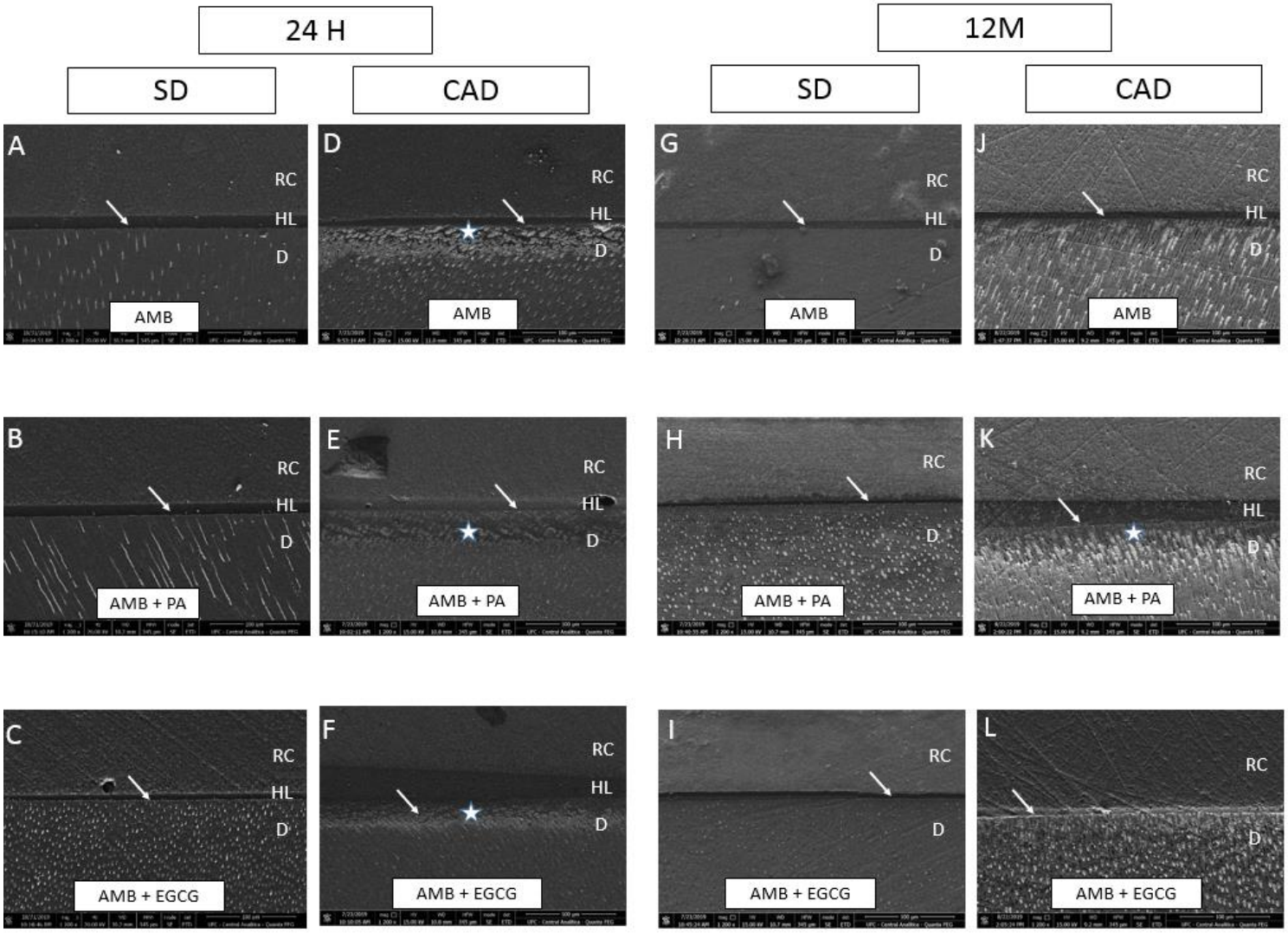

Figure 2. Representative backscattered SEM images of the resin-dentin interfaces for Ambar Universal in sound dentin (A, B, C, G, H, I) or CAD (D, E, F, J, K, L), in initial period (A to F) or after 12 months of water storage (F to L), with PA incorporation (B, E, H, K) or EGCG incorporation (C, F, I, L) or none (A, D, G, J) Usually CAD surface was irregular (star) while sound dentin was flat and uniform. The hybrid layer was generally thicker in CAD than in sound dentin. All groups showed silver nitrate infiltration (White arrows). Legend: RC: resin composite; HL: hybrid layer; D: dentin; AMB: Ambar; PA: proanthocyanidin; EGCG: Epigallocatechin-3-gallate; SD: sound dentin; CAD: caries-affected dentin. Arrows indicate silver nitrate penetration (magnification: 1200x)

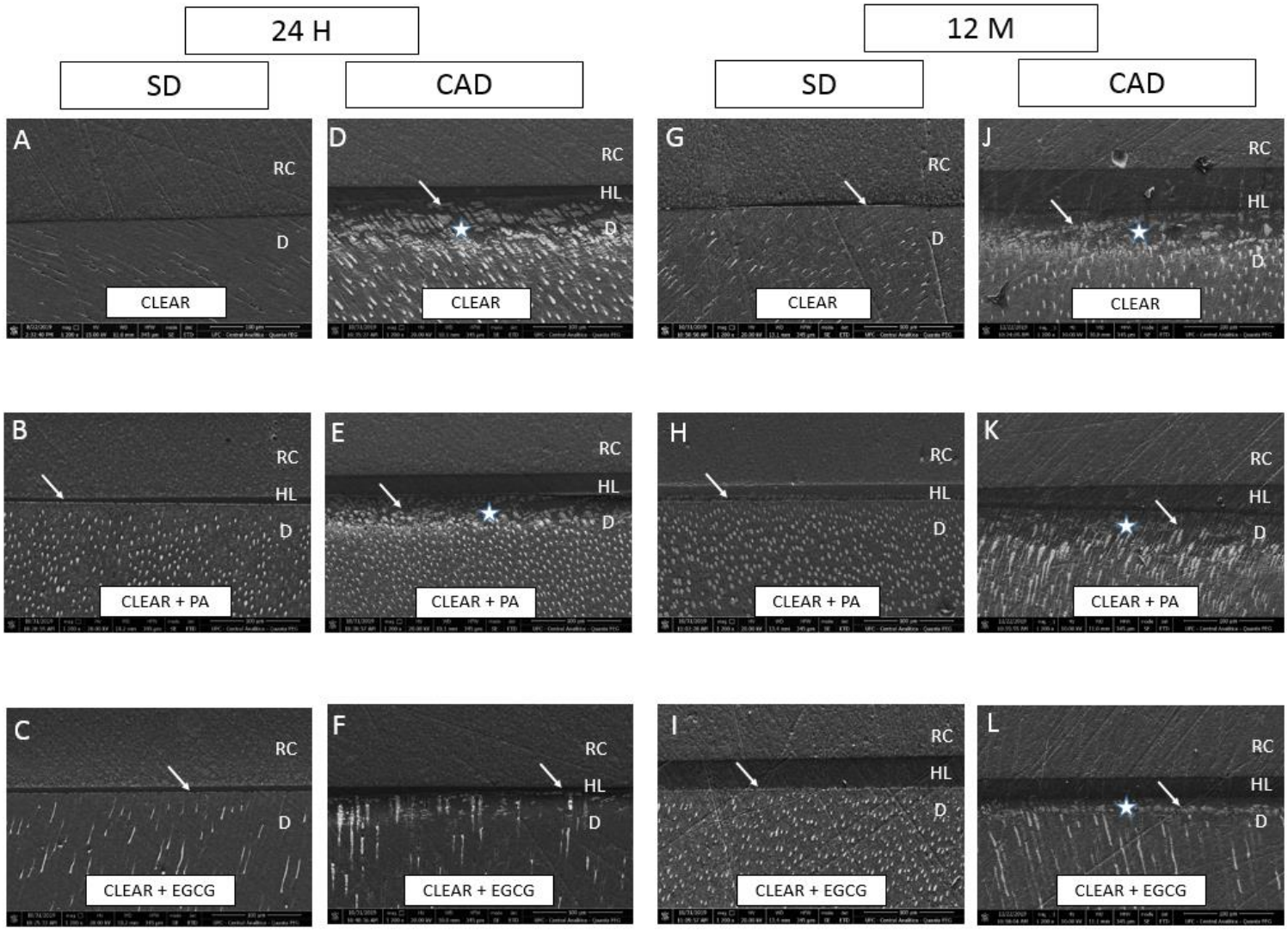

Figure 3. Representative backscattered SEM images of the resin-dentin interfaces for Clearfil SE Bond in sound dentin (A,B,C,G,H,I) or CAD (D,E,F,J,K,L), in initial period (A to F) or after 12 months of water storage (F to L), with PA incorporation (B,E,H,K) or EGCG incorporation (C,F,I,L) or none (A,D,G,J) Usually CAD surface was irregular (star) while sound dentin was flat and uniform. The hybrid layer was generally thicker in CAD than in sound dentin. All groups showed silver nitrate infiltration (White arrows). Legend: RC: resin composite; HL: hybrid layer; D: dentin; AMB: Ambar; PA: proanthocyanidin; EGCG: Epigallocatechin-3-gallate; SD: sound dentin; CAD: caries-affected dentin. Arrows indicate silver nitrate penetration (magnification: 1200x)

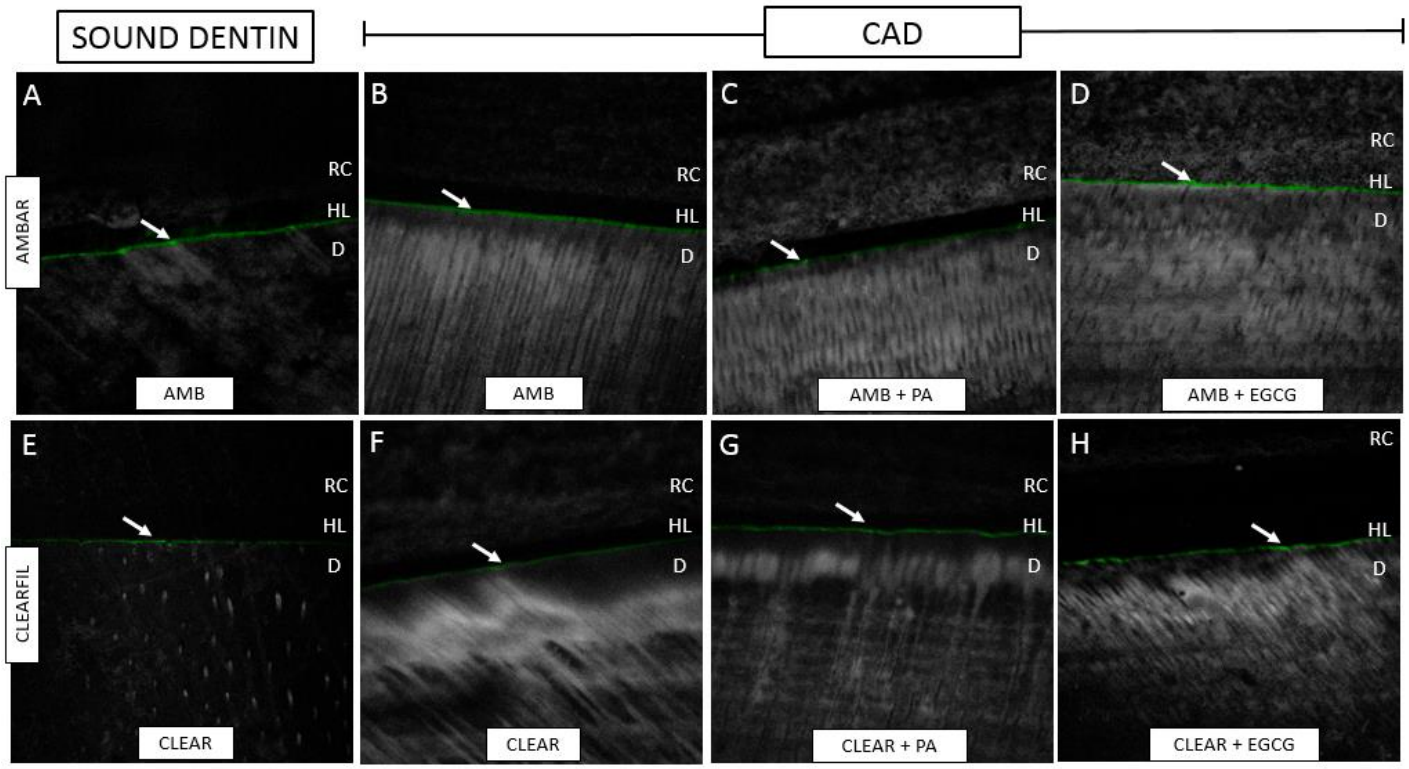

Figure 4. Representative confocal laser scanning microscopy images of the resin-dentin adhesive interface formed by experimental adhesives and SD (A and E) and CAD (B, C, D, F, G and H). Green fluorescence was observed at all representative groups and represents MMP´s activity (White arrows). It can be noticed that CAD groups have naturally increased the intensity of MMP´s activity due to their activation by acidic products released during caries stimulation. Original images were edited by Photoshop aiming better illustration of MMP´s activity at bond-interface. Legend: RC: resin composite; HL: hybrid layer; D: dentin; AMB: Ambar; PA: proanthocyanidin; EGCG: Epigallocatechin-3-gallate; SD: sound dentin; CAD: caries-affected dentin.

TABLES

Table 1. Main materials, composition, groups and application mode

Materials # batch number	Composition	Groups	Application mode
Ambar Universal – FGM / #260917 **pH = 2.5 – 3.0**	Methacrylate monomers (UDMA and 10-MDP), photoinitiators, co-initiators, stabilizers, inert sílica nanoparticles and ethanol	Amb - Control – SD Amb - Control – CAD Amb - PA – SD Amb - PA – CAD Amb - EGCG – SD Amb - EGCG – CAD	1. Apply two coats vigorously by rubbing the adhesive for 20 s (10 s each); 2. Gently air-dry for 10 s; 3. Light cure for 10 s
Clearfil SE Bond – Kuraray/ #9L0509 **pH = 2.0**	Primer: MDP, HEMA, water, photoinitiator Bond: MDP, BisGMA, HEMA, TEGDMA, hydrophobics dimethacrylates, photoinitiator	Clear - Control – SD Clear - Control – CAD Clear - PA – SD Clear - PA – CAD Clear - EGCG – SD Clear - EGCG – CAD	1. Actively apply the primer for 20 s; 2. Gently air-dry; 3. Apply bond; 4. Light cure for 10 s
Proanthocyanidin from grape seed (Vitis vinifera, >90%, Meganatural Gold, Madera, USA)		Amb - PA – SD Amb - PA – CAD Clear - PA – SD Clear - PA – CAD	
Epigallocathequin-3-gallate from green tea (95%,	Epigallocathequin-3-gallate (C22H18O11)	Amb - EGCG – SD Amb - EGCG – CAD	

Sigma Aldrich, Saint Louis, MO, USA)		Clear - EGCG – SD Clear - EGCG – CAD	

Abbreviations: BisGMA: bisphenol-A-diglycidylmethacrylate; HEMA: hydroxyethylmethacrylate; MDP: 10-methacryloyloxi-decyl-phophate; TEGDMA: triethylene-glycol-dimethacrylate; SD – Sound dentin; CAD: Caries-affected dentin.

Table 2. Means and standard deviation of μTBS (MPa) for all experimental groups.

	SD			CAD			SD vs CAD	
	24 H	12M	p-Value[a]	24 H	12M	p-Value[a]	24 H p-Valor[a]	12M p-Value[a]
Ambar Universal								
Control	17,52±10,09	25,15±6,06	0,185	21,23±4,96	29,44±5,69	0,108	0,446	0,360
PA	22,10±5,81	28,52±8,87	0,169	23,97±7,53	24,09±9,57	0,982	0,640	0,451
EGCG	29,05±13,41	40,18±9,38*	0,126	21,59±7,79	23,68±4,36	0,608	0,266	***0,006***
p-Valor[b]	0,206	***0,022***		0,758	0,516			
Clearfil SE Bond								
Control	32,82±13,21	40,74±12,31	0,308	24,26±10,09	35,65±10,18	0,174	0,254	0,560
PA	35,37±11,24	50,95±15,54	0,075	27,11±5,12	26,75±6,57	0,918	0,132	***0,010***
EGCG	31,63±13,15	42,42±14,18	0,223	32,83±10,15	23,61±4,72	0,096	0,863	***0,038***
p-Valor[b]	0,871	0,430		0,275	0,092			

*p<0,05 versus Conventional; [a]t Student´s test; [b]ANOVA-1-way/Bonferroni

Table 3. The number of specimens according to fracture mode for experimental groups.

	SD								CAD							
	24 H				12 M				24 H				12 M			
	TS	A/M	C	PF	TS	A/M	C	PF	TS	A/M	C	PF	TS	A/M	C	PF
Ambar																
Control	35	71%	14%	14%	29	72%	7%	21%	46	76%	13%	11%	54	67%	17%	17%
PA	42	79%	5%	17%	41	78%	12%	10%	57	81%	5%	14%	46	74%	0%	26%
EGCG	39	79%	15%	5%	38	87%	11%	3%	49	78%	4%	18%	40	68%	5%	28%
Clearfil																
Control	48	69%	25%	6%	38	50%	50%	0%	48	73%	15%	13%	35	71%	14%	14%
PA	46	76%	24%	0%	42	45%	55%	0%	69	97%	1%	1%	66	80%	15%	5%
EGCG	55	71%	29%	0%	45	60%	27%	13%	71	87%	4%	8%	63	81%	6%	13%

Abbreviations: SD: sound dentin; CAD: caries-affected dentin; TS: total sticks; A/M: adhesive/mixed failure; C: Cohesive fracture; PF: premature failures; PA: proanthocyanidin; EGCG: epigallocatechin-3-gallate

CONCLUSÃO

CONCLUSÃO GERAL

A partir da realização da revisão sistemática, foi possível perceber que a literatura carece de estudos que avaliam a manutenção da resistência de união promovida pela utilização de agentes de ligação cruzada naturais aplicados em dentina cariada.

A incorporação de inibidores naturais - proantocianidina 1% e epigalocatequina-3-galato 1% (em peso) – em adesivos autocondicionantes e universais, aplicados em dentina sadia, mostrou ser uma boa alternativa na manutenção da adesão nesses substratos em longo prazo (12 meses).

A incorporação de EGCG no adesivo Ambar Universal, aplicado em dentina sadia, mostrou os melhores resultados em longo prazo, inclusive aumentando a resistência de união a esse substrato. A incorporação de PA nesse adesivo também se mostrou benéfica, visto que conseguiu manter a adesão, tanto em dentina sadia quanto cariada.

Após 12 meses de armazenamento em água, pôde-se observar que a adesão em dentina sadia obteve melhores resultados que em dentina cariada, sendo a exceção o desempenho da incorporação de PA no adesivo Ambar, que se comportou de forma semelhante nos dois substratos.

As incorporações dos inibidores de MMPs no adesivo Clearfil SE Bond manteve a resistência de união, quando aplicadas em dentina hígida. Porém, em dentina cariada, após o armazenamento em água, houve um descréscimo na adesão, quando comparado aos valores obtidos em dentina sadia sob mesmas condições de incorporação e tempo de estocagem.

REFERÊNCIAS

ALBUQUERQUE N, NERI JR, LEMOS M, YAMAUTI M, DE SOUSA F, SANTIAGO SL. Effect of Polymeric Microparticles Loaded With Catechin on the Physicochemical Properties of an Adhesive System. Oper Dent. 2019 Jul/Aug;44(4):E202-E211. doi: 10.2341/18-112-L. Epub 2019 Mar 8.

BEDRAN-RUSSO AK, PASHLEY DH, AGEE K, DRUMMOND JL, MIESCKE KJ. Changes in stiffness of demineralized dentin following application of collagen crosslinkers. J Biomed Mater Res B Appl Biomater. 2008 Aug;86(2):330-4.

BRESCHI L, MAZZONI A, RUGGERI A, CADENARO M, DI LENARDA R, DE STEFANO DORIGO E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008 Jan;24(1):90-101. Epub 2007 Apr 17.

CARVALHO C, FERNANDES FP, FREITAS VDA P, FRANÇA FM, BASTING RT, TURSSI CP, AMARAL FL. Effect of green tea extract on bonding durability of an etch-and-rinse adhesive system to caries-affected dentin. J Appl Oral Sci. 2016 May-Jun;24(3):211-7. doi: 10.1590/1678-775720150518.

COSTA CAG, PASSOS VF, NERI JR, MENDONÇA JS, SANTIAGO SL. Effect of Metalloproteinase Inhibitors on Bond Strength of a Self-etching Adhesive on Erosively Demineralized Dentin. J Adhes Dent. 2019;21(4):337-344. doi: 10.3290/j.jad.a42930.

SILVA AP, GONÇALVES RS, BORGES AF, BEDRAN-RUSSO AK, SHINOHARA MS. Effectiveness of plant-derived proanthocyanidins on demineralization on enamel and dentin under artificial cariogenic challenge. J Appl Oral Sci. 2015 May-Jun;23(3):302-9. doi: 10.1590/1678-775720140304.

DU X, HUANG X, HUANG C, WANG Y, ZHANG Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent. 2012 Jun;40(6):485-92. doi: 10.1016/j.jdent.2012.02.013. Epub 2012 Mar 13.

EPASINGHE DJ, YIU CK, BURROW MF, TAY FR, KING NM. Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength. J Dent. 2012 Mar;40(3):173-80. doi: 10.1016/j.jdent.2011.11.013. Epub 2011 Nov 28.

DE MACEDO FAA, SOUZA NO, LEMOS MVS, DE-PAULA DM, SANTIAGO SL, FEITOSA VP. Dentin bonding and physicochemical properties of adhesives incorporated with epigallocatechin-3-gallate. Odontology. 2019 Jan;107(1):23-28. doi: 10.1007/s10266-018-0367-0. Epub 2018 May 23.

FANG M, LIU R, XIAO Y, LI F, WANG D, HOU R, CHEN J. Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds. J Dent. 2012 Jun;40(6):458-66. doi: 10.1016/j.jdent.2012.02.008. Epub 2012 Feb 22.

FIALHO MPN, HASS V, NOGUEIRA RP, FRANÇA FMG, TURSSI CP, BASTING RT, AMARAL FLB. Effect of epigallocatechin-3- gallate solutions on bond durability at the adhesive interface in caries-affected dentin. J Mech Behav Biomed Mater. 2019 Mar;91:398-405. doi: 10.1016/j.jmbbm.2018.11.022. Epub 2018 Nov 30.

FONSECA BM, BARCELLOS DC, SILVA TMD, BORGES ALS, CAVALCANTI BDN, PRAKKI A, OLIVEIRA HPM, GONÇALVES SEP. Mechanical-physicochemical properties and biocompatibility of catechin-incorporated adhesive resins. J Appl Oral Sci. 2019 Jan 7;27:e20180111. doi: 10.1590/1678-7757-2018-0111.

HASS V, DE PAULA AM, PARREIRAS S, GUTIÉRREZ MF, LUQUE-MARTINEZ I, DE PARIS MATOS T, BANDECA MC, LOGUERCIO AD, YAO X, WANG Y, REIS A. Degradation of dentin-bonded interfaces treated with collagen cross-linking agents in a cariogenic oral environment: An in situ study. J Dent. 2016 Jun;49:60-7. doi: 10.1016/j.jdent.2016.02.009. Epub 2016 Apr 19.

ISLAM S, HIRAISHI N, NASSAR M, YIU C, OTSUKI M, TAGAMI J. Effect of natural cross-linkers incorporation in a self-etching primer on dentine bond strength. J Dent. 2012 Dec;40(12):1052-9. doi: 10.1016/j.jdent.2012.08.015. Epub 2012 Sep 11

ISLAM MS, HIRAISHI N, NASSAR M, YIU C, OTSUKI M, TAGAMI J. Effect of hesperidin incorporation into a self-etching primer on durability of dentin bond. Dent Mater. 2014 Nov;30(11):1205-12. doi: 10.1016/j.dental.2014.08.371. Epub 2014 Sep 5.

ISOLAN CP, SARKIS-ONOFRE R, LIMA GS, MORAES RR. Bonding to Sound and Caries-Affected Dentin: A Systematic Review and Meta-Analysis. J Adhes Dent. 2018;20(1):7-18. doi: 10.3290/j.jad.a39775.

JOVES GJ, INOUE G, NAKASHIMA S, SADR A, NIKAIDO T, TAGAMI J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. Dent Mater J. 2013;32(1):138-43.

KHAMVERDI Z, REZAEI-SOUFI L, ROSTAMZADEH T.The Effect of Epigallocatechin Gallate on the Dentin Bond Durability of Two Self-etch Adhesives. J Dent (Shiraz). 2015 Jun;16(2):68-74.

KIM GE, LEME-KRAUS AA, PHANSALKAR R, VIANA G, WU C, CHEN SN, PAULI GF, BEDRAN-RUSSO A. Effect of Bioactive Primers on Bacterial-

Induced Secondary Caries at the Tooth-Resin Interface. Oper Dent. 2017 Mar/Apr;42(2):196-202. doi: 10.2341/16-107-L. Epub 2016 Nov 28.

LIU Y, TJÄDERHANE L, BRESCHI L, MAZZONI A, LI N, MAO J, PASHLEY DH, TAY FR.
Limitations in bonding to dentin and experimental strategies to prevent bond degradatio n. J Dent Res. 2011 Aug;90(8):953-68. doi: 10.1177/0022034510391799. Epub 2011 Jan 10.

MARSHALL GW JR, MARSHALL SJ, KINNEY JH, BALOOCH M.
The dentin substrate: structure and properties related to bonding. J Dent. 1997 Nov;25(6):441-58.

MASKE TT, ISOLAN CP, VAN DE SANDE FH, PEIXOTO AC, FARIA-E-SILVA AL, CENCI MS, MORAES RR.
A biofilm cariogenic challenge model for dentin demineralization and dentin bonding a nalysis. Clin Oral Investig. 2015 Jun;19(5):1047-53. doi: 10.1007/s00784-014-1331-1. Epub 2014 Oct 17.

MASKE TT, VAN DE SANDE FH, ARTHUR RA, HUYSMANS MCDNJM, CENCI MS. In vitro biofilm models to study dental caries: a systematic review. Biofouling. 2017 Sep;33(8):661-675. doi: 10.1080/08927014.2017.1354248. Epub 2017 Aug 9.

MAZZONI A, APOLONIO FM, SABOIA VP, SANTI S, ANGELONI V, CHECCHI V, CURCI R, DI LENARDA R, TAY FR, PASHLEY DH, BRESCHI L.
Carbodiimide inactivation of MMPs and effect on dentin bonding. J Dent Res. 2014 Mar;93(3):263-8. doi: 10.1177/0022034513516465. Epub 2013 Dec 11.

MAZZONI A, MANNELLO F, TAY FR, TONTI GA, PAPA S, MAZZOTTI G, DI LENARDA R, PASHLEY DH, BRESCHI L.
Zymographic analysis and characterization of MMP-2 and - 9 forms in human sound dentin. J Dent Res. 2007 May;86(5):436-40.

MAZZONI A, ANGELONI V, APOLONIO FM, SCOTTI N, TJÄDERHANE L, TEZVERGIL-MUTLUAY A, DI LENARDA R, TAY FR, PASHLEY DH, BRESCHI L. Effect of carbodiimide (EDC) on the bond stability of etch-and-rinse adhesive systems. Dent Mater. 2013 Oct;29(10):1040-7. doi: 10.1016/j.dental.2013.07.010. Epub 2013 Aug 3.

MAZZONI A, PASHLEY DH, NISHITANI Y, BRESCHI L, MANNELLO F, TJÄDERHANE L, TOLEDANO M, PASHLEY EL, TAY FR.
Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials. 2006 Sep;27(25):4470-6. Epub 2006 May 9.

NAKABAYASHI N, KOJIMA K, MASUHARA E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res. 1982 May;16(3):265-73.

NISHITANI Y, YOSHIYAMA M, WADGAONKAR B, BRESCHI L, MANNELLO F, MAZZONI A, CARVALHO RM, TJÄDERHANE L, TAY FR, PASHLEY DH. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci. 2006 Apr;114(2):160-6.

PEIXOTO AC, BICALHO AA, ISOLAN CP, MASKE TT, MORAES RR, CENCI MS, SOARES CJ, FARIA-E-SILVA AL. Bonding of Adhesive Luting Agents to Caries-affected Dentin Induced by a Microcosm Biofilm Model. Oper Dent. 2015 May-Jun;40(3):E102-11. doi: 10.2341/14-169-L. Epub 2015 Jan 9.

PERDIGÃO J, REIS A, LOGUERCIO AD. Dentin adhesion and MMPs: a comprehensive review. J Esthet Restor Dent. 2013 Aug;25(4):219-41. doi: 10.1111/jerd.12016. Epub 2013 Feb 19.

PINNA R, MAIOLI M, ERAMO S, MURA I, MILIA E. Carious affected dentine: its behaviour in adhesive bonding. Aust Dent J. 2015 Sep;60(3):276-93. doi: 10.1111/adj.12309. Epub 2015 Jul 29.

SANON K, SANCHAVANAKIT N, SRISAWASDI S. Grape Seed Extract Reduces Active Gelatinases Using an Etch-and-Rinse Mode Universal Adhesive. J Adhes Dent. 2019;21(2):159-165. doi: 10.3290/j.jad.a42306.

SANTIAGO SL, OSORIO R, NERI JR, CARVALHO RM, TOLEDANO M. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. J Adhes Dent. 2013 Dec;15(6):535-40. doi: 10.3290/j.jad.a29532.

SIGNORI C, VAN DE SANDE FH, MASKE TT, DE OLIVEIRA EF, CENCI MS. Influence of the Inoculum Source on the Cariogenicity of in vitro Microcosm Biofilms. Caries Res. 2016;50(2):97-103. doi: 10.1159/000443537. Epub 2016 Feb 27.

TJÄDERHANE L, LARJAVA H, SORSA T, UITTO VJ, LARMAS M, SALO T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res. 1998 Aug;77(8):1622-9.

TJÄDERHANE L, NASCIMENTO FD, BRESCHI L, MAZZONI A, TERSARIOL IL, GERALDELI S, TEZVERGIL-MUTLUAY A, CARRILHO MR, CARVALHO RM, TAY FR, PASHLEY DH. Optimizing dentin bond durability: control of collagen degradation by matrix metallopr oteinases and cysteine cathepsins. Dent Mater. 2013 Jan;29(1):116-35. doi: 10.1016/j.dental.2012.08.004. Epub 2012 Aug 16.

TJÄDERHANE L, NASCIMENTO FD, BRESCHI L, MAZZONI A, TERSARIOL IL, GERALDELI S, TEZVERGIL-MUTLUAY A, CARRILHO M, CARVALHO RM, TAY FR, PASHLEY DH. Strategies to prevent hydrolytic degradation of the hybrid layer-A review. Dent Mater. 2013 Oct;29(10):999-1011. doi: 10.1016/j.dental.2013.07.016. Epub 2013 Aug 14.

VAN DE SANDE FH, AZEVEDO MS, LUND RG, HUYSMANS MC, CENCI MS. An in vitro biofilm model for enamel demineralization and antimicrobial dose-response studies. Biofouling. 2011 Oct;27(9):1057-63. doi: 10.1080/08927014.2011.625473.

VISSE R, NAGASE H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003 May 2;92(8):827-39.

ANEXOS

ANEXO A – PARECER DE APROVAÇÃO CONSUBSTANCIADO NO COMITÊ DE ÉTICA EM PESQUISA DA UFC – COMEPE

UFC - UNIVERSIDADE FEDERAL DO CEARÁ /

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do efeito do uso de agentes crosslinkers na dentina cariada por modelo multiespécie

Pesquisador: Deborah Cavalcante Magalhães Rolim

Área Temática:

Versão: 2

CAAE: 99289218.0.0000.5054

Instituição Proponente: Departamento de Odontologia Restauradora

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.048.195

Apresentação do Projeto:

O projeto trata-se de uma pesquisa laboratorial e quantitativa que envolverá coleta de saliva e dentes de seres humano. Visando diminuir a degradação da interface de união resina-dentina, muitos estudos buscam métodos para estabilizar e preservar a integridade dessa interface. Agentes crosslinkers, tais como a proantocianidina (PA) e a epigalocatequina-3-galato (EGCG), têm sido objeto de diversas pesquisas atuais devido seu potencial efeito inibidor de MMPs, mostrando efeitos positivos na resistência à biodegradação, propriedades mecânicas e estabilidade estrutural da dentina. Contudo, a maioria dos estudos são realizados em dentina hígida, substrato raramente encontrado na prática restauradora. Um modelo de indução de cárie in vitro intitulado microcosmo torna a condição do substrato dentinário mais semelhante às demandas clínicas. Sendo assim, o presente estudo tem como objetivo avaliar in vitro o efeito dos agentes crosslinkers PA 1% e EGCG 0,1%, incorporados aos adesivos Clearfil SE Bond e Ambar Universal, na estabilidade de restaurações adesivas em dentina previamente cariada por modelo microcosmo, imediatamente e após 6 meses de armazenamento em água destilada. Serão selecionados 72 terceiros molares humanos, os quais terão sua dentina superficial exposta. Os dentes serão aleatorizados e distribuídos em grupos definidos de acordo com os seguintes critérios: aplicação de adesivo conforme as recomendações do fabricante ou aplicação de adesivo incorporado com PA 1% ou aplicação de adesivo incorporado com EGCG 0,1%; adesivo Ambar Universal ou adesivo Clearfil SE Bond; dentina hígida ou dentina afetada por cárie induzida por modelo de biofilme microcosmo.

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Esse biofilme será cultivado dentro de condições de atmosfera anaeróbica por 14 dias em meio enriquecido de mucina. O mesmo meio acrescido de 1% de sacarose será alternado com o meio original durante 4h por dia. Após a aplicação do adesivo, serão construídos platôs de resina composta Z350XT (3M-ESPE) de forma incremental. Após 24 horas de armazenamento em água destilada a 37ºC os dentes serão seccionados para o teste de microtração. Os palitos de cada grupo serão subdivididos em dois subgrupos: teste imediato e teste após 6 meses de armazenamento em água destilada. Será observado, então, o padrão de fratura dos espécimes. 2 palitos aleatórios por grupo serão selecionados para análise de nanoinfiltração. Um dente por grupo será preparado para zimografia in situ da interface resina-dentina. Os dados serão tabulados e avaliados segundo o teste estatístico ANOVA Three-way pareado e post-hoc de Bonferroni (p 0,05). Com base nos trabalhos in vitro já publicados onde a incorporação de PA e EGCG no primer resultaram em melhor desempenho dos sistemas adesivos utilizados, espera-se que o mesmo efeito seja observado no modelo de dentina afetada por cárie. Caso fique comprovado que o uso destes agentes melhora a estabilidade das restaurações nesse substrato adverso, testes in vivo utilizando a mesma metodologia deverão ser realizados.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar in vitro o efeito da incorporação de agentes de ligação cruzada em dois sistemas adesivos autocondicionantes na resistência de união da interface formada por esses adesivos e dentina afetada por cárie, gerada por modelo microcosmo, imediatamente após a restauração ou após o envelhecimento por armazenamento em água.

Objetivo Secundário:

• Comparar a resistência de união entre resina e dentina afetada por cárie com a resistência de união entre resina e dentina hígida, após 24 horas.

• Comparar a resistência de união entre resina e dentina afetada por cárie com a resistência de união entre resina e dentina hígida, após 6 meses de armazenamento em água destilada.

• Comparar a resistência de união entre resina e dentina afetada por cárie na qual foi aplicado adesivo autocondicionante incorporado com agentes de ligação cruzada com a resistência de união entre resina e dentina afetada por cárie na qual foi aplicado adesivo autocondicionante original, após 24 horas e após 6 meses de armazenamento em água destilada.

• Quantificar a inibição da atividade proteolítica das MMPs pelos adesivos incorporados com os agentes de ligação cruzada através de zimografia in situ da interface de união.

• Avaliar o padrão de fratura dos espécimes após o teste de resistência ao microcisalhamento.

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• Comparar os graus de nanoinfiltração entre os grupos controle e os grupos tratamento, após 24 horas e após 6 meses de armazenamento em água destilada.

Avaliação dos Riscos e Benefícios:

Riscos: Este estudo implica em risco mínimo para a saúde do participante. Há risco dos indivíduos doadores de saliva permanecerem por 24h sem higiene oral.

Benefício:Essa pesquisa poderá contribuir como benefício indireto, com a melhor compreensão de agentes adesivos utilizados na clínica odontológica restauradora.

A incorporação dos agentes de ligação cruzada PA 1% e EGCG 0,1% em dois sistemas adesivos autocondicionantes (Clearfil SE Bond e Ambar Universal) pode conferir a esses materiais potencial efeito inibidor da atividade das MMPs, diminuindo a degradação das fibrilas de colágeno, preservando a interface adesiva e aumentando a resistência de união resina-dentina afetada por cárie.

Comentários e Considerações sobre a Pesquisa:

Trata-se de uma pesquisa laboratorial com a utilização de saliva e dentes humanos extraídos(terceiros molares hígidos), a qual irá avaliar in vitro o efeito dos agentes crosslinkers PA 1% e EGCG 0,1%, incorporados aos adesivos Clearfil SE Bond e Ambar Universal, na estabilidade de restaurações adesivas em dentina previamente cariada por modelo microcosmo, imediatamente e após 6 meses de armazenamento em água destilada.

Considerações sobre os Termos de apresentação obrigatória:

Os termos de apresentação obrigatória foram apresentados. Conforme solicitado a pesquisadora atualizou cronograma e esclareceu metodologia.

Recomendações:

Não se aplica.

Conclusões ou Pendências e Lista de Inadequações:

Não se aplica.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1060085.pdf	07/11/2018 16:34:11		Aceito

Projeto Detalhado / Brochura Investigador	Projeto_Deborah_versao2_06112018.docx	07/11/2018 13:04:48	Deborah Cavalcante Magalhães Rolim	Aceito
Cronograma	Cronograma_Deborah_versao2_06112018.PDF	07/11/2018 13:03:45	Deborah Cavalcante Magalhães Rolim	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Deborah_19092018.docx	19/09/2018 14:27:13	Deborah Cavalcante Magalhães Rolim	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Deborah.docx	19/09/2018 14:18:42	Deborah Cavalcante Magalhães Rolim	Aceito
Cronograma	Cronograma_Deborah.pdf	19/09/2018 14:18:29	Deborah Cavalcante Magalhães Rolim	Aceito
Outros	termo_doacao_dentes_humanos_Deborah.pdf	30/08/2018 13:14:43	Deborah Cavalcante Magalhães Rolim	Aceito
Outros	Deborah_cartadesolicitacao.pdf	13/08/2018 13:56:05	Deborah Cavalcante Magalhães Rolim	Aceito
Outros	Deborah_declaracaodeconcordancia.pdf	13/08/2018 13:52:13	Deborah Cavalcante Magalhães Rolim	Aceito
Declaração de Pesquisadores	Deborah_termodecompromisso.pdf	13/08/2018 13:47:31	Deborah Cavalcante Magalhães Rolim	Aceito
Orçamento	Deborah_declaracaodeorcamento.pdf	13/08/2018 13:47:00	Deborah Cavalcante Magalhães Rolim	Aceito
Folha de Rosto	Deborah_folhaderosto.pdf	13/08/2018 13:43:33	Deborah Cavalcante Magalhães Rolim	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

FORTALEZA, 30 de Novembro de 2018

Assinado por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador(a))

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