UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia

Tese

União adesiva à dentina sadia e dentina afetada por cárie

Cristina Pereira Isolan

Pelotas, 2016

Cristina Pereira Isolan

União adesiva à dentina sadia e dentina afetada por cárie

Tese apresentada, como requisito parcial, para obtenção do grau de Doutora em Odontologia (Materiais Odontológicos), Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas.

Orientador: Prof.Dr. Rafael Ratto de Moraes

Pelotas, 2016

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

I85u Isolan, Cristina Pereira

União adesiva à dentina sadia e dentina afetada por cárie / Cristina Pereira Isolan ; Rafael Ratto de Moraes, orientador. — Pelotas, 2016.

109 f.

Tese (Doutorado) — Programa de Pós-Graduação em Materiais Odontológicos, Faculdade de Odontologia, Universidade Federal de Pelotas, 2016.

1. Adesão. 2. Dentina hígida. 3. Dentina cariada. 4. Adesivos autocondicionantes. 5. Resistência de união. I. Moraes, Rafael Ratto de, orient. II. Título.

Black : D151

Elaborada por Fabiano Domingues Malheiro CRB: 10/1955

Cristina Pereira Isolan

União adesiva à dentina sadia e dentina afetada por cárie

Tese aprovada, como requisito parcial, para obtenção do grau de Doutora em Odontologia (Materiais Odontológicos), Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas.

Data da defesa: 25/02/2016

Banca examinadora:

Prof. Dr. Rafael Ratto de Moraes (Orientador)
Doutor em Materiais Dentários pela Universidade Estadual de Campinas

Prof. Dr. Eliseu Aldrighi Münchow
Doutor em Odontologia (Dentística) pela Universidade Federal de Pelotas

Prof[a]. Dr[a]. Francine Cardozo Madruga
Doutora em Odontologia (Materiais Odontológicos) pela Universidade Federal de Pelotas

Prof[a]. Dr[a]. Giana da Silveira Lima
Doutora em Odontologia (Dentística) pela Universidade Federal de Pelotas

Prof. Dr. Maximiliano Sérgio Cenci
Doutor em Odontologia (Cariologia) pela Universidade Estadual de Campinas

Prof. Dr. Fábio Garcia Lima (suplente)
Doutor em Odontologia (Dentística) pela Universidade Federal de Pelotas

Prof. Dr. Rafael Guerra Lund (suplente)
Doutor em Odontologia (Dentística) pela Universidade Federal de Pelotas

Agradecimentos

A Deus pela oportunidade de existir e me sentir abençoada, por tantas dádivas recebidas até hoje.

Aos meus pais, Tânia e Tomaz Isolan, exemplos profissionais e de amor incondicional. Deus foi muito bom comigo ao colocar dois grandes facilitadores no caminho da minha felicidade. Agradeço pelos ensinamentos de ética e respeito ao próximo, não só profissionalmente, mas no âmbito pessoal; aprendi com vocês a valorizar a qualidade das pessoas, sabendo enxergar o que cada uma tem de especial e único. Sou muito feliz e grata por ter a presença de pessoas amorosas e inspiradoras como vocês na minha vida.

Aos meus irmãos Cyro, Bruno e Camila, amigos que a vida me deu de brinde e com os quais tenho repetidos momentos de felicidade. Agradeço por se fazerem sempre presentes na minha vida.

À Universidade Federal de Pelotas (UFPel), por meio de seu Magnífico Reitor, Prof. Dr. Mauro Augusto Burkert Del Pino.

À Faculdade de Odontologia, na pessoa da Diretora Profa. Dra. Adriana Etges.

Ao Programa de Pós-Graduação em Odontologia, na pessoa do Coordenador do Curso Prof. Dr. Maximiliano Sérgio Cenci. Admiro sua dedicação ao programa, que se reflete nas conquistas de todo grupo.

À Capes pela bolsa durante o período de doutoramento.

Aos laboratórios que me acolheram e me possibilitaram executar esta tese. Inicialmente, agradeço ao Centro de Desenvolvimento e Controle de Biomateriais (CDC-Bio), localizado nas dependências da Faculdade de Odontologia da UFPel, por ter sido meu segundo lar e local de trabalho diário. À técnica do laboratório e amiga Tatiana Ramos, meu muito obrigada pela colaboração de todos os dias.

Ao laboratório de Microbiologia. Ao Prof. Dr. Rafael Guerra Lund que possibilitou a realização da minha pesquisa. Às funcionárias Carmem Lúcia Machado Lopes e Lizângela Ferreira sempre dispostas a me ajudar.

Ao Centro de Diagnóstico das doenças da boca, onde fiz toda a parte dos corantes. Às professoras do departamento de Patologia e à técnica do laboratório Ivana Hannemann Neves.

Ao Departamento de Morfologia, do Instituto de Biologia da UFPel, localizado no campus da Faculdade de Medicina desta Universidade.

Também agradeço à Universidade Federal do Rio Grande- FURG por possibilitar a realização das imagens de microscópio desta tese. Agradeço ao Centro de Microscopia Eletrônica da Zona Sul CEME-SUL.

Ao meu Orientador, Prof. Dr. Rafael Ratto de Moraes, agradeço primeiramente por ter aceitado o desafio de me orientar, sabendo da minha falta de experiência na área escolhida. Obrigada pelos ensinamentos, pela confiança, pelas oportunidades, pelo exemplo de profissional, pela paciência, pela delicadeza ao sanar minhas dúvidas e/ou curiosidades. Admiro e respeito muito sua capacidade de simplificar as coisas, tornando-as mais fáceis de entender. Obrigada por ter tornado este período de doutoramento menos estressante do que o esperado.

À Profª. Drª . Ana Carolina Uchoa, meus agradecimentos, pelos ensinamentos, pela dedicação e principalmente pelo convívio e exemplo de entrega à profissão.

À Diovana Dellagostin, minha querida professora de inglês de longa data, agradeço os ensinamentos, a dedicação e principalmente o incentivo frente a todos os desafios que me foram apresentados.

Aos professores com os quais convivi e com os quais tive a oportunidade de vivenciar o magistério nos estágios docentes. Agradeço à Profa. Dra. Giana da Silveira Lima, Prof. Dr. Cesar Henrique Zanchi, Prof. Dr. Evandro Piva e à técnica do laboratório de pré- clínica Dona Leda Caetano, sempre muito diponível.

Prof. Dr.Fabrício Ogliari, pela disponibilidade e exemplo profissional - levo na lembrança suas sínteses no laboratório, sempre realizadas com muito entusiasmo e afinco, grata pela inspiração.

Ao Rafael Sarkis Onofre, colega e amigo, obrigada pela sua dedicação em me ajudar na Revisão Sistemática. Foi um trabalho em equipe muito prazeiroso.

Aos meus colegas Adauê Oliveira, Aline Oliveira-Ogliari, Fernanda Leal, Hellen Lacerda, Mario Cruzeiro, Carmem Lemes, Carine Meereis, Wellington da Rosa, Sônia Peralta, Simone Oliveira, Andressa Goicochea, Ana Paula Gonçalves e tantos mais, por fazerem parte de um período tão importante da minha vida.

À colega Tamires Maske, foram muitas horas no laboratório, mas todas muito agradáveis e valiosas, graças à sua dedicação e amor ao que faz. Com você aprendi que, quando a gente faz o que gosta, o esforço sempre vale muito a pena.

À Gabriela Romanini Basso, colega sempre disponível a ajudar, obrigada pela sua parceria, amizade e pela companhia em nossas memoráveis viagens a Congressos.

À Marina Kaizer, obrigada pela ajuda e disponibilidade de sempre, admiro a sua dedicação e agradeço pelos ensinamentos.

Julia Kaster, aluna de iniciação científica, pelas horas comigo no Laboratório, por sua dedicação, responsabilidade e pontualidade. Espero que você leve para sua vida profissional a disciplina que conseguimos desenvolver no decorrer dos nossos ensaios.

Eliseu Aldrighi Münchow e Lisia Lorea Valente, presentes que o doutorado me deu, amigos para uma vida. Agradeço pelo convívio, pelas oportunidades, pelo aprendizado. Obrigada por tornarem tudo simples, por estarem sempre dispostos a me “salvar” e por fazerem tudo isso de forma delicada e amorosa. Com vocês aprendi e me diverti. Com vocês vivi uma Odontologia agradável e inspiradora e um doutorado que deixa saudade.

Ao Tiago Gadret, que ali do almoxarifado esteve sempre disposto a me ajudar e motivar com suas palavras. Obrigada pelo convívio na faculdade e fora dela.

Gostaria muito de agradecer ao Celaniro Junior, secretário do Programa de Pós-Graduação em Odontologia da UFPel, por estar sempre disposto a ajudar e fazê-lo com competência.

Enfim, a realização do meu trabalho de doutorado não é minha conquista, é de todos aqui listados e também daqueles porventura esquecidos. Muito obrigada!

Eu sei que por algum tempo vou seguir oscilante entre a razão e o desejo. Algumas decisões são tomadas com o coração inquieto e o pensamento tomado por muitas coisas que aconteceram e que acontecem, tudo misturado. Sei também que o tempo vai ser meu amigo para essas coisas da vida. Com coragem eu sigo, nessa velocidade que eu não temo, nem mesmo de ousar ser feliz.

FERNANDO PESSOA

Notas Preliminares

A presente tese foi redigida segundo o Manual de Normas para trabalhos acadêmicos da UFPel, adotando o nível de descrição em capítulos não convencionais. Disponível no endereço eletrônico:
http://sisbi.ufpel.edu.br/arquivos/PDF/Manual_Normas_UFPel_trabalhos_acad%C3%AAmicos.pdf

O projeto de pesquisa referente a esta Tese foi aprovado dia 25 de outubro de 2012, pela Banca Examinadora composta pelos Professores Doutores Maximiliano Sérgio Cenci, Noéli Boscato, Rafael Ratto de Moraes e Patrícia dos Santos Jardim (suplente).

Resumo

ISOLAN, Cristina Pereira. **União adesiva à dentina sadia e dentina afetada por cárie.** 2016. 109f. Tese (Doutorado em Odontologia). Programa de Pós-Graduação em Odontologia, Universidade Federal de Pelotas, Pelotas. 2016.

O objetivo deste trabalho, dividido em três estudos, foi avaliar a resistência de união de sistemas adesivos comerciais e experimentais a diferentes substratos, dando ênfase à dentina afetada por cárie (CAD), por ser um substrato bastante comum na prática clínica e ainda com muitos questionamentos a serem esclarecidos. Materiais e Métodos: No estudo 1, foi comparada a resistência de união entre um Adesivo Universal (Scotchbond Universal, 3 M ESPE) e outros Sistemas adesivos comerciais aplicados a diferentes substratos: esmalte, dentina hígida (SoD), resina composta e porcelana. Espécimes de cada substrato foram confeccionados para o teste de microtração (SoD e resina composta) ou teste de cisalhamento (esmalte e cerâmica). Os dados foram analisados por meio de ANOVA uma via e teste de Tukey (α = 0,05). No estudo 2 foi avaliada, por meio de uma revisão sistemática da literatura, a união de adesivos aplicados à SoD em comparação à CAD. Uma pesquisa no PubMed, Scopus e Web of Science foi realizada por dois revisores independentes. Foram identificados 2.260 artigos únicos e, após avaliação dos títulos/resumos, 65 artigos foram selecionados para leitura completa, dos quais 40 foram incluídos. Os dados foram extraídos e categorizados de acordo com o sistema adesivo utilizado (convencional ou autocondicionante). As médias de resistência de união de ambos substratos foram comparadas utilizando um modelo estatístico de efeitos randômicos (Review Manager Version 5.1). A heterogeneidade estatística foi testada usando teste I^2. Uma análise de subgrupo foi realizada considerando métodos de remoção de tecido cariado. O estudo 3 avaliou a resistência de união imediata, e após período de 6 meses, de adesivos experimentais autocondicionantes à SoD e CAD; tais adesivos continham três diferentes concentrações do monômero ácido GDMA-P. Biofilmes de microcosmos originados de saliva humana foram formados sobre discos de dentina e cultivados em anaerobiose por 14 dias. Doze grupos foram definidos com diferentes concentrações de GDMA-P (5%, 20% e 35%), tipo de dentina e período de estocagem. Discos de dentina bovina para cada grupo (n=10) foram incluídos em resina acrílica e o adesivo foi aplicado. Uma matriz de elastômero foi usada para obtenção de dois cilindros (diâmetro 1,5 mm, espessura 0,5 mm) de resina composta na superfície. Os cilindros foram submetidos ao teste de resistência de união após 24h e após 6 meses em uma máquina de Ensaio Universal. Os dados foram analisados por ANOVA e Student-Newman-Keuls (5%). Resultados: No estudo 1, os resultados foram satisfatórios para adesão a cerâmica, resina composta, esmalte e dentina, esta última tanto com a técnica de condicionamento total, como com a autocondicionante. No estudo 2, os sistemas adesivos convencional e autocondicionante aplicados à SoD apresentaram melhores resultados de resistência de união quando comparados aos da CAD. O método utilizado para a remoção de cárie também afetou a resistência de união. No estudo 3, a concentração de GDMA-P e o tipo de substrato interferiram na resistência de união à dentina. Conclusão: Variáveis na formulação dos adesivos interferem no seu desempenho de união a diferentes substratos. Estudos ainda devem ser realizados para se desenvolver uma formulação tida como ideal para substratos mais complexos como CAD.

Palavras-chave: adesão; adesivos; adesivos autocondicionantes; dentina cariada; dentina hígida; hibridização dentinária; polimerização; resistência de união; técnicas de microscopia.

Abstract

ISOLAN, Cristina Pereira. **Adhesive bonding to sound dentin and caries-affected dentin.** 2016. 109p. Thesis (PhD in Dentistry). Graduate Program in Dentistry. Federal University of Pelotas, Pelotas. 2016.

The main objective of this investigation, divided into three studies, was to assess the bonding strength of commercial and experimental adhesive systems to different substrates, emphasizing caries-affected dentin (CAD), as it is a fairly common substrate in clinical practice and with many doubts to be clarified. Materials and methods: Study 1, to compare the bonding ability of a universal dental adhesive (Scotchbond Universal, 3 M ESPE) to other commercial dental bonding agents applied to different substrates: enamel, sound dentin (SoD), resin composite, and porcelain. Specimens of each substrate were prepared for microtensile bond strength test/μTBS (SoD and composite) or shear bond test/SBS (enamel and porcelain). Data were analyzed using One-Way ANOVA and Tukey's test (α=0.05). In study 2, the adhesive performance (bonding ability) of dental adhesives applied to sound dentin (SoD) in comparison to caries-affected dentin (CAD) was evaluated through a systematic review of dental literature. A structured search in three international databases (Medline/PubMed, Scopus, and Web of Science) was carried out by two independent reviewers. A total of 2.260 unique articles were found. After excluding articles by title and reading of the abstract, 65 articles were selected for full-text reading, and 40 studies were included in the review. Data were extracted and categorized according to the adhesive system used (etch-and-rinse or self-etch adhesives). The bonding strength mean of the two substrates tested were compared using a randomized effects model (Review Manager Version 5.1). Statistical heterogeneity was tested by using I^2 test. A subgroup analysis was carried out to explore the heterogeneity considering the methods used for the removal of caries. Study 3 evaluated immediate and long term shear bonding strength of experimental self-etch adhesives, containing three different concentrations of the acidic monomer GDMA-P, to SoD and CAD. Microcosm biofilms were formed over dentin discs and cultivated under anaerobic conditions for 14 days. Twelve groups were defined by different GDMA-P concentrations (5%, 20%, and 35%), dentin type and storage time. Bovine dentin discs for each group (n=10) were included in acrylic resin and the adhesives were applied. An elastomer mold with cylindrical orifices (1.5 mm diameter) was used to obtain two cylinders of composite resin on the surface. After a period of 24h and 6 months the cylinders were subjected to bond strength test in a mechanical testing machine. Data were analysed by ANOVA and Student-Neuman-Keuls' test (5%). Results: In Study 1, the results were considered satisfactory for adhesion to ceramic, composite, enamel and dentin, and the latter for both the total etching and self-etching technique. In study 2, the conventional and self-etching adhesive systems applied to SoD showed better bond strength results compared to CAD. The method used for removing carious also affected the bond strength. In study 3, the bond strength to dentin varied with the concentration of GDMA-P and type of substrate. Conclusion: Variables in the formulation of adhesives have impact in their bonding performance to different substrates. Further studies are still necessary to develop an ideal formulation for more complex substrates such as CAD.

Key-words: bonding; adhesive; self-etch adhesive; caries-affected dentin; sound dentin; dentin hybridization; polymerization; bonding strength; microscopy techniques.

Sumário

1 Introdução

O sucesso dos procedimentos restauradores adesivos depende, entre outros fatores, da eficiência dos sistemas de união utilizados. As características estruturais e morfológicas dos substratos envolvidos na adesão têm papel importante no desempenho das restaurações, sendo fundamental o conhecimento do mecanismo de ação dos sistemas de união disponíveis no mercado, além do tipo de substrato dentário envolvido. Os sistemas adesivos utilizados em odontologia buscam simplificar a técnica de união e diminuir as dificuldades de adesão à dentina (PASHLEY et al., 2011), havendo inclusive materiais para aplicação em passo único em esmalte e dentina.

Dentro da odontologia minimamente invasiva já está estabelecida a possibilidade da remoção parcial de tecido cariado (MALTZ et al., 2007; MASSARA; ALVES; BRANDÃO, 2002; WAMBIER et al., 2007) sem interferência no sucesso do tratamento restaurador. No entanto, o uso de sistemas adesivos autocondicionantes ainda não é uma unanimidade, especialmente no Brasil. Nestes sistemas adesivos, ao contrário dos sistemas convencionais, não é feita a remoção total da *smear layer*; alternativamente, promove-se integração com a mesma.

Primers autocondicionantes contendo monômeros ácidos são aplicados sobre a dentina coberta pela *smear layer* sem a necessidade de posterior lavagem. Os autocondicionantes de passo único reúnem as etapas de condicionamento e infiltração do substrato em um único procedimento. Apesar destes sistemas de união serem comercializados como simplificados, devido ao menor número de passos de aplicação, eles são na realidade misturas relativamente complexas de monômeros resinosos hidrófilos e hidrófobos, monômeros ácidos, solventes, água e outros aditivos (REIS et al., 2007; TAY; PASHLEY, 2001).

Os testes de adesão em odontologia, em sua maioria, são realizados em dentina hígida (SoD). Sabe-se que na prática clínica, entretanto, o substrato geralmente encontrado é a dentina afetada por cárie (CAD) (PERDIGÃO, 2010). Este substrato, devido às modificações que sofre pelo processo carioso, pode influenciar o desempenho da adesividade dos materiais, resultando em menores valores de resistência de união na CAD em comparação com a SoD de dentes permanentes (SCHOLTANUS et al., 2010). No entanto, em sistemas adesivos convencionais, há relatos de que se consegue uma adequada adesão em dentina afetada por cárie (WEI et al., 2008; YOSHIYAMA et al., 2004; ZANCHI et al., 2010).

A presença da CAD na clínica diária faz dela um relevante substrato a ser estudado, também com sistemas adesivos autocondicionantes. O tratamento tradicional de dentes cariados envolve a remoção de todo o tecido efetivamente comprometido pelo processo carioso, para dar lugar aos materiais restauradores. A escavação da CAD envolvendo remoção além da camada externa infectada, considerada não remineralizada, pode sacrificar mais estrutura do que o necessário (NAKAJIMA et al., 2011; WEI et al., 2008).

Nos preparos cavitários, SoD e dentina alterada pelo processo de cárie coexistem. Materiais que utilizam esses tecidos como substrato para adesão deveriam apresentar satisfatório desempenho de retenção e de selamento a ambos. Entretanto, a resistência de união dos sistemas adesivos à CAD é menor quando comparada à da SoD (PASHLEY; CARVALHO, 1997; SAY et al., 2005).

A dentina acometida por cárie apresenta alterações em suas estruturas morfológica e histológica decorrentes do processo de desmineralização. Os componentes minerais de fosfato e carbonato de cálcio diminuem na região da dentina afetada por cárie, quando comparada à SoD, devido aos ciclos de desmineralização. Estas diferenças de estrutura e composição encontradas não interferem apenas no procedimento de condicionamento ácido, mas também na penetração dos monômeros resinosos na dentina desmineralizada, o que pode induzir a grandes diferenças na interface adesiva quando comparada à encontrada em dentina sadia (WANG; SPENCER; WALKER, 2007).

Após remoção da dentina infectada, o tecido residual, denominado dentina afetada por cárie, (FUSAYAMA et al.,1979; NAKAJIMA et al., 1995) apresenta a dentina intertubular menos mineralizada e com maior número de porosidades (NAKAJIMA et al., 2000; WANG; SPENCER; WALKER, 2007; YOSHIYAMA et al., 2000) além da presença de cristais de fosfato tricálcico ácido-resistentes, decorrentes da recristalização da apatita dissolvida, obliterando a entrada dos túbulos dentinários (OGAWA et al., 1983; WANG; SPENCER; WALKER, 2007).Alterações proteicas de alguma forma significantes para o mecanismo de adesão também podem ocorrer nesse substrato.

Proteoglicanos, localizados na superfície do colágeno e responsáveis pela ligação entre fibrilas adjacentes, são importantes proteínas relacionadas à manutenção da expansão da matriz de dentina na presença de água após sua desmineralização, devido à sua alta afinidade por essas moléculas (BRESCHI et al., 2003; RUGGERI et al., 2007). Na dentina afetada por cárie, entretanto, essas proteínas apresentam alterações moleculares tais que dificultam a manutenção da hidratação da matriz desmineralizada (SUPPA et al., 2006).

As fibrilas de colágeno no substrato cariado apresentam menor concentração de ligações cruzadas quando comparadas às fibrilas da dentina hígida (SPENCER et al., 2005; WANG; SPENCER; WALKER, 2007). Em conjunto, essas características favorecem a formação de zonas de hibridização mais espessas (ERHARDT; OSORIO; TOLEDANO, 2008; PEREIRA et al., 2006), porém com maior número de imperfeições em seu interior (ERHARDT; OSORIO; TOLEDANO, 2008), assim como maior zona de colágeno exposto em sua base devido à incompleta infiltração da resina adesiva (HASHIMOTO et al., 2000; YOSHIYAMA et al., 2002). Além de interferirem negativamente na resistência de união imediata, essas características tornam as interfaces produzidas sobre a CAD mais suscetíveis à degradação hídrica ao longo do tempo (ERHARDT; OSORIO; TOLEDANO, 2008).

Estudos vêm sendo realizados com a intenção de melhorar as propriedades mecânicas dos adesivos autocondicionantes, avaliando a seleção e concentração de monômeros ácidos e monômeros convencionais (LEAL et al., 2011), assim como a quantidade de água (LIMA et al., 2008, 2010) e de co-solvente contidos no sistema (FONTES et al., 2012), os quais podem interferir na longevidade da união adesiva à dentina. Entretanto, a grande maioria das investigações relacionadas à formulação ideal de sistemas de união é realizada considerando a adesão a tecidos hígidos, negligenciando o potencial efeito do substrato alterado na efetividade dos sistemas adesivos.

Portanto, é importante estudar o impacto da formulação de sistemas de união no desempenho destes, quando aplicados à dentina afetada por cárie. Nesse sentido, adesivos autocondicionantes apresentam características bastante adequadas para aplicação em dentina hígida, porém existem poucas certezas sobre o seu desempenho em substratos alterados.

Dessa forma, o objetivo geral do presente estudo foi investigar o comportamento de adesivos simplificados em diferentes substratos. Os objetivos específicos do presente estudo incluem:

1. Avaliar a resistência de união de um Adesivo Universal comercial em diferentes substratos (esmalte, dentina, resina composta, e cerâmica) em comparação com outros sistemas adesivos comerciais;
2. Avaliar, por meio de uma revisão sistemática da literatura, a resistência de união de sistemas adesivos aplicados à SoD em comparação à CAD;
3. Realizar a síntese de um monômero ácido, avaliar sistemas adesivos autocondicionantes experimentais com concentrações diferentes do monômero ácido e investigar o efeito dos adesivos preparados na resistência de união imediata e após período de 6 meses em SoD e CAD.

2 Capítulo 1

Bond strength of a universal bonding agent and other contemporary dental adhesives applied to enamel, dentin, composite, and porcelain[1]

Cristina P Isolan[1], Lisia L Valente[1], Gabriela R Basso[1], Alice H Pimentel[2], Júlia K Schwantz[2], Andreza V da Silva[2], Rafael R Moraes[1,2]

[1] Graduate Program in Dentistry, School of Dentistry, Federal University of Pelotas, Rua Gonçalves Chaves, 457, Pelotas 96015-560, RS, Brazil
[2] Developmental and Control Center of Biomaterials, Federal University of Pelotas, Rua Gonçalves Chaves, 457, Pelotas 96015-560, RS, Brazil

Corresponding author:
Prof. Rafael R. Moraes
Rua Gonçalves Chaves 457, School of Dentistry, Federal University of Pelotas, RS, Brazil, 96015-560. Tel/Fax: +55 53 3225-6741. E-mail: moraesrr@gmail.com

[1] Artigo publicado: Applied Adhesion Science 2014, 2:25, doi:10.1186/s40563-014-0025-x. O texto é aqui apresentado de acordo com as normas do periódico.

2.1 Abstract

The aim of this study was to compare the bonding ability of a universal dental adhesive (Scotchbond Universal/SBU, 3M ESPE) and other contemporary dental bonding agents applied to different substrates: enamel, dentin, resin composite, and porcelain. SBU was tested using both the etch-and-rinse/ER and self-etch/SE bonding approaches. The other adhesives tested were Scotchbond Multipurpose/SBMP (3M ESPE), Single Bond 2/SB (3M ESPE), and Clearfil SE Bond/CLSE (Kuraray). Specimens of each substrate were prepared for microtensile bond strength test/µTBS (dentin and composite) or shear/SBS test (enamel and porcelain). In composite and porcelain, negative (no treatment) and positive (silane+SB) control groups were tested. Data were analyzed using One-Way ANOVA and Tukey's test (α=0.05). In enamel, SBU resulted in similar SBS ($p \geq 0.458$) compared to all other adhesives (SBMP=19.0±10.2^{B}; SB=26.6±9.3^{A}; CLSE=26.0±8.5^{A}; SBU-SE=23.5±8.4^{AB}; SBU-ER=22.6±9.9^{AB}). In dentin, SBU showed similar results to all other materials ($p \geq 0.123$), except SB ($p \leq 0.045$), which showed the highest µTBS (SBMP=35.4±10.5^{AB}; SB=39.4±11.2^{A}; CLSE=36.6±10.9^{AB}; SB-SE=28.1±13.7^{B}; SBU-ER=26.9±7.4^{B}). In resin composite, SBU and the positive control presented similar µTBS ($p=0.963$), and were higher than the negative control ($p \leq 0.001$) (SBU=28.4±9.9^{A}; positive control=29.5±11.7^{A}; negative control=12.1±8.7^{B}). In porcelain, SBU had higher SBS than the positive control ($p=0.001$), which showed higher SBS ($p<0.001$) than the negative control (SBU=29.0±6.9^{A}; positive control=21.0±7.0^{B}; negative control=5.3±2.7^{C}). Equilibrium of adhesive and mixed failures occurred in dentin and resin composite, whereas a predominance of adhesive failures was observed in enamel and porcelain. In conclusion, the bonding ability of the universal adhesive was comparable to the other contemporary bonding agents tested, although it was dependent on the substrate evaluated. Universal adhesives seem to have potential applicability in adhesive dentistry.

2.2 Background

Adhesive bonding in dentistry is a process dependent on several factors, such as the type of substrate [1], type of adhesive substance(s) [2], humidity of the environment [3,4], and operator's ability in performing the bonding procedure [5]. With regard to the dental substrates, adhesive procedures are usually performed to achieve bond to dental enamel and dentin. Enamel is a highly-mineralized substrate constituted of almost 100 wt% of hydroxyapatite crystals, which do not require a wet surface during adhesive procedures for proper bonding. By contrast, dentin is a more complex substrate constituted of both mineral and organic phases (e.g., collagen fibrils), as well as water. Consequently, bonding to dentin is challenging because an ideal moisture condition should be maintained to avoid collapse of the collagen matrix and allow proper adhesive infiltration of the adhesive into the demineralized substrate [1,6].

Dental adhesive systems are commonly characterized by the application of three different substances that fill three distinct clinical steps: etching, priming, and bonding [7]. Etching corresponds to the application of an acid substance to demineralize the surface; priming is the preparation of the etched surface before application of the adhesive, and it is usually applied to dentin alone. Bonding is the application of the hydrophobic resin bond adhesive over enamel and dentin. Acid-etching might be a separate clinical step (etch-and-rinse technique approach [1]), or it might be produced by acidic functional monomers (self-etch materials) [2]. Despite their differences, both techniques have demonstrated long-lasting dental bonding results [1,2].

One of the most recent novelties in adhesive dentistry was the introduction of ‘universal’ or ‘multi-mode’ adhesives. These materials are simplified adhesives, usually containing all bonding components in a single bottle. Universal adhesives may be applied either in etch-and-rinse or self-etching bonding approaches, according to manufacturers’ claims. In addition, some universal adhesives may contain silane in their formulation, potentially eliminating the silanization step when bonding to glass ceramics or resin composites, for instance. Nevertheless, it is known that simplified materials are associated with lower *in vitro* bond strength results and poorer *in vivo* longevity of restorations [8-10]. These findings are probably a result of the complex formulation of simplified adhesives and their high content of solvents, which may impair complete solvent volatilization and consequently lead to poorer adhesive polymerization [11,12].

The aim of this study was to investigate the bonding ability of a universal dental adhesive to different dental substrates (enamel, dentin, composite, and porcelain) in comparison to other contemporary dental bonding agents. The hypothesis tested was that the universal adhesive would have similar bond strength results to the other adhesives irrespective of the substrate tested.

2.3 Methods

2.3.1 Study design

The design of this *in vitro* study is shown in Figure 1. Dental substrates (enamel and dentin) and material substrates (resin composite and porcelain) were used to investigate the bond strength performance of distinct bonding agents. The bonding agents tested were: the universal adhesive Scotchbond Universal/SBU (3M ESPE, St. Paul, MN, USA), the 3-step, etch-and-rinse Scotchbond Multipurpose/SBMP (3M ESPE), the 2-step, etch-and-rinse Single Bond 2/SB (3M ESPE), and the 2-step, self-etch Clearfil SE Bond/CLSE (Kuraray, Osaka, Japan). SBU was tested using both the etch-and-rinse and self-etch bonding approaches. When testing resin composite and porcelain, only SBU was investigated and compared to positive and negative control groups: the positive control was comprised of the application of silane (Silane, Dentsply, York, PA, USA) and SB, whereas the negative control was characterized by no prior treatment of substrates. Information about the pH (which was measured in triplicate using a pHmeter – Analion, model FM 608, Ribeirão Preto, SP, Brazil), manufacturer, lot number, composition, and directions of application of the bonding agents used are presented in Table 1. The response variables tested were bond strength (MPa) and failure mode, and the number of specimens tested in each group was 20.

2.3.2 Preparation of tooth substrates

Enamel and dentin specimens were obtained from fifty bovine incisors, which were properly cleaned, disinfected in 0.5% chloramine-T solution for seven days, and cut to remove the roots. All teeth specimens were randomly allocated into two groups according to the substrate to be tested: enamel or dentin. Enamel specimens were prepared for shear bond strength/SBS testing, i.e., the specimens were embedded in acrylic resin and then wet-ground at the buccal face using 600-grit silicon carbide (SiC) paper in order to standardize the smear layer [1]. Dentin specimens were prepared for microtensile bond strength/µTBS testing, i.e., the specimens were wet-ground using 600-grit SiC paper until exposure of medium dentin. Both enamel and dentin were acid etched with 37% phosphoric acid (Condac 37; FGM, Joinville, SC, Brazil) for 30 s and 15 s, respectively and rinsed with water for the same period of the acid-etching. Enamel was completely dried with compressed air, while dentin was kept moist (i.e., neither dry nor wet).

2.3.3 Preparation of resin composite and porcelain

Fifteen resin composite specimens were prepared by placing a microhybrid composite (Opallis; FGM – shade A3) into a silicone rectangular mold (18 × 10 mm; 3 mm thickness) using an incremental technique. Each increment was light-activated for 20 s with a light-emitting diode (LED) light-curing unit (Radii, SDI, Bayswater, VIC, Australia). The specimens were then prepared for µTBS testing following the same procedures described for dentin specimens.

Fifteen porcelain specimens (12 × 10 mm; 2.5 mm thickness) were obtained from feldspathic porcelain blocks for CAD/CAM (Vitablocks Mark II, Vita Zahnfabrik, Bad Säckingen, Germany). The blocks were cut using a water-cooled diamond saw (Isomet 1000, Buheler Ltd, Lake Bluff, IL, USA) at low speed. The specimens were then prepared for SBS testing following the same protocol described for the preparation of enamel specimens, except for the acid-etching step which was carried out using 10% hydrofluoric acid for 90 s (Condac Porcelana, FGM).

2.3.4 Bonding protocol

The bonding agents were applied according to the manufacturers' directions of application, which are shown in Table 1. Specimens prepared for SBS testing were prepared by inserting resin composite into a silicone mold containing four cylindrical orifices (1.5 mm in diameter, 0.5 mm in thickness) followed by light-activation for 20 s. The adhesive was light-activated for 20 s after positioning the mold onto the surfaces in order to delimitate the bonding area. Specimens prepared for µTBS testing were prepared by placing three increments of resin composite over the surfaces and light-activation for 20 s each increment. All specimens were stored in distilled water at 37°C, for 24 h, and then sectioned in two perpendicular directions to the bonded interface, resulting in beam-shaped specimens with approximately 0.8 mm^2 of transverse-sectional area.

2.3.5 Bond strength testing and failure mode analysis

After storage of all specimens in distilled water, for 24 h, the shear and microtensile bonding tests were carried out using a mechanical testing machine (DL500; São José dos Pinhais, PR, Brazil). While the specimens for SBS test were looped with a thin wire and tested under shear stress, the specimens for µTBS test were positioned in a specific jig and then tested under tensile stress [13]. Both SBS and μTBS tests were performed at a crosshead speed of 1 mm/min until failure, and the bond strength data were calculated in MPa.

After the test, all surfaces were examined using a light stereomicroscope at 40× magnification in an attempt to identify the failure patterns obtained after each bond strength test performed. Failure modes were classified as adhesive, cohesive in the substrate (enamel, dentin, original composite, or porcelain), cohesive in the composite restoration ('fresh composite' for resin composite substrate), or mixed.

2.3.6 Statistical analysis

The pH of adhesives as well as the bond strength data were analyzed with the statistical program SigmaPlot version 12 (Systat Software Inc., San Jose, CA, USA) using One-Way Analysis of Variance and Tukey's *post hoc* test for multiple comparisons ($\alpha=0.05$).

2.4 Results

2.4.1 pH of the adhesives

The pH of the four adhesives evaluated is shown in Table 1. The pH has decreased significantly in the following order: SB > SBMP (Primer) > SBU > CLSE ($p<0.001$).

2.4.2 Bond strength to enamel

The results of bond strength to enamel are presented in Table 2. SB and CLSE resulted in higher bond strength than SBMP ($p\leq0.018$), although similar to SBU and regardless of the etching approach used ($p\geq0.458$). SBU demonstrated similar SBS compared to all other adhesives ($p\geq0.145$).

2.4.3 Bond strength to dentin

The results of bond strength to dentin are shown in Table 3. SB had the highest bond strength, which was similar to CLSE and SBMP ($p\geq0.848$) and higher than SBU applied under both ER and SE techniques ($p\leq0.045$). SBU resulted in similar μTBS to CLSE and SBMP ($p\geq0.123$).

2.4.4 Bond strength to resin composite

The results of bond strength to resin composite are displayed in Table 3. SBU and the positive control resulted in similar µTBS results ($p=0.963$), which were higher than the negative control ($p \leq 0.001$).

2.4.5 Bond strength to porcelain

The results of bond strength to porcelain are shown in Table 2. SBU had higher SBS than the positive control ($p \leq 0.001$), and both showed higher bond strength than the negative control ($p \leq 0.001$).

2.4.6 Failure analysis

The failure modes results for all bond strength tests performed in the study is shown in Figure 2. In enamel, predominance of adhesive failures was observed in all groups (Figure 2a). In dentin, equilibrium of adhesive and mixed failures was detected (Figure 2b). In resin composite, while the negative control showed only adhesive failures, the positive control and SBU groups presented similar percentages of adhesive and mixed failures (Figure 2c). In porcelain, virtually all failures were adhesive in the negative control and in lower frequency in the other groups (Figure 2d).

2.5 Discussion

The type of substrate is one of the most important factors affecting the bonding performance of adhesives in dentistry [1]. The chemistry of the substrates, that might be dental tissues or restorative materials, may request the application of specific materials to allow a satisfactory and long-lasting bonding. Dentin, for instance, is naturally a complex and wet substrate, requiring the application of both hydrophilic and hydrophobic materials; enamel, on the other hand, requires the application of a hydrophobic material only, since its composition is almost exclusively inorganic [1,2]. In contrast, restorative materials such as resin composites and porcelains have a low reactive structure after curing/sintering, thus requiring the application of specific components to make their surface active again and prone to adhesion [14]. Some universal adhesives present a versatile formulation that may enable adhesion to any type of substrate, although the performance of universal adhesives tested to different substrates still needs further investigation.

Universal adhesives have the versatility of being applied to dental tissues either using etch-and-rinse/ER or self-etch/SE bonding approaches. Although SE adhesives are easier to apply and commonly less technique-sensitive than ER versions [2], it has been shown that both techniques may lead to appropriates dental bonding [1,2]. Results of the present study corroborate with those previous findings, since groups SBU-SE and SBU-ER had similar enamel and dentin bond strengths. Taking into consideration that the acid-etching with 37% phosphoric acid was the only difference between the groups, it can be suggested that the application of the acid as a separate clinical step is not essential to improve the bond strength results when using the universal adhesive tested herein. This may be due to the unique composition of SBU (Table 1): first, it is constituted of 10-MDP, which is a phosphate monomer that renders the adhesive an acidic character (in Table 1, SBU and CLSE, which are both 10-MDP-based adhesives, showed the lowest pH values), enabling simultaneous demineralization and monomer infiltration [2]; second, 10-MDP is a recognized monomer able to chemically interact with tooth minerals [2], improving the long-term stability of the adhesion formed; lastly, SBU is also comprised of a polyalkenoic acid copolymer (Vitrebond™ copolymer), which, according to the manufacturer, provides satisfactory bonding to dentin under moist or dry conditions [11].

In enamel, the universal adhesive showed similar bond strength to all the other adhesive systems investigated (Table 2), demonstrating that it would be a good option to promote adhesion between resin composites and enamel. Special attention should be addressed to the SBU-SE group, which involved in a single adhesive step of application, differently from the other adhesives. Indeed, the possibility of using an easy and faster bonding agent to satisfactorily bond to enamel and without compromising the adhesion outcome is still important and desired in dentistry [2]. However, it should be highlighted that the selective enamel etching clinical technique is still regarded as the most reliable approach to bond to dental enamel when using self-etch adhesives [15].

In dentin, the bond strength of the universal adhesive was similar to all bonding agents except SB. Considering that dentin is a challenging substrate for adhesion and that the universal SBU is comprised of a heterogeneous composition that mixes various different components into the same solution (e.g., acidic and non-acidic monomers, solvents, fillers, initiators, and silane – Table 1), the combination of these factors may have probably decreased the bonding ability of SBU to dentin. SB, on the other hand, has a less complex composition than SBU, thus allowing satisfactory adhesion, which is corroborated by several previous studies [16-19]. However, this study tested only the immediate bond strength to dentin, and it is known that etch-and-rinse adhesives tend to generate less stable dentin bonding as compared with self-etch adhesives [8].

In resin composite, SBU resulted in similar bond strength when compared to the positive control (i.e., the conventional protocol used to repair resin composite restorations – application of silane and adhesive). In porcelain, SBU showed the highest bond strength, which was higher than the positive control (i.e., application of silane and adhesive) as well as the negative control (no treatment). The repair process of restorative materials such as resin composites and porcelains can be performed by using several chemical substances and physical methods [14,20,21], although the most common procedure performed by dental practitioners is the application of silane prior to the adhesive material. Silane is a coupling agent that interacts with the inorganic glass fillers of resin composites [22]. Consequently, silane is usually applied on the surface of the composites during repairs, for instance. Silane could make the surface of the restorative active again and thus able to adhesively interact with the fresh repairing composite. In a similar fashion, silane is also used for bonding or repairing porcelains, but only after the prior application of hydrofluoric acid, which produces micro-retentions on the surface [23]. In the present study, SBU resulted in higher or similar bond strength when compared to the positive controls, irrespective of the substrate tested. This finding is likely a result of the silane molecule presented in SBU formulation, allowing proper chemical interaction with the glass phases of porcelain and composite.

The present findings demonstrated that the universal dental adhesive tested herein allowed satisfactory adhesion to different substrates of application as compared to the other contemporary agents tested. Findings of the failure analysis corroborate in showing similar performance between the adhesives investigated (Figure 2). It is important to note that SBU performed differently depending on the substrate, thus allowing only the partial acceptance of the study hypothesis.The present study had some limitations, including the immediate (24 h) testing only and absence of scanning electron microscopy analysis, which would have contributed to the understanding of the quality of the adhesive interfaces. Furthermore, the bonding ability of other universal adhesives on additional substrates (e.g., metals, sclerotic dentin, different types of ceramics, among others) still needs evaluation to confirm the universal applicability of these materials.

2.6 Conclusion

The bonding ability of the universal adhesive was comparable to the other contemporary dental bonding agents tested, although it was dependent on the substrate evaluated. Universal adhesives seem to have potential applicability in different areas of the adhesive dentistry.

2.7 Acknowledgements

The authors thank FGM Produtos Odontológicos for donation of the composite resin used in the study.

2.8 References

1. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A (2011) State of the art etch-and-rinse adhesives. Dent Mater 27:1-16.
2. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL (2011) State of the art of self-etch adhesives. Dent Mater 27:17-28.
3. Kanca J, 3rd (1997) One step bond strength to enamel and dentin. Am J Dent 10:5-8.
4. Münchow EA, Valente LL, Bossardi M, Priebe TC, Zanchi CH, Piva E (2014) Influence of surface moisture condition on the bond strength to dentin of etch-and-rinse adhesive systems. Braz J Oral Sci 13:182-186.
5. Giachetti L, Scaminaci Russo D, Bertini F, Pierleoni F, Nieri M (2007) Effect of operator skill in relation to microleakage of total-etch and self-etch bonding systems. J Dent 35:289-293.
6. Münchow EA, de Barros GD, Alves LS, Valente LL, Cava SS, Piva E, Ogliari FA (2014) Effect of elastomeric monomers as polymeric matrix of experimental adhesive systems: degree of conversion and bond strength characterization. Appl Adhes Sci 2. doi:10.1186/2196-4351-2-3.
7. Alex G (2012) Is total-etch dead? Evidence suggests otherwise. Compend Contin Educ Dent 33:12-4, 16-22, 24-25.

8. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B (2005) A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res 84:118-132.
9. Munoz MA, Sezinando A, Luque-Martinez I, Szesz AL, Reis A, Loguercio AD, Bombarda NH, Perdigao J (2014) Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives. J Dent 42:595-602.
10. Tuncer D, Yazici AR, Ozgunaltay G, Dayangac B (2013) Clinical evaluation of different adhesives used in the restoration of non-carious cervical lesions: 24-month results. Aust Dent J 58:94-100.
11. 3M ESPE. Scotchbond™ Universal Adhesive. Date access: November 25, 2014. Available from: http://http://multimedia.3m.com/mws/media/754753O/scotchbond-universal-adhesive.pdf?fn=scotchbond_uni_brochure.pdf.
12. Spencer P, Wang Y (2002) Adhesive phase separation at the dentin interface under wet bonding conditions. J Biomed Mater Res 62:447-456.
13. Münchow EA, Bossardi M, Priebe TC, Valente LL, Zanchi CH, Ogliari FA, Piva E (2013) Microtensile versus microshear bond strength between dental adhesives and the dentin substrate. Int J Adhes Adhes 46:95-99.
14. Costa TR, Ferreira SQ, Klein-Junior CA, Loguercio AD, Reis A (2010) Durability of surface treatments and intermediate agents used for repair of a polished composite. Oper Dent 35:231-237.
15. Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, Van Meerbeek B (2010) Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching. Dent Mater 26:1176-1184.
16. Arrais CA, Giannini M, Nakajima M, Tagami J (2004) Effects of additional and extended acid etching on bonding to caries-affected dentine. Eur J Oral Sci 112:458-64.
17. Pereira PN, Nunes MF, Miguez PA, Swift EJ, Jr. (2006) Bond strengths of a 1-step self-etching system to caries-affected and normal dentin. Oper Dent 31:677-681.
18. Singh UP, Tikku A, Chandra A, Loomba K, Boruah LC (2011) Influence of caries detection dye on bond strength of sound and carious affected dentin: An in-vitro study. J Conserv Dent 14:32-35.

19. Yoshiyama M, Urayama A, Kimochi T, Matsuo T, Pashley DH (2000) Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin. Oper Dent 25:163-169.
20. Hickel R, Brushaver K, Ilie N (2013) Repair of restorations--criteria for decision making and clinical recommendations. Dent Mater 29:28-50.
21. Valente LL, Münchow EA, Silva MF, Manso IS, Moraes RR (2014) Experimental methacrylate-based primers to improve the repair bond strength of dental composites - a preliminary study. Appl Adhes Sci 2. doi:10.1186/2196-4351-2-6
22. Matinlinna JP, Lassila LV, Ozcan M, Yli-Urpo A, Vallittu PK (2004) An introduction to silanes and their clinical applications in dentistry. Int J Prosthod 17:155-164.
23. Tian T, Tsoi JK, Matinlinna JP, Burrow MF (2014) Aspects of bonding between resin luting cements and glass ceramic materials. Dent Mater 30:e147-e162.

Table 1. Information of pH, manufacturer, lot number, composition, and directions of application of the adhesive materials investigated in the study.

Material pH [§]	**Manufacturer (Lot number)**	**Composition**	**Directions of application***
SBU pH=2.6 [c]	3M ESPE (1302800437)	MDP phosphate monomer, dimethacrylate resins, HEMA, polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	e; c; f (10 s)
SBMP pH=3.9 [b] (*Primer*)	3M ESPE (205453)	*Primer:* Polyalkenoic acid copolymer HEMA, water *Bond:* Bis-GMA, HEMA, tertiary amines, photo-initiator	a; b; c; d (20 s); c; e (10 s); f (10 s)
SB pH=4.2 [a]	3M ESPE (330843 BR)	Dimethacrylate resins, HEMA, polyalkenoic acid copolymer, filler, ethanol, water, initiators	a; b; c; e (10 s); c; (repeat 2-3 times steps “e” and “c”); f (10 s)
CLSE pH=1.4 [d]	Kuraray (01714-A)	*Primer:* MDP, dimethacrylate monomer, HEMA, silica, N,N-diethanol-p-toluidine, CQ *Bond:* HEMA, dimethacrylate monomer, Bis-GMA, N,N-diethanol-p-toluidine, silica, CQ	d; c; e; c; f (10 s)
Silane	Dentsply (802197F)	Silane, ethanol, acetic acid	g (15 s); h; i; c; (repeat steps “i” and “c”)

SBU: Scotchbond Universal; SBMP: Scotchbond Multipurpose; SB: Single Bond 2; CLSE: Clearfil SE Bond; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol A glycidyl methacrylate; CQ: camphorquinone.

* a: acid-etching (15 s in dentin/resin composite and 30 s in enamel); b: (rinsing with water for the same period of time of acid-etching); c: drying with compressed air; d: primer application; e: resin bond/adhesive application; f: light-activation; g: mix one drop of the primer and one drop of the activator; h: let the mixture rest for 5 minutes; i: silane application.

[§] Distinct superscript letters indicate statistically significant differences in pH ($p<0.05$).

Table 2. Shear bond strength means and standard deviation (±SD) for enamel and porcelain.

Substrate	SBMP	SB	CLSE	SBU		Positive control	Negative control
				SE	ER		
Enamel	19.0[b] (±10.2)	26.6[a] (±9.3)	26.0[a] (±8.5)	23.5[ab] (±8.4)	22.6[ab] (±9.9)		
Porcelain					29.0[a] (±6.9)	21.0[b] (±7.0)	5.3[c] (±2.7)

SBMP: Scotchbond Multipurpose; SB: Single Bond 2; CLSE: Clearfil SE Bond; SBU: Scotchbond Universal; SE: self-etch technique; ER: etch-and-rinse technique; Positive control: Silane plus SB; Negative control: no material.

Distinct letters in the same row indicate statistically significant differences ($p<0.05$).

Table 3. Microtensile bond strength means and standard deviation (±SD) for dentin and resin composite.

Substrate	SBMP	SB	CLSE	SBU		Positive control	Negative control
				SE	ER		
Dentin	35.4[ab] (±10.5)	39.4[a] (±11.2)	36.6[ab] (±10.9)	28.1[b] (±13.7)	26.9[b] (±7.4)		
Resin composite					28.4[a] (±9.9)	29.5[a] (±11.7)	12.1[b] (±8.7)

SBMP: Scotchbond Multipurpose; SB: Single Bond 2; CLSE: Clearfil SE Bond; SBU: Single Bond Universal; SE: self-etch technique; ER: etch-and-rinse technique; Positive control: Silane plus SB; Negative control: no treatment.

Distinct letters in the same row indicate statistically significant differences ($p<0.05$).

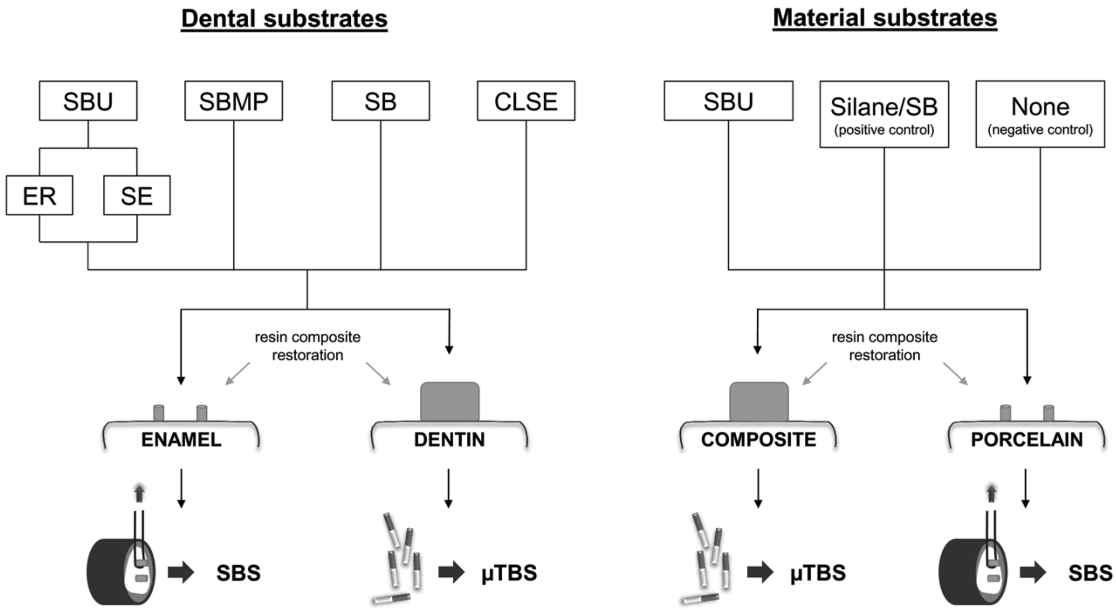

Figure 1. Experimental design of the study. SBU – Scotchbond Universal; ER – etch-and-rinse; SE – self-etch; SBMP – Scotchbond Multipurpose; SB – Single Bond 2; CLSE – Clearfil SE Bond; SBS – shear bond strength; and μTBS – microtensile bond strength.

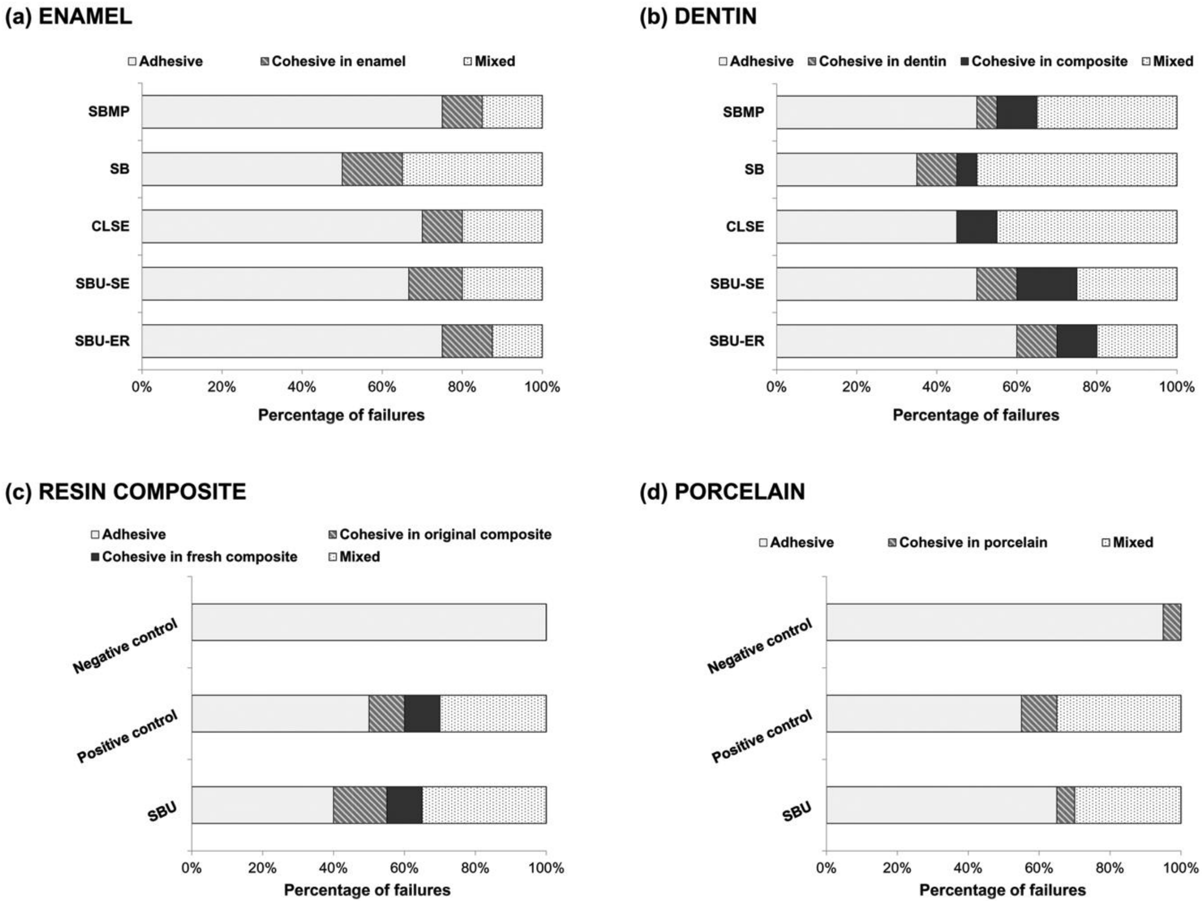

Figure 2. Failure patterns obtained after bond strength evaluation of the adhesive systems applied in enamel (a), dentin (b), resin composite (c), and porcelain (d).

3 Capítulo 2

Bonding to sound and caries-affected dentin: systematic review and meta-analyses[2]

Short title: Bonding to sound vs. caries-affected dentin

Cristina P. Isolan[a]/Rafael Sarkis-Onofre[a]/
Rafael R. Moraes[b]

[a]PhD student, Graduate Program in Dentistry, School of Dentistry, Federal University of Pelotas, Pelotas/RS, Brazil. Participated in the study design, data collection and analysis, drafted and proofread the manuscript.
[b]Assistant Professor, Graduate Program in Dentistry, Federal University of Pelotas, Pelotas/RS, Brazil. Participated in the study design and data analysis, drafted and proofread the manuscript.

Corresponding author:
Prof. Rafael R. Moraes
Rua Gonçalves Chaves 457, School of Dentistry, Federal University of Pelotas, RS, Brazil, 96015-560. Tel/Fax: +55 53 3225-6741. E-mail: moraesrr@gmail.com

[2] Artigo formatado segundo as normas do periódico Journal of Adhesive Dentistry.

3.1 Abstract

Purpose: This study systematically reviewed the literature to compare the bonding ability of dental adhesives applied to sound dentin (SoD) *vs.* caries-affected dentin (CAD).

Materials and Methods: Three international databases (Medline/PubMed, Scopus, and Web of Science) were searched. Eligible studies that evaluated the bond strength to both SoD and CAD were included. Random effects meta-analyses were conducted to calculate pooled mean difference between substrates, separately for etch-and-rinse and self-etch adhesives. Subgroup analyses were carried out to explore heterogeneity considering the methods used for removal of infected carious dentin. A comparison between etch-and-rinse and self-etch adhesives restricted to CAD was also carried out. Statistical heterogeneity was considered using I^2 test. Risk of bias of all included studies was assessed.

Results: In total, 2,260 articles were found, 65 were selected for full-text reading, and 40 studies were included. The meta-analyses favored SoD over CAD for both etch-and-rinse (effect size: -10.04; 95% confidence interval (CI):-11.94,-8.14; I^2=95%) and self-etch adhesives (effect size: -6.76; 95% CI:-8.23,-5.30; I^2=89%). In the subgroup analyses, SoD was favored irrespective of the method used for caries removal (effect size ≤-4.86; I^2≥28%): excavation (manual or with burs), grinding with abrasive papers, combination of more than one method, and when the method was not mentioned. The meta-analysis restricted to CAD favored etch-and-rinse over self-etch adhesives (effect size: 3.13; 95% CI:1.82,4.44; I^2=72%). Most included studies were judged as having unclear risk of bias.

Conclusion: Bonding to SoD is better compared to CAD. The performance of Etch-and-rinse adhesives is preferable to self-etch adhesives when applied to CAD.

3.2 Introduction

Structural and morphological characteristics of dental substrates involved in the adhesion of resin-based materials play important roles on the performance of dental restorations. Restorative treatments might last long clinically when the bonding mechanism of adhesive systems to dental tissues is known and procedures are carried out properly. Current dental adhesive approaches seek to simplify the bonding technique and reduce difficulties of bonding to dentin,[54] which is still considered the weakest link in dental adhesion.

In vitro testing of dental adhesives usually involves the use of sound dentin (SoD) as bonding substrate. It is known, however, that caries-affected dentin (CAD) is a more frequent substrate for bonding in the clinical practice. Changes caused by the caries process, such as loss of mineral content, increased porosity of intertubular dentin,[44] dissolution of apatite mineral crystals,[5,52] and degradation of unprotected collagen by bacterial and host-mediated enzymes[28,70] might negatively impact the performance of the adhesives applied to CAD. These morphological alterations might result in poorer dentin hybridization[1,50] and reduced mechanical performance of the bonded restorations.[35] Taking into account that the dental substrate usually used in *in vitro* bonding tests is less challenging for adhesion than the substrate found clinically, it might be assumed that the actual performance of dental adhesives is generally overestimated.

Little evidence is available from clinical studies on the performance of dental adhesives comparing different dentin substrates to base clinical decisions. Clinicians have to rely on their own clinical judgement or in *in vitro* data for choosing the best approach to bond to CAD. Pooled *in vitro* data could aid in drawing more solid conclusions on which strategy might work better for CAD. A recent systematic review on bonding to CAD[9] showed that from 40% to 85% of studies reported increased bond strengths to SoD, depending on the adhesives tested. However, the authors did not conduct a meta-analysis on bond strength data for comparing the bonding potential between substrates. A meta-analysis might additionally allow testing other factors associated with bonding to SoD *vs.* CAD, such as the method used for removal of infected carious dentin, for instance.

This study was designed to evaluate by means of a systematic review of the literature the bond strength of different adhesive approaches (etch-and-rinse and self-etch) applied to SoD *vs.* CAD dentin. Attention was also given to the effect that methods used for removal of infected carious dentin might have on bonding to CAD. The hypothesis tested was that the bond strength to CAD is lower than to SoD.

3.3 Materials and Methods

This systematic review was carried out according to the guidelines of Cochrane Handbook for Systematic Reviews of Interventions[21] and followed the four-phase flow diagram based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.[39] The present report is based on the PRISMA Statement.

3.3.1 Study selection and search strategy

In vitro studies that compared the bond strength of adhesive systems to SoD and CAD were selected. The study should have reported at least one comparison between substrates (SoD vs. CAD) for inclusion, irrespective of the caries detection method, method used for removal of carious infected dentin, bond strength test, and storage time of specimens before testing. Articles assessing only the bond strength of adhesives to SoD or CAD without comparing the substrates were excluded.

Studies were identified through Medline/PubMed, Scopus, and Web of Science databases. The last search was carried out in March 2015 with no language or date restrictions. References of all included studies were also hand-searched. The following search strategy was used in the three databases: dentin* AND (bond* OR adhes*) AND (caries* OR carious OR decay*). Literature search results were de-duplicated using EndNote X7 software (Thomson Reuters, New York, NY, USA). Two independent reviewers (C.P.I. and R.S.O) initially screened the titles of all identified studies. If the title indicated possible inclusion, the abstract was evaluated. After the abstracts were carefully appraised, manuscripts considered eligible for the review (or in case of doubt) were selected for full-text reading. Discrepancies were resolved by discussion with a third reviewer (R.R.M.).

3.3.2 Data collection

A standardized outline was used for data extraction based on the characteristics of studies and groups tested: sample size, carious dentin type (e.g. natural, artificially-induced), caries detection method (e.g. visual examination, hardness, dye staining), method used for removal of carious infected dentin (e.g. excavation, grinding), dental substrate used (e.g. human molars, bovine incisors), bond strength test, adhesive system type and brand. Dentin bond strength means and standard deviations were also extracted. The authors of studies were contacted in case of missing or any unpublished data; these studies were only included if the authors provided the missing information.

3.3.3 Assessment of risk of bias

The risk of bias was assessed based on previous studies[40,41,58] and The Cochrane Collaboration's tool for assessing risk of bias.[20] The following parameters were considered: teeth randomization, materials used according to manufacturers' instructions, sample size calculation, blinding of the operator of the testing machine, and caries detection method. The reporting or not of each item was evaluated as high, low, or unclear risk of bias. The parameters used were discussed by the researchers involved and judgment was carried out by a single researcher (R.S.O). Assessment of risk of bias was conducted using Review Manager 5.3 software (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

3.3.4 Data analysis

Characteristics of studies were summarized descriptively. When sufficient data were available, a random effects meta-analysis was conducted to calculate pooled mean difference between SoD and CAD. Analyses were carried out separately for self-etch and etch-and-rinse adhesives. As a *post hoc* decision, a subgroup analysis was carried out to explore the heterogeneity considering the caries removal methods used in CAD group (excavation, grinding, more than one method, or unknown). An additional comparison between etch-and-rinse and self-etch adhesives restricted to CAD was carried out. In order to avoid overestimation of results, bond strength data included in this additional analysis were restricted to those from studies in which self-etch or etch-and-rinse adhesives were compared under same conditions (e.g. same method for removal of carious infected dentin) and when a pairwise comparison was feasible. Statistical heterogeneity was considered using I^2 test (>75% indicates high heterogeneity). The analyses were conducted using Review Manager 5.3 software.

3.4 Results

After screening 2,260 unique titles, 121 abstracts, and 65 full-text articles, 40 studies were included in this review. Details of articles selection and reasons for exclusions are shown in Figure 1. In total, 26 studies were excluded from the review.[2,6,12,17-19,22,24,25,27,29,30,33,36,42,43,51,57,59,61,62,67-69,71,76] and one study was included after reading the references of the included articles.[48] Characteristics of the studies included are summarized in Table 1.

From the 40 studies included in the meta-analyses, 39 studies used human teeth (usually third molars), 36 studies tested natural caries lesions, and only 4 studies tested artificially induced caries lesions. Regarding the caries detection method, 60% of studies combined staining with a dye for caries detection and visual examination. Most studies (87.5%) used surface grinding as method for removal of infected carious dentin, sometimes combining grinding with other methods; excavation alone (manual or with burs) or combined with other method was used in 47.5% of studies. Most studies used microtensile bond strength testing and stored specimens in water at 37°C for 24 h. As regards the comparison of failure modes between SoD and CAD, although the majority of studies (57.1%) reported no appreciable differences between these substrates, 25% of studies observed increased occurrence of cohesive failure within dentin for CAD compared to SoD groups.

Figures 2 and 3 show the results for the meta-analyses and subgroup analyses comparing SoD and CAD. In studies that tested etch-and-rinse adhesives, the meta-analysis favored SoD, with effect size of -10.04, 95% confidence interval (CI) between -11.94 and -8.14, and I^2=95% (Figure 2). In the subgroup analysis for studies using excavation for removal of infected carious dentin, SoD was favored with effect size of -9.34 (95% CI: -12.00, -6.67) and I^2=94%. For grinding as removal method, the results favored SoD with effect size of -10.67 (95% CI: -14.34, -6.99) and I^2=97%. For studies using more than one method for removal of infected carious dentin, the results favored SoD with effect size of -4.86 (95% CI: -9.73, 0.00) and I^2=84%. When analyzing studies that did not mention the removal method, SoD was again favored with effect size of -13.77 (95% CI: -16.25, -11.29) and I^2=70%.

When studies testing self-etch adhesives were considered (Figure 3), the meta-analysis favored SoD with effect size of -6.76 (95% CI: -8.23, -5.30) and I^2=89%. In the subgroup analysis for studies using excavation for removal of infected carious dentin, the result favored SoD with effect size of -5.61 (95% CI: -7.78, -23.45) and I^2=89%. For studies using grinding as removal method, SoD was favored with effect size of -7.34 (95% CI: -9.97, -4.70) and I^2=90%. SoD was again favored in studies that used more than one method for removal of infected carious dentin (effect size: -7.45; 95% CI: -9.92, -4.98; I^2=68%) and in studies that did not informed the method (effect size: -13.21; 95% CI: -16.95, -9.46; I^2=28%).

Figure 4 shows the results for the meta-analysis comparing the bond strength between adhesives restricted to CAD. The resulted favored etch-and-rinse adhesives over self-etch adhesives with effect size of 3.13 (95% CI: 1.82, 4.44) and I^2=72%. Results for the judgment of risk of bias in the studies included in all meta-analyses are presented in Figures 5 and 6. Only 2 studies did not report the method used for caries detection[1,81] and none of the included studies reported sample size calculation or blinding of the operator of the testing machine. Randomization of specimens was reported in more than 50% of the studies assessed. Almost 100% of studies reported that used adhesive materials according to the manufacturers' instructions.

3.5 Discussion

This review is one of the first to summarize data from *in vitro* literature on bonding to SoD and CAD, and the first to provide meta-analyses on bond strength data comparing these substrates. The meta-analyses indicated that bonding to SoD was always significantly higher than bonding to CAD, irrespective of materials and techniques tested, confirming the hypothesis tested. These results corroborate the observations of a recent review on the same topic.[9] In our systematic review and meta-analyses, 40 studies were included, whereas 29 studies were included in that previous review.[9] The present study covers 79% of papers addressed by Ekambaram et al. (2015),[9] whereas their article covers about 57% of the papers included here. These findings highlight the fact that a systematic review is hardly an ultimate, definitive conclusion on a subject or research question; there is usually room for new contributions, particularly when the literature is abundant on a topic and large variability exists between studies. Differences in inclusion and exclusion criteria change between studies, often leading to different sets of included papers, and sometimes perhaps even to different conclusions.

The present review was able to meta-analyze the bond strength results to compare SoD and CAD, having an additional focus (subgroup analyses) on the methods used for removal of infected carious dentin before bonding. Different methods for removal of caries could lead to different extents of tissue removal and deeper dentin exposure, for instance. Methods such as grinding or bur excavation could be less conservative in removing infected carious dentin, exposing the harder dentin tissue beneath the lesion. In contrast, methods such as laser ablation or biochemical caries removal could lead to altered surface topography. Irrespective of the methods used for caries removal, SoD was always favored in the analyses for either etch-and-rinse or self-etch adhesives, with different effect sizes only. This might be explained by the fact that at least a minimum surface flattening is needed for bond strength measurements, regardless of the caries removal method employed. Conditions of the dental surfaces were thus probably not that different between studies, since removal of caries could not be simply restricted to necrotic tissue.

The meta-analyses were carried out separately for etch-and-rinse and self-etch adhesives, since most of studies did not compare these two adhesive approaches with appropriate controls for each condition. An additional meta-analysis comparing the bond strength of etch-and-rinse *vs.* self-etch adhesives applied to CAD alone was carried out. Although not many comparisons were included (11 in total), this additional analysis favored etch-and-rinse over self-etch adhesives, meaning that the previous application of phosphoric acid seems beneficial for bonding to CAD. This result is corroborated by the previous systematic review on bonding to CAD, which indicated that 3-step etch-and-rinse adhesives seemed to perform better in CAD,[9] although the authors were cautious in interpreting their results because few studies had been addressed. General explanation for the better performance of etch-and-rinse over self-etch adhesives is two-fold. Acid etching is more effective in dissolving the superficial tissue for mechanical keying in the altered CAD[35] than self-etch adhesives, which have a less acidic composition, reducing their potential to demineralize and create microporosity.[54] In addition, bonding of self-etch monomers relies on chemical interaction with calcium ions,[16] which are usually in lower concentration in CAD.[5,44,52]

One of the shortcomings of most studies included here is that only immediate bond strengths (i.e. after 24 h storage in water) were measured. This means that only the initial bonding potential of materials and techniques addressed in the papers should be taken into account. The mentioned better performance of etch-and-rinse in CAD over self-etch adhesives, for instance, could be different in case long-term storage was tested. Another variable to be mentioned is that 4 studies testing artificially induced CAD dentin were included, in order to broader the investigation and cover one important aspect that is sometimes ignored in bond strength tests: artificial caries lesions tend to be more homogeneous and controlled than natural caries lesions.[36] The use of artificially-induced CAD might allow testing dental adhesives in an altered substrate rather than always focusing adhesion to sound, unaltered dentin substrate. The number of included studies addressing artificial caries lesions was quite low, with probably negligible effect on the overall meta-analysis. However, it should be mentioned that artificial CAD is histologically different from natural CAD, particularly due to possible presence of reparative dentin in natural lesions, which usually take longer to be produced.

Almost all statistical analyses carried out here presented high heterogeneity, and the subgroup analyses were performed to identify factors possibly influencing the results. Reasons and variables that influenced the high heterogeneity were hardly identified since the studies present a high number of covariates involved. The parameters assessed by the risk of bias tool showed a high prevalence of unclear judgment, indicating possible problems with reporting in the included studies. Reporting problems is unfortunately a common place in laboratory studies, especially because there are no consensus guidelines or orientation on how to conduct and report studies in the dental *in vitro* literature. It is also likely that the present results may have been influenced by publication bias, as studies with poor or negative results could simply have not been published. This last aspect is in fact a concern in all types of literature, not only *in vitro*. A broad search was used to aid in minimizing this problem, with additional no restriction to language or publication date.

Current concepts and techniques for caries excavation and adhesion to residual dentin present a number of alternative materials and techniques for application. The dental substrate left after excavation, with remaining caries degradative phenomena, is still a challenge for the bonding of resin-based restorative materials. Results of the present systematic review and meta-analyses corroborate a study[7] that indicated that irrespective of the caries excavation method chosen, it is safer to finish the cavity margins in clean/sound tooth tissue in order to obtain the best performance of dental adhesives. However, this should be accomplished while being the least invasive possible with regard to caries excavation and as most conservative as feasible with regard to sound tissue preservation.

Reasons for the observed lower bond strength to CAD have been abundantly addressed in the literature. These include lower mineral content and deeper demineralized zone in CAD, changes in morphological and other chemical characteristics of mineralized tissues,[13,44,71] changes in the secondary structure of collagen,[71] as well as thicker hybridization in CAD as compared to SoD. A study[72] that analyzed the effect of dentin type on bond strength after removing the variance for which hardness accounted as a covariate indicated that the condition of dentin had a significant effect on bond strength: even if SoD and CAD had similar intertubular hardness, the bond strength to CAD would still be significantly lower than to SoD. Reduction in the cohesive strength of CAD has been also linked with poor bonding,[78] which corroborates the 25% of the articles included in the systematic review that observed that more cohesive failures within CAD than SoD. It is clear that CAD is a more challenging substrate for bonding compared to SoD, and this effect should be taken into account when evaluating dental adhesives *in vitro* or when developing new bonding agents, which usually are tested only using sound dentin in pre-clinical tests.

3.6 Clinical relevance

Caries-affected dentin is a more challenging substrate for bonding than sound dentin, irrespective of the adhesive approach used. When bonding to caries-affected dentin, the *in vitro* literature indicates that the use of etch-and-rinse adhesives seems preferable over self-etch materials.

3.7 References

1. Arrais CAG, Giannini M, Nakajima M, Tagami J. Effects of additional and extended acid etching on bonding to caries-affected dentine. Eur J Oral Sci 2004;112:458-464.
2. Banerjee A, Kellow S, Mannocci F, Cook RJ, Watson TF. An in vitro evaluation of microtensile bond strengths of two adhesive bonding agents to residual dentine after caries removal using three excavation techniques. J Dent 2010;38:480-489.
3. Burrow MF, Bokas J, Tanumiharja M, Tyas MJ. Microtensile bond strengths to caries-affected dentine treated with Carisolv®. Aust Dent J 2003;48:110-114.
4. Ceballos L, Camejo DG, Fuentes MV, Osorio R, Toledano M, Carvalho RM, Pashley DH. Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine. J Dent 2003;31:469-477.
5. Daculsi G, LeGeros RZ, Jean A, Kerebel B. Possible physico-chemical processes in human dentin caries. J Dent Res 1987;66:1356-1359.
6. de-Melo MAS, Goes DD, de-Moraes MDR, Santiago SL, Rodrigues LKA. Effect of chlorhexidine on the bond strength of a self-etch adhesive system to sound and demineralized dentin. Braz Oral Res 2013;27:218-224.

7. de Almeida Neves A, Coutinho E, Cardoso MV, Lambrechts P, Van Meerbeek B. Current concepts and techniques for caries excavation and adhesion to residual dentin. J Adhes Dent 2011;13:7-22.
8. Doi J, Itota T, Torii Y, Nakabo S, Yoshiyama M. Micro-tensile bond strength of self-etching primer adhesive systems to human coronal carious dentin. J Oral Rehabil 2004;31:1023-1028.
9. Ekambaram M, Yiu CK, Matinlinna JP. Bonding of resin adhesives to caries-affected dentin - A systematic review. Int J Adhes Adhes 2015;61:23-34.
10. Ekambaram M, Yiu CK, 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;42:709-719.
11. Ergucu Z, Celik EU, Unlu N, Turkun M, Ozer F. Effect of Er,Cr:YSGG Laser on the Microtensile Bond Strength of Two Different Adhesives to the Sound and Caries-affected Dentin. Oper Dent 2009;34:460-466.
12. Erhardt MC, Lobo MM, Goulart M, Coelho-de-Souza FH, Valentino TA, Pisani-Proenca J, Conceicao EN, Pimenta LA. Microtensile bond strength of etch-and-rinse and self-etch adhesives to artificially created carious dentin. Gen Dent 2014;62:56-61.
13. Erhardt MC, Toledano M, Osorio R, Pimenta LA. Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure. Dent Mater 2008;24:786-798.
14. Erhardt MCG, Osorio R, Toledano M. Dentin treatment with MMPs inhibitors does not alter bond strengths to caries-affected dentin. J Dent 2008;36:1068-1073.
15. Erhardt MCG, Rodrigues JA, Valentino TA, Ritter AV, Pimenta LAF. In vitro μTBS of one-bottle adhesive systems: Sound versus artificially-created caries-affected dentin. J Biomed Mater Res B Appl Biomater 2008;86:181-187.
16. Fujita K, Ma S, Aida M, Maeda T, Ikemi T, Hirata M, Nishiyama N. Effect of reacted acidic monomer with calcium on bonding performance. J Dent Res 2011;90:607-612.
17. Giriyappa RH, Chandra BS. Comparative evaluation of self-etching primers with fourth and fifth generation dentin-bonding systems on carious and normal dentin substrates: An in vitro shear bond strength analysis. J Conserv Dent 2008;11:154-158.

18. Haak R, Wicht MJ, Noack MJ. Does chemomechanical caries removal affect dentine adhesion? Eur J Oral Sci 2000;108:449-455.
19. Hamama HH, Yiu CK, Burrow MF. Effect of chemomechanical caries removal on bonding of self-etching adhesives to caries-affected dentin. J Adhes Dent 2014;16:507-516.
20. Higgins JPT, Altman DG, Sterne JAC. Assessing risk of bias in included studies. In: Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 510 (updated March 2011) 2011;8: www.cochrane-handbook.org.
21. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 Cochrane Library 2011; www.cochrane-handbook.org.
22. Hsu KW, Marshall SJ, Pinzon LM, Watanabe L, Saiz E, Marshall GW. SEM evaluation of resin-carious dentin interfaces formed by two dentin adhesive systems. Dent Mater 2008;24:880-887.
23. Huang XQ, Li L, Huang C, Du XJ. Effect of ethanol-wet bonding with hydrophobic adhesive on caries-affected dentine. Eur J Oral Sci 2011;119:310-315.
24. 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 B Apllied Biomater 2005;72:109-116.
25. 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;32:138-143.
26. Kimochi T, Yoshiyama M, Urayama A, Matsuo T. Adhesion of a new commercial self-etching/self-priming bonding resin to human caries-infected dentin. Dent Mater J 1999;18:437-443.
27. Kita T, Ishii K, Yoshikawa K, Yasuo K, Yamamoto K, Awazu K. In vitro study on selective removal of bovine demineralized dentin using nanosecond pulsed laser at wavelengths around 5.8 μm for realizing less invasive treatment of dental caries. Lasers Med Sci 2015;30:961-967.
28. Kleter GA, Damen JJ, Buijs MJ, Ten Cate JM. Modification of amino acid residues in carious dentin matrix. J Dent Res 1998;77:488-495.

29. Komori PCP, Pashley DH, Tjaderhane L, Breschi L, Mazzoni A, de Goes MF, Wang L, Carrilho MR. Effect of 2% chlorhexidine digluconate on the bond strength to normal versus caries-affected dentin. Oper Dent 2009;34:157-165.
30. Koyuturk AE, Ozmen B, Cortcu M, Tokay U, Tosun G, Erhan Sari M. Effects of Er:YAG laser on bond strength of self-etching adhesives to caries-affected dentin. Microsc Res Tech 2014;77:282-288.
31. Koyuturk AE, Sengun A, Ozer F, Sener Y, Gokalp A. Shear bond strengths of self-etching adhesives to caries-affected dentin on the gingival wall. Dent Mater J 2006;25:59-65.
32. Kunawarote S, Nakajima M, Foxton RM, Tagami J. Effect of pretreatment with mildly acidic hypochlorous acid on adhesion to caries-affected dentin using a self-etch adhesive. Eur J Oral Sci 2011;119:86-92.
33. Lenzi TL, Braga MM, Raggio DP. Shortening the etching time for etch-and-rinse adhesives increases the bond stability to simulated caries-affected primary dentin. J Adhes Dent 2014;16:235-241.
34. Macedo GV, Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res 2009;88:1096-1100.
35. Marshall GW, Habelitz S, Gallagher R, Balooch M, Balooch G, Marshall SJ. Nanomechanical properties of hydrated carious human dentin. J Dent Res 2001;80:1768-1771.
36. Maske TT, Isolan CP, van de Sande FH, Peixoto AC, Faria ESAL, Cenci MS, Moraes RR. A biofilm cariogenic challenge model for dentin demineralization and dentin bonding analysis. Clin Oral Invest 2015;19:1047-1053.
37. 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;36:649-660.
38. Mobarak EH, El-Badrawy WH. Microshear bond strength of self-etching adhesives to caries-affected dentin identified using the dye permeability test. J Adhes Dent 2012;14:245-250.
39. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.

40. 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;93:733-743.
41. Moraes AP, Sarkis-Onofre R, Moraes RR, Cenci MS, Soares CJ, Pereira-Cenci T. Can silanization increase the retention of glass-fiber posts? a systematic review and meta-analysis of in vitro studies. Oper Dent 2015;doi:10.2341/14-330-O.
42. Munoz MA, Luque I, Hass V, Reis A, Loguercio AD, Bombarda NHC. Immediate bonding properties of universal adhesives to dentine. J Dent 2013;41:404-411.
43. Naik SV, Shashikiran ND, Chaitra NL, Syed G. A microtensile bond strength evaluation of a single-bottle adhesive to caries-affected dentin in conventional versus minimal invasive caries removal techniques: An in-vitro study. Indian J Dent 2014;5:127-131.
44. Nakajima M, Kitasako Y, Okuda M, Foxton RM, Tagami J. Elemental distributions and microtensile bond strength of the adhesive interface to normal and caries-affected dentin. J Biomed Mater Res B Appl Biomater 2005;72:268-275.
45. Nakajima M, Ogata M, Okuda M, Tagami J, Sano H, Pashley DH. Bonding to caries-affected dentin using self-etching primers. Am J Dent 2000;13:72-72.
46. Nakajima M, Sano H, Burrow MF, Tagami J, Yoshiyama M, Ebisu S, Ciucchi B, Russell CM, Pashley DH. Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives. J Dent Res 1995;74:1679-1688.
47. Nakajima M, Sano H, Urabe I, Tagami J, Pashley DH. Bond strengths of single-bottle dentin adhesives to caries-affected dentin. Oper Dent 2000;25:2-10.
48. Nakajima M, Sano H, Zheng L, Tagami J, Pashley DH. Effect of moist vs. dry bonding to normal vs. caries-affected dentin with Scotchbond Multi-Purpose Plus. J Dent Res 1999;78:1298-1303.
49. Neves AA, Coutinho E, Cardoso MV, de Munck J, Van Meerbeek B. Micro-tensile bond strength and interfacial characterization of an adhesive bonded to dentin prepared by contemporary caries-excavation techniques. Dent Mater 2011;27:552-562.
50. Nikaido T, Kunzelmann KH, Ogata M, Harada N, Yamaguchi S, Cox CF, Hickel R, Tagami J. The in vitro dentin bond strengths of two adhesive systems in class I cavities of human molars. J Adhes Dent 2002;4:31-39.

51. Nishitani Y, Yoshiyama M, Tay FR, Wadgaonkar B, Waller J, Agee K, Pashley DH. Tensile strength of mineralized/demineralized human normal and carious dentin. J Dent Res 2005;84:1075-1078.
52. Ogawa K, Yamashita Y, Ichijo T, Fusayama T. The ultrastructure and hardness of the transparent layer of human carious dentin. J Dent Res 1983;62:7-10.
53. Omar H, El-Badrawy W, El-Mowafy O, Atta O, Saleem B. Microtensile bond strength of resin composite bonded to caries-affected dentin with three adhesives. Oper Dent 2007;32:24-30.
54. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1-16.
55. Perdigao J, Swift EJ, Jr., Denehy GE, Wefel JS, Donly KJ. In vitro bond strengths and SEM evaluation of dentin bonding systems to different dentin substrates. J Dent Res 1994;73:44-55.
56. Pereira PN, Nunes MF, Miguez PA, Swift EJ, Jr. Bond strengths of a 1-step self-etching system to caries-affected and normal dentin. Oper Dent 2006;31:677-681.
57. Piva E, Ogliari FA, Moraes RR, Cora F, Henn S, Correr-Sobrinho L. Papain-based gel for biochemical caries removal: influence on microtensile bond strength to dentin. Braz Oral Res 2008;22:364-370.
58. Sarkis-Onofre R, Skupien JA, Cenci MS, Moraes RR, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. Oper Dent 2014;39:E31-44.
59. Sattabanasuk V, Burrow MF, Shimada Y, Tagami J. Resin adhesion to caries-affected dentine after different removal methods. Aust Dent J 2006;51:162-169.
60. Say EC, Nakajima M, Senawongse P, Soyman M, Ozer F, Tagami J. Bonding to sound vs caries-affected dentin using photo- and dual-cure adhesives. Oper Dent 2005;30:90-98.
61. Scheffel DL, Ricci HA, de Souza Costa CA, Pashley DH, Hebling J. Effect of reducing acid etching time on bond strength to noncarious and caries-affected primary and permanent dentin. Pediatr Dent 2013;35:199-204.
62. Schmidlin PR, Siebenmann J, Kocher P, Seemann R, Attin T, Bindl A. Effects of de- and remineralization of dentin on bond strengths yielded by one-, three-, and four-step adhesives. J Adhes Dent 2008;10:119-126.

63. Scholtanus JD, Purwanta K, Dogan N, Kleverlaan CJ, Feilzer AJ. Microtensile bond strength of three simplified adhesive systems to caries-affected dentin. J Adhes Dent 2010;12:273-278.
64. Sengun A, Koyuturk AE, Sener Y, Ozer F. Effect of desensitizers on the bond strength of a self-etching adhesive system to caries-affected dentin on the gingival wall. Oper Dent 2005;30:430-435.
65. Sengun A, Unlu N, Ozer F, Ozturk B. Bond strength of five current adhesives to caries-affected dentin. J Oral Rehabil 2002;29:777-781.
66. Singh UP, Tikku A, Chandra A, Loomba K, Boruah LC. Influence of caries detection dye on bond strength of sound and carious affected dentin: An in-vitro study. J Conserv Dent 2011;14:32-35.
67. Sonoda H, Banerjee A, Sheriff M, Tagami J, Watson TF. An in vitro investigation of microtensile bond strengths of two dentine adhesives to caries-affected dentine. J Dent 2005;33:335-342.
68. Swift EJ, Jr., Linden JJ, Wefel JS. Effects of the XR-bonding system on in vitro caries. Am J Dent 1991;4:157-161.
69. Tak O, Usumez A. Diffusion of HEMA through human carious and non-carious dentin in vitro. J Prosthodont 2013;22:184-189.
70. Tjaderhane 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 Rs 1998;77:1622-1629.
71. Wang Y, Spencer P, Walker MP. Chemical profile of adhesive/caries-affected dentin interfaces using Raman microspectroscopy. J Biomed Mater Res Part A 2007;81:279-286.
72. Wei S, Sadr A, Shimada Y, Tagami J. Effect of caries-affected dentin hardness on the shear bond strength of current adhesives. J Adhes Dent 2008;10:431-440.
73. Xie JX, Flaitz CM, Hicks MJ, Powers JM. Bond strength of composite to sound and artificial carious dentin. Am J Dent 1996;9:31-33.
74. Xuan W, Hou BX, Lu YL. Bond strength of different adhesives to normal and caries-affected dentins. Chin Med J (Engl) 2010;123:332-336.
75. Yazici AR, Akca T, Ozgunaltay G, Dayangac B. Bond strength of a self-etching adhesive system to caries-affected dentin. Oper Dent 2004;29:176-181.

76. Yildiz E, Sirinkaraarslan E, Yegin Z, Cebe MA, Tosun G. Effect of caries removal techniques on the bond strength of adhesives to caries-affected primary dentin in vitro. Eur J Paediatr Dent 2013;14:209-214.
77. Yoshiyama M, Doi J, Nishitani Y, Itota T, Tay FR, Carvalho RM, Pashley DH. Bonding ability of adhesive resins to caries-affected and caries-infected dentin. J Appl Oral Sci 2004;12:171-176.
78. Yoshiyama M, Tay FR, Doi J, Nishitani Y, Yamada T, Itou K, Carvalho RM, Nakajima M, Pashley DH. Bonding of self-etch and total-etch adhesives to carious dentin. J Dent Res 2002;81:556-560.
79. Yoshiyama M, Tay FR, Torii Y, Nishitani Y, Doi J, Itou K, Ciucchi B, Pashley DH. Resin adhesion to carious dentin. Am J Dent 2003;16:47-52.
80. Yoshiyama M, Urayama A, Kimochi T, Matsuo T, Pashley DH. Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin. Oper Dent 2000;25:163-169.
81. Zanchi CH, D'Avila OP, Rodrigues SA, Burnett LH, Demarco FF, Del Pinto FAB. Effect of additional acid etching on bond strength and structural reliability of adhesive systems applied to caries-affected dentin. J Adhes Dent 2010;12:109-115.
82. Zanchi CH, Lund RG, Perrone LR, Ribeiro GA, del Pino FAB, Pinto MB, Demarco FF. Microtensile bond strength of two-step etch-and-rinse adhesive systems on sound and artificial caries-affected dentin. Am J Dent 2010;23:152-156.

Table 1. Characteristics of the studies included in this systematic review

Author (year)	Sample size*	Dental substrate and carious dentin type	Caries detection method	Method for removal of carious infected dentin	Bond strength test	Modes of failure	Conclusion
Arrais et al. (2004)[1]	9 teeth	Human third molars with coronal natural caries lesions	Visual examination and surface hardness using a dental explorer	Excavation and grinding (400-, 600-grit SiC papers)	µTBS	n.i.	SoD had higher bond strength than CAD. Additional and extended acid-etching times improved the bond strength to CAD
Burrow et al. (2003)[3]	4-11 specimens	Human molars with natural caries lesions	Biochemical solution	Excavation	µTBS	CAD generally had more cohesive failures in dentin than SoD	Similar bond strengths were observed for SoD and CAD
Ceballos et al. (2003)[4]	4 teeth	Human molars with natural coronal caries lesions	Staining and visual examination	Grinding	µTBS	No major differences between substrates	SoD had higher bond strength than CAD depending on the material tested
Doi et al. (2004)[8]	5 teeth	Human molars with natural coronal caries lesions	Staining	Grinding (diamond saw and 600-grit SiC paper)	µTBS	Cohesive failures in dentin were observed only for CAD	SoD had higher bond strength than CAD
Ekambaram et al. (2014)[10]	16 specimens	Human molars with natural coronal caries lesions	Staining	Excavation (manual)	µTBS	Cohesive failures in dentin were observed only for CAD	SoD generally had higher bond strength than CAD. Use of chlorhexidine preserved the bond strength of hydrophobic adhesive to SoD and CAD
Ergüçü et al. (2009)[11]	4 teeth (5 specimens/tooth)	Human molars with natural coronal caries lesions	Staining and visual and tactile examination	Laser (Er,Cr:YSGG) and excavation (bur)	µTBS	No major differences between substrates	SoD had higher bond strength than CAD
Erhardt et al. (2008)[13]	17-19 specimens	Human molars with natural coronal caries lesions	Staining and visual examination	Grinding (180- to 600-grit SiC papers)	µTBS	No major differences between substrates.	SoD had higher bond strength than CAD. Increased exposed collagen zone and decreased
Erhardt et al. (2008)[14]	5 teeth	Human molars with natural coronal caries lesions	Staining	Excavation and grinding (600-grit SiC paper)	µTBS	No major differences between substrates.	SoD had higher bond strength than CAD after acid-etching

Erhardt et al. (2008)[15]	6 teeth (4 specimens/tooth)	Bovine incisors with artificial caries	Microhardness testing	Grinding (180- to 600-grit SiC papers)	μTBS	No major differences between substrates	SoD had higher bond strength than CAD
Huang et al. (2011)[23]	15 specimens	Human molars with natural coronal caries lesions	Staining	Excavation and grinding (600-grit SiC paper)	μTBS	n.i.	SoD had higher bond strength than CAD
Kimochi et al. (1999)[26]	6-8 teeth	Human molars with natural coronal caries lesions	Staining and visual examination	Grinding (600-grit SiC paper)	μTBS	CAD had more cohesive failures in dentin than SoD	SoD had higher bond strength than CAD
Koyuturk et al. (2006)[31]	14 teeth	Human molars with natural coronal caries lesions	Staining, visual examination, and surface hardness using a sharp excavator	Grinding (320-grit SiC paper)	SBS	No major differences between substrates	Three adhesives had higher bond strength to SoD and two other adhesives had higher bond strength to CAD
Kunawarote et al. (2011)[32]	10 teeth (4-5 specimens/ tooth)	Human molars with natural coronal caries lesions	Staining, radiography and visual examination	Excavation and grinding (600-grit SiC paper)	μTBS	SoD had more cohesive failures within the restorative composite than CAD	SoD had higher bond strength than CAD
Macedo et al. (2009)[34]	6 teeth (8 specimens/ tooth)	Human molars with natural occlusal caries lesions	Staining, visual examination, and surface hardness	Grinding (600-grit SiC paper)	μTBS	No major differences between substrates	SoD had higher bond strength than CAD
Mobarak et al. (2011)[37]	20 teeth	Human molars with natural occlusal caries lesions	Staining and visual examination	Excavation and grinding (600-grit SiC paper)	μSBS	No major differences between substrates	Similar bond strengths were observed for SoD and CAD. Use of chlorhexidine preserved the bond strength to CAD
Mobarak & El-Badrawy (2012)[38]	20 teeth (2 specimens/ tooth)	Human molars with natural coronal caries lesions	Visual and tactile examination and microhardness testing	Grinding	μSBS	No major differences between substrates	Differences in bond strength between SoD and CAD depended on the adhesive system

Nakajima et al. (1995)[46]	10 specimens	Human molars with coronal caries lesions	Staining, visual examination and surface hardness using a dental explorer	Grinding (320-, 600-grit SiC papers)	μTBS	No major differences between substrates	SoD generally had higher bond strength than CAD
Nakajima et al. (1999)[48]	9-14 specimens	Human molars with natural coronal caries lesions	Staining and visual examination	Grinding (320-, 600-grit SiC papers)	μTBS	n.i.	Similar bond strengths were observed for SoD and CAD
Nakajima et al. (2000)[45]	12-19 specimens	Human molars with natural coronal caries lesions	Staining and visual examination	Grinding (180-, 600-grit SiC papers)	μTBS	CAD had more mixed failures than SoD	SoD generally had higher bond strength than CAD
Nakajima et al. (2000)[47]	6 teeth (4-5 slices/ tooth)	Human third molars with natural coronal caries lesions	Staining and visual examination	Grinding (600-grit SiC paper)	μTBS	No major differences between substrates	SoD had higher bond strength than CAD
Nakajima et al. (2005)[44]	26 specimens	Human molars with natural coronal caries lesions	Staining and visual examination	Grinding (600-grit SiC paper)	μTBS	CAD had more cohesive failures in dentin than SoD	SoD had higher bond strength than CAD. The demineralized zone of the CAD-resin interface (8 μm thick) was thicker than that of SoD (3 μm thick)
Neves et al. (2011)[49]	5 teeth (~35 specimens/ group)	Human molars with natural coronal	Digital radiography	Grinding, laser (Er:YAG), biochemical solution, excavation (bur)	μTBS	CAD had more cohesive failures in dentin than SoD	SoD had higher bond strength than CAD
Omar et al. (2007)[53]	5 teeth	Human molars with natural occlusal caries lesions	Visual and microscopy examination	Excavation (bur) and grinding (diamond saw)	μTBS	n.i.	SoD had higher bond strength than CAD, but not for all adhesives tested
Perdigão et al. (1994)[55]	10 teeth	Human molars with Artificial lesions induced by acidogenic challenge	Visual examination	Grinding (240-, 400-, 600-grit SiC papers)	SBS	No major differences between substrates	SoD had higher bond strength than hypermineralized and demineralized dentin groups
Pereira et al. (2006)[56]	5 teeth (5-8 specimens/ tooth)	Human molars with natural coronal caries lesions	Staining	Grinding (600-grit SiC paper)	μTBS	n.i.	SoD generally had higher bond strength than CAD

Say et al. (2005)[60]	3 teeth (80 specimens / SoD and 40 specimens / CAD)	Human third molars with natural coronal caries lesions	Staining and visual examination	Grinding (600-grit SiC paper)	μTBS	CAD generally had more cohesive failures in dentin than SoD	SoD had higher bond strength than CAD. There were no significant differences between self-etch and etch-and-rinse adhesives in CAD
Scholtanus et al. (2010)[63]	10-12 specimens	Human molars with natural occlusal caries lesions	Staining, visual and tactile examination	Excavation	μTBS	No major differences between substrates	SoD generally had higher bond strength than CAD
Sengün et al. (2002)[65]	12 teeth	Human molars with natural coronal caries lesions	Staining and visual examination	Excavation and grinding (diamond saw)	SBS	No major differences between substrates	SoD had higher bond strength than CAD, but not for all adhesives tested
Sengün et al. (2005)[64]	15 teeth	Human molars with natural coronal caries lesions	Staining and visual examination	Excavation and grinding (320-grit SiC paper)	SBS	No major differences between substrates	Differences in bond strength between SoD and CAD depended on the sensitizer used before bonding
Singh et al. (2011)[66]	10 teeth	Human mandibular molars with natural caries lesion	Staining and visual examination	Grinding (220-, 600-grit SiC papers)	μTBS	n.i.	SoD had higher bond strength than CAD
Taniguchi et al. (2009)	12 specimens /group	Human molars with natural coronal caries lesions	Staining and visual examination	Grinding (600-grit SiC paper)	μTBS	No major differences between substrates	SoD generally had higher bond strength than CAD
Xie et al. (1996)[73]	11 teeth	Human third molars with Artificial lesions induced by acidogenic challenge	Visual examination	n.i.	μTBS	CAD had more adhesive failures than SoD	Similar bond strengths were observed for SoD and CAD
Xuan et al. (2010)[74]	10 beam-shaped specimens/ group	Human third molars with natural coronal caries lesions	Staining and visual examination	Excavation and grinding (600-grit SiC paper)	μTBS	n.i.	SoD generally had higher bond strength than CAD
Yazici et al. (2004)[75]	3 teeth (10-12 specimens/ tooth)	Human mandibular molars with natural coronal caries lesions	Staining and visual examination	Excavation (bur) and grinding (600-grit SiC paper)	μTBS	n.i.	SoD had higher bond strength than CAD without additional acid-etching. Additional acid-etching did not improve the bond strength to CAD
Yoshiyama et al. (2000)[80]	10-12 specimens	Human molars with natural coronal caries lesions	Staining and visual examination	Excavation (bur) and grinding	μTBS	n.i.	SoD generally had higher bond strength than CAD

Yoshiyama et al. (2002)[78]	7-9 specimens	Human molars with natural coronal caries lesions	Staining and visual examination	Excavation (manual) and grinding (600-grit SiC paper)	μTBS	n.i.	SoD had higher bond strength than CAD. There were no significant differences between self-etch and etch-and-rinse adhesives in CAD
Yoshiyama et al. (2003)[79]	15 specimens	Human third molars with natural coronal caries lesions	Staining and visual examination	Excavation (manual) and grinding (600-grit SiC paper)	μTBS	n.i.	SoD had higher bond strength than CAD
Yoshiyama et al. (2004)[77]	7 teeth	Human third molars with natural coronal caries lesions	n.i.	Grinding	μTBS	n.i.	SoD had higher bond strength than CAD
Zanchi et al. (2010)[81]	30 specimens	Human molars with Natural coronal caries lesions	Visual examination and surface hardness using a dental explorer	Excavation and grinding (600-grit SiC paper)	μTBS	CAD generally had more adhesive failures than SoD	SoD generally had higher bond strength than CAD. Additional acid-etching generally improved the bond strength to CAD and reduced to SoD
Zanchi et al. (2010)[82]	15 specimens	Human molars with Artificial lesions induced by pH cycling	Morphologic evaluation	Grinding	μTBS	No major differences between substrates	SoD generally had higher bond strength than CAD

*Number of teeth or specimens *per* group; specimens refer to resin-dentin beams for microtensile bond strength (μTBS) or composite cylinders for shear or microshear bond strength (μSBS) tests. n.i.: not informed.

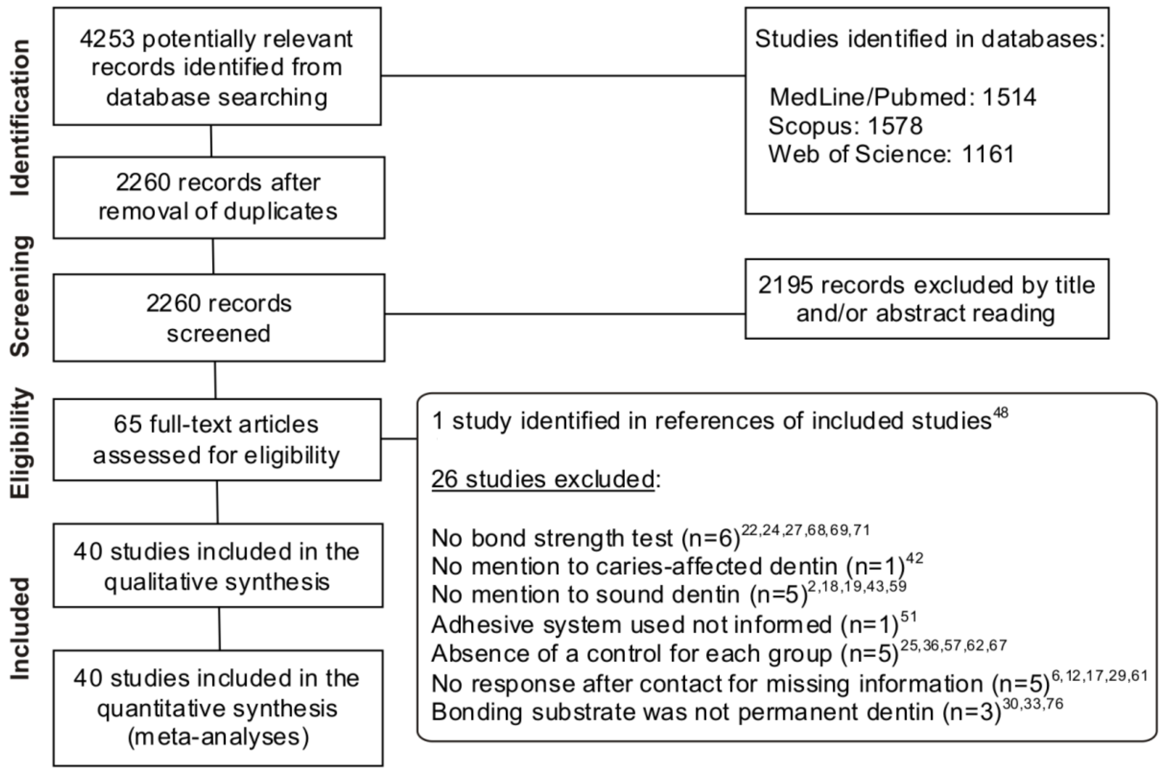

Fig 1 Flow diagram of the systematic review.

Study or Subgroup	Carious dentin Mean	Carious dentin SD	Carious dentin Total	Sound dentin Mean	Sound dentin SD	Sound dentin Total	Weight	Mean Difference IV, Random, 95% CI
1.4.1 Excavation								
Arrais et al 2004	23.58	9.18	9	50.69	10.81	9	1.4%	-27.11 [-36.38, -17.84]
Arrais et al 2004	33.97	12.18	9	43.74	8.97	9	1.4%	-9.77 [-19.65, 0.11]
Ekambaram et al 2014	8.03	3.1	16	18.27	4	16	2.1%	-10.24 [-12.72, -7.76]
Ekambaram et al 2014	33.61	4.2	16	51.78	3.9	16	2.0%	-18.17 [-20.98, -15.36]
Ekambaram et al 2014	33.93	3.3	16	52.99	4.4	16	2.0%	-19.06 [-21.75, -16.37]
Ekambaram et al 2014	19.06	3.5	16	26.56	1.9	16	2.1%	-7.50 [-9.45, -5.55]
Ekambaram et al 2014	23.45	4.8	16	26.64	3.8	16	2.0%	-3.19 [-6.19, -0.19]
Ekambaram et al 2014	20.52	3.6	16	25.24	4.1	16	2.0%	-4.72 [-7.39, -2.05]
Ekambaram et al 2014	28.78	3.6	16	40.83	5.7	16	2.0%	-12.05 [-15.35, -8.75]
Ekambaram et al 2014	31.76	5.2	16	50.37	4.1	16	2.0%	-18.61 [-21.85, -15.37]
Erhardt et al 2008b	26.2	3.5	30	31.9	3.3	30	2.1%	-5.70 [-7.42, -3.98]
Erhardt et al 2008b	24.9	5.8	30	28.1	4.2	30	2.0%	-3.20 [-5.76, -0.64]
Erhardt et al 2008b	23.8	5.5	30	27.3	4.7	30	2.0%	-3.50 [-6.09, -0.91]
Omar et al 2007	18.6	2.89	25	22.19	4.6	25	2.1%	-3.59 [-5.72, -1.46]
Omar et al 2007	16.62	6.23	25	15.7	5.5	25	2.0%	0.92 [-2.34, 4.18]
Scholtanus et al 2010	25	10	10	35	10.6	10	1.4%	-10.00 [-19.03, -0.97]
Segün et al 2002	14.1	7.94	12	10.57	4.56	12	1.8%	3.53 [-1.65, 8.71]
Xuan et al 2010	28.98	5.44	10	32.44	5.59	10	1.9%	-3.46 [-8.29, 1.37]
Yoshiyama et al 2000	18.1	2.1	10	26.4	4.8	10	2.0%	-8.30 [-11.55, -5.05]
Yoshiyama et al 2000	27.1	6.5	9	46	10.5	9	1.5%	-18.90 [-26.97, -10.83]
Yoshiyama et al 2002	28.8	6.3	7	50.9	3.9	7	1.8%	-22.10 [-27.59, -16.61]
Zanchi et al 2010a	26.64	10.3	30	47.5	11	30	1.8%	-20.86 [-26.25, -15.47]
Zanchi et al 2010a	35.29	12	30	35.36	12.7	30	1.7%	-0.07 [-6.32, 6.18]
Zanchi et al 2010a	33.43	11.9	30	40.2	10.2	30	1.8%	-6.77 [-12.38, -1.16]
Subtotal (95% CI)			**434**			**434**	**45.1%**	**-9.34 [-12.00, -6.67]**
Heterogeneity: Tau² = 38.56; Chi² = 357.86, df = 23 (P < 0.00001); I² = 94%								
Test for overall effect: Z = 6.87 (P < 0.00001)								
1.4.2 Grinding								
Ceballos et al 2003	36.3	12.2	10	43.9	11.4	10	1.3%	-7.60 [-17.95, 2.75]
Ceballos et al 2003	41.3	10.7	10	56.3	11.1	10	1.4%	-15.00 [-24.56, -5.44]
Erhardt et al 2008a	16.3	5.2	24	25.6	6	24	2.0%	-9.30 [-12.48, -6.12]
Erhardt et al 2008a	16	2.3	24	20.8	3.2	24	2.1%	-4.80 [-6.38, -3.22]
Huang et al 2011	29.5	3.1	15	68.3	4.1	15	2.0%	-38.80 [-41.40, -36.20]
Macedo et al 2009	36.75	8.5	48	59.62	20	48	1.7%	-22.87 [-29.02, -16.72]
Macedo et al 2009	37.38	14.8	48	65.22	20.04	48	1.6%	-27.84 [-34.89, -20.79]
Nakajima et al 1995	13.01	3.64	10	26.9	8.83	10	1.8%	-13.89 [-19.81, -7.97]
Nakajima et al 1995	18.49	4.04	10	20.32	5.55	10	1.9%	-1.83 [-6.08, 2.42]
Nakajima et al 1999	30.2	13.4	13	24.9	17.5	12	1.1%	5.30 [-6.99, 17.59]
Nakajima et al 2000a	36.9	8	11	47.7	6.8	10	1.7%	-10.80 [-17.13, -4.47]
Nakajima et al 2000a	45	7.2	9	49.7	6.1	13	1.8%	-4.70 [-10.46, 1.06]
Nakajima et al 2000a	41.2	0.6	9	51.7	4.3	13	2.1%	-10.50 [-12.87, -8.13]
Nakajima et al 2000a	40.2	11.1	16	49.5	9.3	12	1.6%	-9.30 [-16.87, -1.73]
Nakajima et al 2000b	48.2	3.9	9	42.4	9	14	1.8%	5.80 [0.44, 11.16]
Pereira et al 2006	36.1	8.2	12	43.3	14.1	12	1.4%	-7.20 [-16.43, 2.03]
Say et al 2005	28.5	5	10	38.7	8.9	20	1.9%	-10.20 [-15.18, -5.22]
Say et al 2005	10.5	3.9	10	17.2	4.7	20	2.0%	-6.70 [-9.88, -3.52]
Singh et al 2011	13.19	1.24	10	22.42	1.37	10	2.1%	-9.23 [-10.38, -8.08]
Singh et al 2011	9.45	1.1	10	18.55	0.83	10	2.1%	-9.10 [-9.95, -8.25]
Subtotal (95% CI)			**318**			**345**	**35.5%**	**-10.67 [-14.34, -6.99]**
Heterogeneity: Tau² = 61.22; Chi² = 624.62, df = 19 (P < 0.00001); I² = 97%								
Test for overall effect: Z = 5.69 (P < 0.00001)								
1.4.3 More than one method								
Erguçu et al 2009	17.71	3.78	20	18.25	5.5	20	2.0%	-0.54 [-3.46, 2.38]
Erguçu et al 2009	16.33	6.76	20	17.32	6.1	20	1.9%	-0.99 [-4.98, 3.00]
Erhardt et al 2008c	34.5	6.8	18	42.6	6.2	18	1.9%	-8.10 [-12.35, -3.85]
Erhardt et al 2008c	27.8	7.4	18	38.4	6.9	18	1.9%	-10.60 [-15.27, -5.93]
Subtotal (95% CI)			**76**			**76**	**7.8%**	**-4.86 [-9.73, 0.00]**
Heterogeneity: Tau² = 20.51; Chi² = 18.74, df = 3 (P = 0.0003); I² = 84%								
Test for overall effect: Z = 1.96 (P = 0.05)								
1.4.4 Method not mentioned								
Perdigão et al 1994	1.96	2.41	10	17.47	4.59	10	2.0%	-15.51 [-18.72, -12.30]
Perdigão et al 1994	4.58	2.92	10	11.4	5.45	10	2.0%	-6.82 [-10.65, -2.99]
Perdigão et al 1994	2.93	1.67	10	16.84	4.06	10	2.0%	-13.91 [-16.63, -11.19]
Perdigão et al 1994	1.51	1.04	10	16.14	2.21	10	2.1%	-14.63 [-16.14, -13.12]
Zanchi et al 2010b	23.66	5.4	15	38.63	8.6	15	1.8%	-14.97 [-20.11, -9.83]
Zanchi et al 2010b	15.05	5.3	15	33.73	12.6	15	1.7%	-18.68 [-25.60, -11.76]
Subtotal (95% CI)			**70**			**70**	**11.6%**	**-13.77 [-16.25, -11.29]**
Heterogeneity: Tau² = 6.03; Chi² = 16.88, df = 5 (P = 0.005); I² = 70%								
Test for overall effect: Z = 10.88 (P < 0.00001)								
Total (95% CI)			**898**			**925**	**100.0%**	**-10.04 [-11.94, -8.14]**
Heterogeneity: Tau² = 44.29; Chi² = 1140.72, df = 53 (P < 0.00001); I² = 95%								
Test for overall effect: Z = 10.34 (P < 0.00001)								
Test for subgroup differences: Chi² = 12.39, df = 3 (P = 0.006), I² = 75.8%								

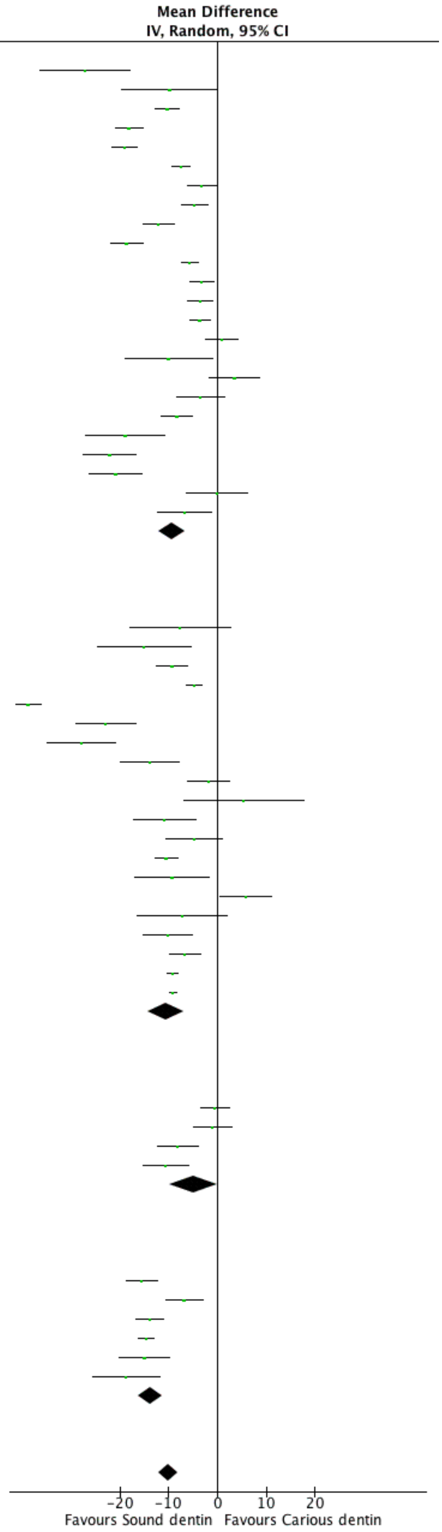

Fig 2 Summary of findings of the meta-analysis comparing the bond strength of etch-and-rinse adhesives to sound vs. caries-affected dentin, according to the methods used for removal of infected carious dentin (subgroup analyses). All analyses favored sound dentin.

Study or Subgroup	Carious dentin Mean	Carious dentin SD	Carious dentin Total	Sound dentin Mean	Sound dentin SD	Sound dentin Total	Weight	Mean Difference IV, Random, 95% CI
1.3.1 Excavation								
Arrais et al 2004	23.06	7.84	9	41.82	10.05	9	1.0%	-18.76 [-27.09, -10.43]
Arrais et al 2004	30.76	8.16	9	48.7	9.93	9	1.0%	-17.94 [-26.34, -9.54]
Burrow et al 2003	27.4	6.4	11	29.4	7.9	4	0.9%	-2.00 [-10.62, 6.62]
Burrow et al 2003	28.7	6.9	6	31.6	7.1	8	1.0%	-2.90 [-10.30, 4.50]
Erguçu et al 2009	9.96	4.55	20	9.59	2.9	20	1.3%	0.37 [-1.99, 2.73]
Erguçu et al 2009	9.99	4.07	20	11.38	5.81	20	1.3%	-1.39 [-4.50, 1.72]
Kunawarote et al 2011	39.28	5.27	11	41.93	4.93	11	1.2%	-2.65 [-6.91, 1.61]
Kunawarote et al 2011	26.77	3.47	13	41.56	5.39	12	1.3%	-14.79 [-18.38, -11.20]
Kunawarote et al 2011	34.16	6.58	11	41.24	7.1	11	1.2%	-7.08 [-12.80, -1.36]
Kunawarote et al 2011	31.62	6.62	12	40.87	5.33	12	1.2%	-9.25 [-14.06, -4.44]
Mobarak et al 2011	20.59	5.1	10	25.94	6.4	10	1.2%	-5.35 [-10.42, -0.28]
Mobarak et al 2011	21.73	6	10	24.33	5.1	10	1.2%	-2.60 [-7.48, 2.28]
Mobarak et al 2011	14.67	4.5	10	10.98	3.3	10	1.3%	3.69 [0.23, 7.15]
Mobarak et al 2011	20.84	6.2	10	23.79	5.9	10	1.2%	-2.95 [-8.25, 2.35]
Mobarak et al 2011	9.97	3.5	10	9.46	3.4	10	1.3%	0.51 [-2.51, 3.53]
Mobarak et al 2011	9.99	3.4	10	8.74	3.2	10	1.3%	1.25 [-1.64, 4.14]
Omar et al 2007	20.23	6.1	25	22.3	6.7	25	1.3%	-2.07 [-5.62, 1.48]
Omar et al 2007	15.45	6.62	25	21.43	7.6	25	1.3%	-5.98 [-9.93, -2.03]
Omar et al 2007	20.7	5.5	25	24.25	5.7	25	1.3%	-3.55 [-6.65, -0.45]
Omar et al 2007	14.8	3.89	25	18.3	7.11	25	1.3%	-3.50 [-6.68, -0.32]
Scholtanus et al 2010	21	11.2	12	35	8.5	12	1.0%	-14.00 [-21.96, -6.04]
Scholtanus et al 2010	39	5.2	12	33	9.2	12	1.1%	6.00 [0.02, 11.98]
Segün et al 2002	21.19	9.17	12	17.45	6.21	12	1.1%	3.74 [-2.53, 10.01]
Segün et al 2002	24.49	5.38	12	29.91	8.95	12	1.1%	-5.42 [-11.33, 0.49]
Segün et al 2002	17.43	9.78	12	11.99	10.15	12	1.0%	5.44 [-2.53, 13.41]
Segün et al 2002	21.49	9.15	12	21.17	5.41	12	1.1%	0.32 [-5.69, 6.33]
Segün et al 2005	22.67	5.85	15	18.51	5.88	15	1.3%	4.16 [-0.04, 8.36]
Segün et al 2005	25.15	5.58	15	20.44	7.11	15	1.2%	4.71 [0.14, 9.28]
Segün et al 2005	21.98	7.92	15	29.25	6.4	15	1.2%	-7.27 [-12.42, -2.12]
Segün et al 2005	16.95	5.78	15	20.23	6.08	15	1.2%	-3.28 [-7.53, 0.97]
Xuan et al 2010	14.5	3.37	10	20.77	4.73	10	1.3%	-6.27 [-9.87, -2.67]
Xuan et al 2010	15.13	3.08	10	24.36	5.7	10	1.3%	-9.23 [-13.25, -5.21]
Xuan et al 2010	21.18	4.96	10	35.41	5.62	10	1.2%	-14.23 [-18.88, -9.58]
Yoshiyama et al 2000	17.5	2.1	10	28.2	6.1	11	1.3%	-10.70 [-14.53, -6.87]
Yoshiyama et al 2002	25.3	5	7	44.9	14.6	7	0.8%	-19.60 [-31.03, -8.17]
Yoshiyama et al 2003	30	10	7	45	10	7	0.8%	-15.00 [-25.48, -4.52]
Zanchi et al 2010a	29.31	9.1	30	46.59	9.9	30	1.2%	-17.28 [-22.09, -12.47]
Zanchi et al 2010a	34.18	10.6	30	51.28	8.5	30	1.2%	-17.10 [-21.96, -12.24]
Zanchi et al 2010a	23.02	7.1	30	42.24	8.3	30	1.3%	-19.22 [-23.13, -15.31]
Subtotal (95% CI)			**558**			**553**	**45.8%**	**-5.61 [-7.78, -3.45]**
Heterogeneity: $Tau^2 = 40.08$; $Chi^2 = 346.90$, df = 38 ($P < 0.00001$); $I^2 = 89\%$								
Test for overall effect: Z = 5.08 ($P < 0.00001$)								
1.3.2 Grinding								
Ceballos et al 2003	21.5	5.5	10	35.5	11.6	10	1.0%	-14.00 [-21.96, -6.04]
Ceballos et al 2003	13.4	1.9	10	18.2	9.6	10	1.1%	-4.80 [-10.87, 1.27]
Doi et al 2004	21.5	5.3	7	41.2	10	8	1.0%	-19.70 [-27.66, -11.74]
Doi et al 2004	19.6	6	7	27.2	3.9	7	1.2%	-7.60 [-12.90, -2.30]
Doi et al 2004	20.2	5.8	7	35	8.9	7	1.0%	-14.80 [-22.67, -6.93]
Kimochi et al 1999	11	5.8	6	33.4	6.3	8	1.1%	-22.40 [-28.77, -16.03]
Koyuturk et al 2006	29.25	4.28	14	34.09	5.49	14	1.3%	-4.84 [-8.49, -1.19]
Koyuturk et al 2006	12.28	4.03	14	7.02	2.02	14	1.3%	5.26 [2.90, 7.62]
Koyuturk et al 2006	10.91	3.09	14	16.02	4.29	14	1.3%	-5.11 [-7.88, -2.34]
Koyuturk et al 2006	11.15	2.91	14	7.44	3.2	14	1.3%	3.71 [1.44, 5.98]
Koyuturk et al 2006	22.95	7.91	14	29.25	6.39	14	1.2%	-6.30 [-11.63, -0.97]
Mobarak et al 2012	17.31	10.3	10	17.21	6.8	10	1.0%	0.10 [-7.55, 7.75]
Mobarak et al 2012	18.7	4.09	10	22.34	6.4	10	1.2%	-3.64 [-8.35, 1.07]
Mobarak et al 2012	18.31	4.9	10	24.52	4.9	10	1.2%	-6.21 [-10.50, -1.92]
Mobarak et al 2012	18.97	9.4	10	24.49	8	10	1.0%	-5.52 [-13.17, 2.13]
Mobarak et al 2012	7.31	2.4	10	13.67	4.4	10	1.3%	-6.36 [-9.47, -3.25]
Nakajima et al 1995	13.97	4.3	10	29.52	10.9	10	1.0%	-15.55 [-22.81, -8.29]
Nakajima et al 1999	29.7	10.3	12	45.2	13.9	17	0.9%	-15.50 [-24.31, -6.69]
Nakajima et al 1999	39.1	8.9	12	57.4	10.4	19	1.1%	-18.30 [-25.17, -11.43]
Nakajima et al 2005	29.4	7.5	12	43.5	11.1	14	1.0%	-14.10 [-21.30, -6.90]
Pereira et al 2006	37.3	9.7	12	52	17.5	15	0.8%	-14.70 [-25.12, -4.28]
Say et al 2005	29.2	4.3	10	44.2	7.7	20	1.2%	-15.00 [-19.30, -10.70]
Say et al 2005	13.5	3.3	10	18.3	6.1	20	1.3%	-4.80 [-8.17, -1.43]
Taniguchi et al 2009	27.9	6.2	12	40.9	6	12	1.2%	-13.00 [-17.88, -8.12]
Taniguchi et al 2009	35.6	9.1	12	36.8	3.9	12	1.2%	-1.20 [-6.80, 4.40]
Taniguchi et al 2009	29.8	4.6	12	44	4	12	1.3%	-14.20 [-17.65, -10.75]
Taniguchi et al 2009	39.9	6.7	12	37.5	4.4	12	1.2%	2.40 [-2.14, 6.94]
Taniguchi et al 2009	41.6	6.6	12	42	3.6	12	1.2%	-0.40 [-4.65, 3.85]
Taniguchi et al 2009	31.4	8.5	12	30.4	7.7	12	1.1%	1.00 [-5.49, 7.49]
Taniguchi et al 2009	40.6	7.7	12	43.7	9.9	12	1.1%	-3.10 [-10.20, 4.00]
Taniguchi et al 2009	33.3	5.2	12	34.4	3.5	12	1.3%	-1.10 [-4.65, 2.45]
Subtotal (95% CI)			**341**			**381**	**35.7%**	**-7.34 [-9.97, -4.70]**
Heterogeneity: $Tau^2 = 47.43$; $Chi^2 = 303.88$, df = 30 ($P < 0.00001$); $I^2 = 90\%$								
Test for overall effect: Z = 5.46 ($P < 0.00001$)								
1.3.3 More than one method								
Erhardt et al 2008c	24.2	7	18	28.2	6.3	18	1.2%	-4.00 [-8.35, 0.35]
Erhardt et al 2008c	28.7	5	18	39.2	5.2	18	1.3%	-10.50 [-13.83, -7.17]
Erhardt et al 2008c	24.5	5.6	18	39.5	7.6	18	1.2%	-15.00 [-19.36, -10.64]
Erhardt et al 2008c	17.2	5.1	18	27.2	7.7	18	1.2%	-10.00 [-14.27, -5.73]
Neves et al 2011	29.8	9.9	39	40.7	12.1	39	1.2%	-10.90 [-15.81, -5.99]
Neves et al 2011	36.1	12.3	47	40.1	12.9	47	1.2%	-4.00 [-9.10, 1.10]
Neves et al 2011	37.4	13.2	31	39.3	13	31	1.1%	-1.90 [-8.42, 4.62]
Neves et al 2011	37.1	13.7	28	46	11.5	28	1.1%	-8.90 [-15.53, -2.27]
Neves et al 2011	31.7	10.1	41	38	9.5	41	1.2%	-6.30 [-10.54, -2.06]
Neves et al 2011	41.3	13.9	31	41.7	11.7	31	1.1%	-0.40 [-6.80, 6.00]
Neves et al 2011	33.7	9.2	29	39.8	11.2	29	1.2%	-6.10 [-11.38, -0.82]
Yazici et al 2004	16.3	5.7	10	19.2	5.8	12	1.2%	-2.90 [-7.72, 1.92]
Yazici et al 2004	15.9	7	10	32.9	13.7	11	0.9%	-17.00 [-26.19, -7.81]
Subtotal (95% CI)			**338**			**341**	**15.3%**	**-7.45 [-9.92, -4.98]**
Heterogeneity: $Tau^2 = 13.48$; $Chi^2 = 37.70$, df = 12 ($P = 0.0002$); $I^2 = 68\%$								
Test for overall effect: Z = 5.91 ($P < 0.00001$)								
1.3.4 Method not mentioned								
Xie et al 1996	11.8	4.1	11	22.2	6.8	11	1.2%	-10.40 [-15.09, -5.71]
Xie et al 1996	8.8	3.5	11	23.1	5.8	11	1.3%	-14.30 [-18.30, -10.30]
Yoshiyama et al 2004	25.5	5	7	44.9	14.6	7	0.8%	-19.40 [-30.83, -7.97]
Subtotal (95% CI)			**29**			**29**	**3.2%**	**-13.21 [-16.95, -9.46]**
Heterogeneity: $Tau^2 = 3.22$; $Chi^2 = 2.78$, df = 2 ($P = 0.25$); $I^2 = 28\%$								
Test for overall effect: Z = 6.91 ($P < 0.00001$)								
Total (95% CI)			**1266**			**1304**	**100.0%**	**-6.76 [-8.23, -5.30]**
Heterogeneity: $Tau^2 = 40.11$; $Chi^2 = 741.92$, df = 85 ($P < 0.00001$); $I^2 = 89\%$								
Test for overall effect: Z = 9.04 ($P < 0.00001$)								
Test for subgroup differences: $Chi^2 = 11.86$, df = 3 ($P = 0.008$), $I^2 = 74.7\%$								

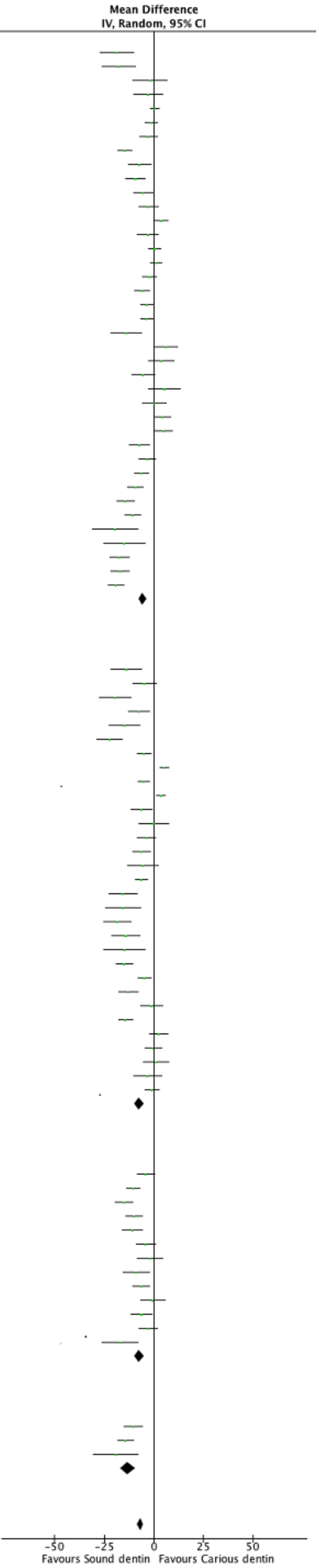

Fig 3 Summary of findings of the meta-analysis comparing the bond strength of self-etch adhesives to sound vs. caries-affected dentin, according to the methods used

for removal of infected carious dentin (subgroup analyses). All analyses favored sound dentin.

	Etch-and-rinse			Self-etch				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
Arrais et al 2004	33.97	12.18	9	30.76	8.16	9	1.9%	3.21 [-6.37, 12.79]
Arrais et al 2004	23.58	9.18	9	23.06	7.84	9	2.8%	0.52 [-7.37, 8.41]
Erguçu et al 2009	16.33	6.76	20	9.99	4.07	20	14.4%	6.34 [2.88, 9.80]
Erguçu et al 2009	17.71	3.78	20	9.96	4.55	20	25.6%	7.75 [5.16, 10.34]
Erguçu et al 2009	28.5	5	10	29.2	4.3	10	10.3%	-0.70 [-4.79, 3.39]
Pereira et al 2006	36.1	8.2	12	37.3	9.7	12	3.3%	-1.20 [-8.39, 5.99]
Say et al 2005	10.5	3.9	10	13.5	3.3	10	17.1%	-3.00 [-6.17, 0.17]
Yoshiyama et al 2002	28.8	6.3	7	25.3	5	7	4.8%	3.50 [-2.46, 9.46]
Zanchi et al 2010a	35.29	12	30	34.18	10.6	30	5.2%	1.11 [-4.62, 6.84]
Zanchi et al 2010a	33.43	11.9	30	29.31	9.1	30	6.0%	4.12 [-1.24, 9.48]
Zanchi et al 2010a	26.64	10.3	30	23.02	7.1	30	8.6%	3.62 [-0.86, 8.10]
Total (95% CI)			**187**			**187**	**100.0%**	**3.13 [1.82, 4.44]**

Heterogeneity: $Chi^2 = 35.76$, df = 10 ($P < 0.0001$); $I^2 = 72\%$
Test for overall effect: $Z = 4.67$ ($P < 0.00001$)

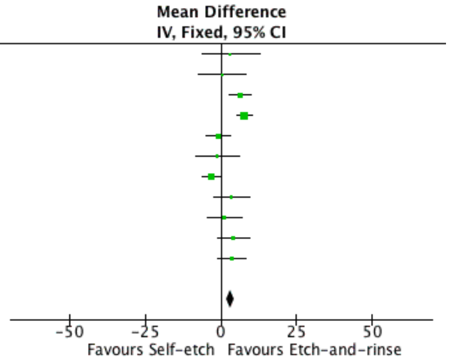

Fig 4 Meta-analysis comparing the bond strength of etch-and-rinse vs. self-etch adhesives applied to caries-affected dentin. The analysis favored etch-and-rinse adhesives.

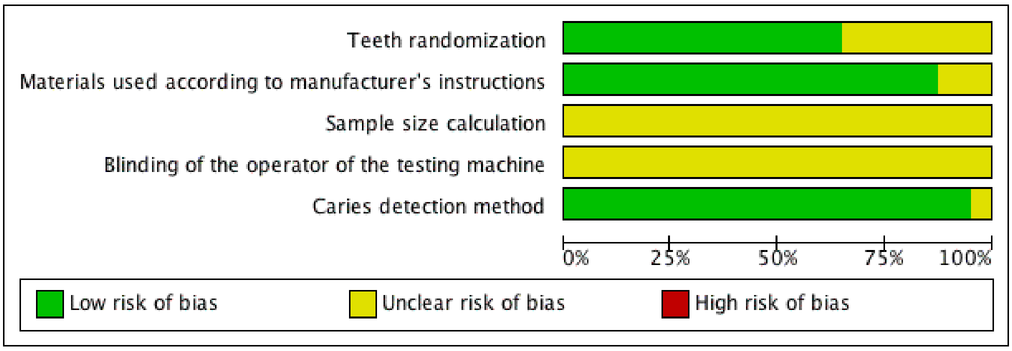

Fig 5 Risk of bias graph: proportion of studies with low, unclear, or high risk of bias for each item according to the authors' judgements.

	Teeth randomization	Materials used according to manufacturer's instructions	Sample size calculation	Blinding of the operator of the testing machine	Caries detection method
Arrais et al 2004	+	+	?	?	?
Burrow et al 2003	?	+	?	?	+
Ceballos et al 2003	+	+	?	?	+
Doi et al 2004	?	+	?	?	+
Ekambaram et al 2014	+	?	?	?	+
Erguçu et al 2009	+	+	?	?	+
Erhardt et al 2008a	+	+	?	?	+
Erhardt et al 2008b	?	+	?	?	+
Erhardt et al 2008c	?	+	?	?	+
Huang et al 2011	+	+	?	?	+
Kimochi et al 1999	?	?	?	?	+
Koyuturk et al 2006	+	+	?	?	+
Kunawarote et al 2011	+	+	?	?	+
Macedo et al 2009	+	+	?	?	+
Mobarak et al 2011	+	+	?	?	+
Mobarak et al 2012	?	+	?	?	+
Nakajima et al 1995	+	+	?	?	+
Nakajima et al 1999	+	+	?	?	+
Nakajima et al 2000a	+	+	?	?	+
Nakajima et al 2000b	+	+	?	?	+
Nakajima et al 2005	+	+	?	?	+
Neves et al 2011	+	+	?	?	+
Omar et al 2007	+	+	?	?	+
Perdigão et al 1994	+	+	?	?	+
Pereira et al 2006	?	+	?	?	+
Say et al 2005	+	?	?	?	+
Scholtanus et al 2010	+	+	?	?	+
Segun et al 2002	+	+	?	?	+
Segun et al 2005	+	+	?	?	+
Singh et al 2011	?	+	?	?	+
Taniguchi et al 2009	?	+	?	?	+
Xie et al 1996	?	+	?	?	+
Xuan et al 2010	+	+	?	?	+
Yazici et al 2004	+	+	?	?	+
Yoshiyama et al 2000	?	+	?	?	+
Yoshiyama et al 2002	?	?	?	?	+
Yoshiyama et al 2003	?	?	?	?	+
Yoshiyama et al 2004	?	+	?	?	+
Zanchi et al 2010a	+	+	?	?	?
Zanchi et al 2010b	+	+	?	?	+

Fig 6 Risk of bias summary: authors' judgements on each item for each included study.

4 Capítulo 3

Bonding effectiveness of experimental one-step, self-etch adhesives to sound and caries-affected dentin[3]

Short title: One-step adhesives and caries-affected dentin

Cristina P. Isolan[1], Ana Carolina U. Vasconcelos[1], Victor P. Feitosa[2], Rafael R. Moraes[1]

[1]Graduate Program in Dentistry, Federal University of Pelotas, Brazil
[2]Graduate Program in Dentistry, Federal University of Ceará, Brazil

Corresponding author:
Prof. Rafael Moraes
Graduate Program in Dentistry, Federal University of Pelotas
Rua Gonçalves Chaves 457 room 505
96015-560, Pelotas-RS, Brazil
Tel/Fax: 55 53 3225.6741 ext. 135
E-mail: moraesrr@gmail.com

Acknowledgments

CAPES/Brazil for a scholarship (C.P.I.). Esstech Inc. for donation of reagents used in the study. CEME-Sul – Centro de Microscopia Eletrônica da Zona Sul at Federal University of Rio Grande, Brazil for SEM equipment support.

[3] Artigo formatado segundo as normas do periódico Dental Materials.

4.1 Abstract

Objectives: This study evaluated the shear bond strength (SBS) of experimental one-step, self-etch adhesives containing three different concentrations of acidic monomer (GDMA-P) to sound dentin (SoD) and caries-affected dentin (CAD).

Methods: Bovine teeth were used to prepare disc-shaped dentin specimens. Microcosm biofilms were formed over half of the specimens and cultivated under anaerobic conditions (14 days) for CAD preparation (CAD group). The other specimens were separated in the SoD group. Each group was then divided into six subgroups according to the type of adhesive used (AD5, AD20, and AD35, depending on the concentration of GDMA-P) and period of water storage (24 h and 6 months), representing a 2 × 3 × 2 study design (n=10). The specimens were included in acrylic resin and treated with the adhesives. An elastomer mold with cylindrical orifices (1.5 mm diameter) was used to obtain two cylinders of composite resin on the surface. After 24 h and 6 months of water storage, the cylinders were subjected to SBS testing using a mechanical testing machine. Scanning electron microscopy (SEM) was performed to evaluate the hybrid layer formed in all groups, and Masson's trichrome test was carried out to examine the presence of exposed collagen. Data were analyzed with ANOVA and Student-Newman-Keuls test (α=5%).

Results: The acidity of the adhesive influenced the bond strength results, regardless of the substrate. CAD was not always a worse scenario when compared to SoD. SEM images showed that CAD surfaces were more irregular (uneven) than SoD ones. The total amount of exposed collagen increased over time for all groups. The adhesive with 20 wt% of acidic methacrylate yielded stable bond strength values that were generally independent of the dentin substrate tested.

Significance: The adhesives prepared here were effective as dentin bonding agents, although the level of effectiveness was dependent on factors such as the acidity of the adhesive, type of substrate, and period of water storage.

Keywords: Adhesive, dental caries, shear bond strength

4.2 Introduction

Dental caries is one of the most common oral diseases in humans [1], and caries lesions extending to dentin are usually treated by placement of restorations. Under the concepts of minimally invasive dentistry, decayed dental tissue located at the inner layer of the cavity may be only partially removed [2], thus the restorative procedure would include bonding to both sound dentin (SoD) and caries-affected dentin (CAD). It is worth mentioning that, even upon a decision for complete removal of the caries lesion, some CAD may remain in the cavity. Several studies indicate that bonding to CAD is more challenging than bonding to non-altered dentin [3-6] due to morphological and chemical alterations of CAD [7,8] which may result in unfavorable conditions for effective adhesion [9,10].

Despite the nature and state of the substrate, bonding to enamel/dentin may follow two different strategies, i.e, etch-and-rinse or self-etch approaches. Depending on the strategy chosen, the resulting treated substrate might present different characteristics. In dentin, while the etch-and-rinse strategy removes completely the smear layer, thus letting the tubules open for resin infiltration and hybridization, the self-etch strategy only modifies the smear layer, so the adhesive is incorporated to it. Considering the caries-affected scenario, self-etch adhesives would be incorporated to the CAD. Etch-and-rinse adhesives were found to perform better than self-etch compositions when applied to CAD [3,6]; notwithstanding, the use of self-etch materials has grown in dentistry, especially due to their easier application and less sensitive bonding protocol. Consequently, self-etch compositions are currently being considered for the development of new adhesive systems.

Within the self-etch category, one-step adhesives have the simplest protocol of application, but the most complex composition. Taking into consideration that typical components of adhesives, including but not limited to resin monomers (acidic, hydrophilic, hydrophobic), solvents, water, and photoinitiators, are all mixed together, one-step adhesives are usually associated with inferior bond strength performance [11]. One-step adhesives are very hydrophilic, which may potentiate hydrolysis of the bonding layer over time.

Furthermore, and according to some previous studies, the concentration of acidic monomer incorporated to two-step, self-etch adhesives was found to influence the immediate and long-term dentin bond strengths [12,13]. However, there are still few studies investigating the bonding performance of one-step, self-etch adhesives, especially on CAD [14]. Hence, the objectives of this study were: a) to formulate experimental one-step, self-etch adhesive systems containing different contents of acidic monomer and b) to investigate the effect of these adhesives on the effectiveness of bonding to SoD and CAD substrates.

4.3 Materials and methods

4.3.1 Preparation of dentin discs

Bovine incisors were cleaned and stored in 0.5% chloramine-T solution for seven days. Standard enamel-dentin discs (2 mm in thickness, 6 mm in diameter) were cut from the buccal surfaces of the teeth using water-cooled trephine drill. The discs were wet-ground using 80-grit SiC abrasive paper until the dentin was exposed, then wet-polished with 600-grit SiC abrasive paper for 1 min to standardize the smear layer. All discs were inspected with 40X magnification to ensure the absence of enamel. The dentin discs were randomly divided into two groups according to the type of substrate: SoD or CAD. The SoD discs were not subjected to any further treatment, whereas CAD discs were coated with nail varnish, except for the buccal surface, which was left uncoated to undergo the cariogenic challenge further detailed. All discs were sterilized using gamma radiation and kept at 4°C in a humid atmosphere until use.

4.3.2 *Formation of artificial CAD*

The experimental setup used to artificially form CAD was described elsewhere[15]. After approval by the local Research Ethics Committee (protocol 25/2013), 20 mL of fresh saliva stimulated by paraffin film were collected from a healthy volunteer (a 48-year-old female) who had not been under antibiotic therapy for at least six 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 dentin disc (n=84) in a 24-microwell plate, and it remained at rest for 1 h at 37^{o}C. After this period, the saliva was gently aspirated from the bottom of each well and 1.8 mL of defined medium enriched with mucin (DMM) [16,17] containing 1% sucrose was added; the plates were then incubated at 37^{o}C under an anaerobic atmosphere (5–10% CO_2, less than 1% O_2) [18]. 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. The cross-sectional hardness test was performed to determine integrated hardness loss (ΔS) and to confirm formation of artificially-induced CAD, as described before [15]. Briefly, four CAD specimens were longitudinally sectioned using a water-cooled diamond saw, embedded in PVC tubes using poly(methyl)methacrylate (PMMA), and wet polished with 600-, 1200-, 1500-, and 2000-grit SiC abrasive papers, with an 1 µm diamond suspension. Cross-sectional Knoop hardness measurements were carried out with a microindenter (FM-700; FutureTech, Tokyo, Japan) under a load of 5 g and with a dwell time of 5 s. Two columns with eight indentations each were made per specimen (10, 20, 30, 40, 50, 100, 150, and 200 µm from the surface). The ΔS was calculated by subtracting the hardness profile (Knoop hardness number, kgf/mm^2) of the artificial CAD from the hardness values obtained for SoD substrate.

4.3.3 Formulation of experimental one-step, self-etch adhesives

Three one-step, self-etch adhesives were prepared by mixing bisphenol-A glycidyl dimethacrylate (Bis-GMA, hydrophobic monomer), 2-hydroxyethyl methacrylate (HEMA, hydrophilic monomer), 1,3-glycerol dimethacrylate phosphate (GDMA-P, acidic monomer), water and ethanol (solvents), and photoinitiators (0.4 wt% camphorquinone, 0.8 wt% 4-(dimethyl)aminoethyl benzoate). All monomers were obtained from Esstech Inc. (Essington, PA, USA), except for GDMA-P that was synthesized as previously described [12]. The concentration of HEMA and GDMA-P varied according to the adhesive, as shown in Table 1. The adhesives were prepared using two distinct bottles (A and B), which were mixed prior to their application. The concentration of acidic monomer in the mixed adhesives was 5 wt%, 20 wt%, and 35 wt%, thus the materials were labelled as AD5, AD20, and AD35. The pH of mixed adhesives (n=3) was measured using a digital pHmeter (An2000; Analion, Ribeirão Preto, SP, Brazil).

4.3.4 Bond strength test and failure mode analysis

Dentin discs (60 SoD, 60 CAD) were cleaned with a toothbrush and distilled water and embedded in PVC tubes using PMMA [19]. The adhesives were vigorously applied for 20 s to the dentin surface using microbrush and dried with mild air stream. Elastomer molds with two cylindrical orifices (diameter 1.5 mm, thickness 0.5 mm) were placed at the center of the dentin discs. The adhesive was photoactivated for 20 s using a light-emitting diode curing unit (Radii; SDI, Bayswater, Victoria, Australia) with 800 mW/cm^2 irradiance. The orifices were filled with composite resin (Filtek Z350 XT; 3M ESPE, St. Paul, MN, USA), which was photoactivated for 20 s. The specimens were stored in distilled water at 37°C for 24 h or 6 months without renewal of the storage medium. For the bond strength test, a stainless steel wire (0.2 mm in diameter) was looped around each cylinder and aligned with the bonded interface. The shear bond strength test was conducted on a mechanical testing machine (DL500; EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min until failure. In total, 20 cylinder specimens were tested for each adhesive, substrate, and storage time combination. Fractured specimens were observed under 40× magnification using a stereomicroscope to determine the failure mode: adhesive (interfacial) or mixed failure (partially adhesive and partially cohesive within the dentin).

4.3.5 In situ degree of C=C conversion

The adhesives were applied to dentin discs (n=3) and stored in distilled water at 37°C for 24 h. The specimens were sectioned longitudinally across the bonded interfaces to obtain two resin-dentin slices, which were wet-polished with 1200 and 2500-grit SiC paper for 60 s each. After ultrasonically cleaning for 20 min in distilled water, the specimens were air-dried and *in situ* degree of C=C conversion (DC) was measured within the hybrid layer using a micro-Raman spectrometer (Xplora; Horiba, Paris, France). The spectrometer was calibrated for zero and for coefficient values using a standard silicon specimen, and the following parameters were used: 20 mW neon laser with 532 nm wavelength, spatial resolution of 3 mm, spectral 5 cm^{-1}, accumulation time of 10 s with 4 accumulations, and 100× magnification

(Olympus, London, UK) to a 1 mm beam diameter. Polymer spectra were taken at three different sites for each adhesive interface and the values averaged. Spectra of uncured adhesives (monomer) were used as reference. Post-processing of spectra was performed using LabSpec software v.6.1 (Horiba) by means of baseline correction and normalization of the range between 1590 and 1660 cm^{-1}. %DC was calculated as previously described [20].

4.3.6 SEM morphological analysis of the bonded interfaces

Two additional specimens for each substrate and for each group (n=24) were tested. Each adhesive system was applied as described before and the two dentin discs were bonded to each other using a photoactivated composite resin, generating a dentin-composite-dentin sandwiched specimen. The specimens were embedded cross-sectionally in epoxy resin for visualization of the dentin–composite interfaces. After 24 h, the specimens were wet-polished with 600-, 1200-, 1500-, and 2000-grit SiC abrasive papers and polished with 3-, 1-, and 0.5-µm diamond suspensions. The surfaces were etched with a 50% phosphoric acid aqueous solution for 5 s and deproteinized by immersion in 2.5% NaOCl aqueous solution for 10 min. The specimens were ultrasonically cleaned with distilled water and dried in a container with silica gel for 2 h, at room temperature. The polished surfaces were coated with gold and the bonded interfaces examined using scanning electron microscopy – SEM (JSM 6610, JEOL, Tokyo, Japan).

4.3.7 Histological analysis

Two specimens for each substrate and for each group tested (n=24) were separated; each adhesive system was applied as described before. Two dentin discs were bonded to each other using a photoactivated composite resin, generating dentin–composite–dentin sandwiched specimens which were cut in a precision cutting machine to obtain three slices (2 mm thick × 2 mm wide × 5 mm long) per specimen. The slices were fixed in a 10% formalin solution for 48 h and slightly demineralized in a 10% Morse solution for 48 h without agitation. Next, the slices were washed in running tap water for 24 h, neutralized in a 5%

sodium sulfate solution for the same period, washed with water again for 24 h, dehydrated in a series of increasing ethanol solutions (70% to 100%), cleared in xylol, and embedded in paraffin under vacuum. The 4-μm-thick serial sections were cut from the slices with a microtome (820 Spencer Microtome; American Optical, Buffalo, NY, USA) and stained with Goldner's Masson trichrome [21]. In this staining technique, green indicates the mineralized dentin, beige the adhesive layer, orange the collagen-resin hybridized layer, and dark red indicates the exposed collagen.

The histological sections were digitized using a light microscope (Nikon Eclipse E200; Nikon, Tokyo, Japan) connected to a video camera (Moticam 5.0; Motic®, Xiamen, China) and computer operating with Image Pro Capture Kit Platform (Media Cybernetics; Bethesda, MD, USA). The images were captured using 10× objective and stored in Tagged Image File Format. For each slide, as many fields of 540 μm were captured as necessary, in order to include the entire region of interest (Figure 1). Sixty four images were then obtained. The images were analyzed by a calibrated and blinded examiner. The calibration consisted of evaluating a series of 20 histological images, twice, at two different moments. The results of these two evaluations were subjected to a paired t-test and Pearson's correlation coefficient, showing the absence of a significant differences ($P>0.05$) and a strong correlation ($r>0.9$). The exposed collagen was quantified by means of semi-automated segmentation technique [22] in the image software.

4.3.8 Statistical analysis

All data were statistically analyzed with SigmaStat v.3.5 software (Systat Software Inc., San Jose, CA, USA). pH data were analyzed using one-way Analysis of Variance (ANOVA). DC and bond strength data were analyzed using two-way ANOVA (adhesive vs. substrate). Bond strength data between 24 h and 6 months for each adhesive and substrate were compared using t-tests. Data were transformed to ranks when necessary before the analyses. Total counts of exposed collagen mesh were analyzed using ANOVA on Ranks. All pairwise multiple comparison procedures were carried out using the Student-Newman-Keuls' method. A significance level of $\alpha=0.05$ was considered for all analyses.

4.4 Results

The acidic monomer content was associated with lower pH of the adhesives (Table 1). Formation of artificially-induced CAD was confirmed by a ΔS ranging from 2,030 to 2,964 in CAD specimens, with a lesion depth between 100 and 150 µm. Representative light micrographs of bonded interfaces of AD20 stained with Goldner's Masson trichrome are shown in Figure 1. Intertubular demineralized dentin layer in CAD was thicker than in SoD; also, a more evident exposed collagen zone along the base of the hybrid layer was observed for CAD. Table 2 shows the results for in situ DC. Whereas the factor 'adhesive' was significant ($p<0.001$), no significant differences were observed for the factor 'substrate' ($p=0.291$) or the interaction between factors ($p=0.651$). The adhesive AD35 had significantly lower DC than the other adhesives in general.

Results for 24 h and 6 months shear bond strength are also shown in Table 2. At 24 h, the factor 'adhesive' was not significant ($p=0.138$), whereas the factor 'substrate' and the interaction between factors were significant ($p<0.001$). Bonding to SoD vs. CAD at 24 h was always significantly different: bond strength to SoD was higher for AD5 and AD35, but lower for AD20. For SoD, AD35 had better immediate bonding performance than AD20, whereas for CAD the adhesive AD20 had better results than the other materials. At 6 months, both factors and their interaction were significant ($p\leq0.01$). Only AD20 showed significant differences in bonding between the substrates, again with improved bonding to CAD. The bond strengths of AD35 were higher as compared to the other adhesives at 6 months.

Comparisons for each adhesive between 24 h and 6 months are presented in Figure 2. AD20 was the only material showing stable dentin bond strengths overtime, irrespective of the dentin substrate tested. In contrast, AD5 applied to SoD showed lower bond strengths after 6 months whereas, interestingly, AD35 applied to CAD had poorer performance at 24 h. Failure modes (Figure 3) indicated a predominance of adhesive failures for all adhesives, dentin substrates, and storage periods tested. Occurrence of mixed failures seemed to be less frequent when the adhesives were tested after 6 months of storage.

Regarding the SEM analysis of the bonded interfaces (Figure 4), which show the interface formed between the experimental adhesives and SoD or CAD, it can be observed that both substrates were impregnated with the adhesives. SoD always presented a planar dentin surface to interact with the adhesive, whereas the surface of CAD, in some cases, was more irregular. The hybrid layer was generally thicker in CAD than in SoD. Results for the amount of exposed collagen in SoD and CAD bonded substrates are shown in Table 3. No appreciable differences between the substrates were generally observed. The only significant difference between SoD and CAD was observed for AD20 at 24 h, with greater exposed collagen area in CAD. At 24 h, AD35 had greater exposed collagen area in SoD than AD20, which had lower exposed collagen in SoD than the other materials at 6 months. Interestingly, the highest average fold increase in exposed collagen area between 24 h and 6 months was observed for AD20 applied to SoD, whereas AD35, for instance, showed no major changes with time.

4.5 Discussion

The main goal of this study was to investigate the role of acidic monomer concentration in one-step, self-etch adhesives, on their bonding effectiveness to SoD and CAD. The monomer used was GDMA-P, which is an effective acidic-functional methacrylate that demonstrates ability to demineralize dentin, allowing resin infiltration and long-lasting adhesion between resin-based materials and dental substrates [12,23]. One important factor that may affect dental adhesion outcomes is the acidity (pH) of the adhesive [13,24]. Self-etch adhesives are acidic in nature due to the need for demineralizing the dental substrate for infiltration. According to Leal et al. [12], the greater the content of the acidic monomer in self-etch adhesives, the greater their acidity. This corroborates the present findings, in which adhesives with higher GDMA-P content had lower pH. It is worth mentioning that, although the pH values of adhesives AD20 and AD35 were numerically close to each other, i.e., 1.2 and 1.0 respectively, the latter was two times more acidic than the former, since pH is the negative decadic logarithm of the hydrogen ion concentration. This means that even small reductions in pH may correspond to significant gains in acidity [25]. Considering this, AD35 was approximately nine times more acidic than AD5.

Despite the gain in acidity that materials may present upon incorporation of higher amounts of acidic monomer, it has been reported that greater acidity might be associated with poorer polymerization potential [26]. In fact, the adhesive with highest content of GDMA-P (AD35) showed the poorest in situ DC, which is explained by the negative effects of unreacted acidic species over C=C. No significant differences in DC were observed, in contrast, when AD5 and AD20 were compared. A possible explanation for this finding is that AD20 was probably more viscous than AD5, reducing the negative effects of increased acidity [27]. The acidic monomer content in AD35 may be considered too high as regards the C=C conversion within the hybrid layer. It is interesting to note, however, that the more acidic adhesives seem to have produced enhanced adhesion to dentin, although their performance was dependent on both substrate type and water storage period.

It is already well-accepted that acidic methacrylates may not interact with hard dental tissues through an exclusive acid-dependent mechanism, but also through a process known as “Adhesion-decalcification concept” [28,29]. This concept states that any acidic molecule is able to chemically bond to hydroxyapatite, thus forming a calcium salt, and depending on the stability of the salt, the acid may remain bonded to (adhesion) or de-bond (decalcification) from the substrate [30]. To fully understand the effect of the concept on the present findings, two points should be considered: i) SoD is morphologically different from CAD, i.e., the former has a mineralized substrate with open tubules and the latter has a partially demineralized intertubular dentin with mineral deposits occluding most of the tubules [31,32] and ii) the three adhesives prepared in this study were different from each other regarding their acidic potential – AD5, AD20, and AD35 can be accordingly classified as ‘mild’ (pH around 2), ‘intermediately strong’ (pH between 1 and 2), and ‘strong’ (pH ≤ 1.0) self-etch adhesives [30]. At 24 h, while SoD was better hybridized with AD35, this adhesive produced the lowest bonding ability to CAD; conversely, AD20 performed better in CAD than in SoD. It can be suggested that AD35 was too acidic for application in the already demineralized CAD, over-etching (decalcification process) the substrate. By contrast, the use of the ‘moderately strong’ AD20 allowed proper demineralization and resin infiltration of the substrate (adhesion process). Occurrence of more than 20% of mixed failures happened only in SoD treated with AD35 and in CAD treated with AD20, corroborating the bond strength results. The SEM micrographs also confirm the present findings, showing the over-demineralized aspect of CAD treated with AD35, as opposed to AD20, which exhibited the presence of resin tags, indicating resin infiltration through the substrate.

One of the major disadvantages of one-step systems is their excessive hydrophilicity, derived from the presence of acidic species and water. This excessive hydrophilicity makes the adhesives more prone to attract water molecules from dentin, for instance [33]. As the adhesive layer acts as semipermeable membranes even after polymerization, water diffusion through the hybrid layer might occur [34]. Such permeability contributes to polymer hydrolysis and degradation of the resin-dentin interface overtime [35,36]. In contrast to previous studies [37-39], the present findings demonstrated that the adhesion generally stayed stable overtime, except for two groups: SoD treated with AD5 (decreased bond strengths at 6 months), and CAD treated with AD35 (improved bond strengths after 6 months). Composition of the adhesives differed only in the content of GDMA-P, and HEMA, as a consequence. A previous study showed that incorporation of more than 10 wt% of HEMA into self-etch adhesives had no advantageous effects on the adhesive performance [40]. Therefore, considering that adhesives AD5, AD20, and AD35 were constituted of 40, 25, and 10 wt% of HEMA, faster hydrolytic degradation processes could be expected for substrates treated with AD5. The bonding performance of AD5 applied to SoD, comparing 24 h and 6 months results, corroborates this assumption. However, this was true only in SoD, so explanation might rely on other phenomena, such as the total amount of exposed collagen. After 6 months, the amount of exposed collagen for groups treated with AD5 was 9.5 times higher in SoD and only 4.3 times higher in CAD, indicating that more hydrolysis occurred within the former than the latter. It seems that the presence of demineralized dentin in CAD prior to the adhesive application facilitated resin infiltration and interlocking with the exposed collagen fibrils, reducing degradation and consequently the exposure of new collagen fibrils overtime.

Histological staining differences between CAD and SoD are usually dependent on the availability of exposed collagen for reaction with the Goldner’s Masson trichrome stains. The presence of partially demineralized dentin (stained red) in CAD indicates more exposure of collagen fibrils, differently from the underlying intact dentin packed by minerals (stained green). Although the bond strength to CAD treated with AD35 was low at 24 h, it was higher compared to the other adhesives after 6 months of water storage. One can note that the amount of exposed collagen in CAD at 24 h was low upon application of AD35, so it can be expected that most collagen fibrils were impregnated with adhesive. As a consequence, less degradation occurred overtime. In addition, the greater variability and more irregular topography of CAD [31] may contribute to improving the micromechanical interlocking of the adhesive, as well as to increasing the thickness of the hybrid layer. This could also explain the bond strength results obtained.

4.6 Conclusion

In conclusion, the experimental one-step, self-etch adhesive systems synthesized in this study worked effectively as dentin bonding agents. Caries-affected dentin was not always a more challenging bonding substrate when compared to sound dentin. The effectiveness of the adhesives was found dependent on factors such as the concentration of acidic monomer and acidity of the adhesive, in addition to water storage period. The adhesive with 20 wt% acidic methacrylate had the highest C=C conversion and yielded stable bond strengths that were, in general, independent of the dentin substrate tested.

4.7 References

1. Selwitz RH, Ismail AI, Pitts NB. Dental Caries. Lancet 2007;369:51-9.

2. Maltz M, Alves LS, Jardim JJ, Moura Mdos S, de Oliveira EF. Incomplete caries removal in deep lesions: a 10-year prospective study. American Journal of Dentistry 2011;24:211-4.

3. Arrais CAG, Giannini M, Nakajima M, Tagami J. Effects of additional and extended acid etching on bonding to caries-affected dentine. European Journal Oral Sciences 2004;112:458-64.

4. 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. Dental Materials Journal 2013;32:138-43.

5. Xuan W, Hou BX, Lu YL. Bond strength of different adhesives to normal and caries-affected dentins. Chinese Medical Journal (Engl) 2010;123:332-6.

6. Zanchi CH, D'Avila OP, Rodrigues SA, Burnett LH, Demarco FF, Pinto MB. Effect of Additional Acid Etching on Bond Strength and Structural Reliability of Adhesive Systems Applied to Caries-affected Dentin. Journal of Adhesive Dentistry 2010;12:109-15.

7. Daculsi G, LeGeros RZ, Jean A, Kerebel B. Possible physico-chemical processes in human dentin caries. Journal of Dental Research 1987;66:1356-9.

8. Ogawa K, Yamashita Y, Ichijo T, Fusayama T. The ultrastructure and hardness of the transparent layer of human carious dentin. Journal of Dental Research 1983;62:7-10.

9. Marshall GW, Habelitz S, Gallagher R, Balooch M, Balooch G, Marshall SJ. Nanomechanical properties of hydrated carious human dentin. Journal of Dental research 2001;80:1768-71.

10. Nakajima M, Kunawarote S, Prasansuttiporn T, Tagami J. Bonding to caries-affected dentin. Japanese Dental Science Review 2011;47:102-14.

11. De Munck J, Vargas M, Iracki J, Van Landuyt K, Poitevin A, Lambrechts P, et al. One-day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. Operative Dentistry 2005;30:39-49.

12. Leal FB, Madruga FC, Prochnow EP, Lima GS, Ogliari FA, Piva E, et al. Effect of acidic monomer concentration on the dentin bond stability of self-etch adhesives. International Journal of Adhesion and Adhesives 2011;31:571-4.

13. Munchow EA, da Silva AF, da Silveira Lima G, Wulff T, Barbosa M, Ogliari FA, et al. Polypropylene glycol phosphate methacrylate as an alternative acid-functional monomer on self-etching adhesives. Journal of Dentistry 2015;43:94-102.

14. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dental materials : official publication of the Academy of Dental Materials 2011;27:1-16.

15. Maske TT, Isolan CP, van de Sande FH, Peixoto AC, Faria ESAL, Cenci MS, et al. A biofilm cariogenic challenge model for dentin demineralization and dentin bonding analysis. Clinical Oral Investigations 2015;19:1047-53.

16. Wong L, Sissons C. A comparison of human dental plaque microcosm biofilms grown in an undefined medium and a chemically defined artificial saliva. Archives of Oral Biology 2001;46:477-86.

17. Wong L, Sissons CH. Human dental plaque microcosm biofilms: effect of nutrient variation on calcium phosphate deposition and growth. Archives of Oral Biology 2007;52:280-9.

18. 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;27:1057-63.

19. Navarra CO, Cadenaro M, Armstrong SR, Jessop J, Antoniolli F, Sergo V, et al. Degree of conversion of Filtek Silorane Adhesive System and Clearfil SE Bond within the hybrid and adhesive layer: an in situ Raman analysis. Dental materials : official publication of the Academy of Dental Materials 2009;25:1178-85.

20. Moraes RR, Faria-e-Silva AL, Ogliari FA, Correr-Sobrinho L, Demarco FF, Piva E. Impact of immediate and delayed light activation on self-polymerization of dual-cured dental resin luting agents. Acta Biomaterialia 2009;5:2095-100.

21. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence International (Berlin, Germany : 1985) 1999;30:501-7.

22. Vasconcelos AC, Berti-Couto SA, Azambuja AA, Salum FG, Figueiredo MA, da Silva VD, et al. Comparison of effects of clodronate and zoledronic acid on the repair of maxilla surgical wounds - histomorphometric, receptor activator of nuclear factor-kB ligand, osteoprotegerin, von Willebrand factor, and caspase-3 evaluation. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology 2012;41:702-12.

23. Macedo CLR, Münchow EA, Lima GS, Zanchi CH, Ogliari FA, Piva E. Incorporation of inorganic fillers into experimental resin adhesives: Effects on

physical properties and bond strength to dentin. International Journal of Adhesion and Adhesives 2015;62:78-84.

24. Dieng-Sarr F, Sharrock P, Dabsie F, Grégoire G. Modifications of the organic and mineral fractions of dental tissues following conditioning by self-etching adhesives. Journal of Dentistry 2011;39:141-7.

25. Kaaden C, Powers JM, Friedl KH, Schmalz G. Bond strength of self-etching adhesives to dental hard tissues. Clinical Oral Investigations 2002;6:155-60.

26. Moraes RR, Guimaraes GZ, Oliveira AS, Faot F, Cava SS. Impact of acidic monomer type and concentration on the adhesive performance of dental zirconia primers. International Journal of Adhesion and Adhesives 2012;39:49-53.

27. Zhang Y, Wang Y. The effect of hydroxyapatite presence on the degree of conversion and polymerization rate in a model self-etching adhesive. Dental materials : official publication of the Academy of Dental Materials 2012;28:237-44.

28. Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y, et al. Adhesion to and decalcification of hydroxyapatite by carboxylic acids. Journal of Dental Research 2001;80:1565-9.

29. Yoshioka M, Yoshida Y, Inoue S, Lambrechts P, Vanherle G, Nomura Y, et al. Adhesion/decalcification mechanisms of acid interactions with human hard tissues. Journal of Biomedical Materials Research 2002;59:56-62.

30. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dental materials : official publication of the Academy of Dental Materials 2011;27:17-28.

31. Nakajima M, Sano H, Zheng L, Tagami J, Pashley DH. Effect of moist vs. dry bonding to normal vs. caries-affected dentin with Scotchbond Multi-Purpose Plus. Journal of Dental Research 1999;78:1298-303.

32. Tay FR, Pashley DH, Hiraishi N, Imazato S, Rueggeberg FA, Salz U, et al. Tubular occlusion prevents water-treeing and through-and-through fluid movement in a single-bottle, one-step self-etch adhesive model. Journal of Dental Research 2005;84:891-6.

33. Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? Journal (Canadian Dental Association) 2003;69:726-31.

34. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. Journal of Dentistry 2002;30:371-82.

35. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. Biomaterials 2003;24:3795-803.

36. Hashimoto M, Tay FR, Ohno H, Sano H, Kaga M, Yiu C, et al. SEM and TEM analysis of water degradation of human dentinal collagen. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2003;66:287-98.

37. Vanajasan PP, Dhakshinamoorthy M, Rao CS. Factors affecting the bond strength of self-etch adhesives: A meta-analysis of literature. Journal of Conservative Dentistry : JCD 2011;14:62-7.

38. Abdalla AI. Effect of long-term water aging on microtensile bond strength of self-etch adhesives to dentin. American Journal of Dentistry 2010;23:29-33.

39. Erhardt MC, Toledano M, Osorio R, Pimenta LA. Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure. Dental materials : official publication of the Academy of Dental Materials 2008;24:786-98.

40. Van Landuyt KL, Snauwaert J, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. The role of HEMA in one-step self-etch adhesives. Dental Materials : official publication of the Academy of Dental Materials 2008;24:1412-9.

Table 1. Composition of the experimental one-step, self-etch adhesives tested (wt%)

Reagent	AD5			AD20			AD35		
	Bottle A	Bottle B	A+B	Bottle A	Bottle B	A+B	Bottle A	Bottle B	A+B
GDMA-P	10%	-	5%	40%	-	20%	70%	-	35%
HEMA	65%	15%	40%	35%	15%	25%	5%	15%	10%
Bis-GMA	10%	50%	30%	10%	50%	30%	10%	50%	30%
Water	-	20%	10%	-	20%	10%	-	20%	10%
Ethanol	15%	15%	15%	15%	15%	15%	15%	15%	15%
pH (mean ± SD)			1.93 ± 0.15[A]			1.25 ± 0.04[B]			1.05 ± 0.05[C]

Distinct letters indicate statistically significant differences in pH between the adhesives ($p<0.05$).

Table 2. Means ± SD for in situ degree of C=C conversion (DC) and dentin bond strengths at 24 h and 6 months

Adhesive	DC, %		Bond strength, MPa*			
			24 h		6 months	
	SoD	CAD	SoD	CAD	SoD	CAD
AD5	66.6 ± 8.1 ^{A,ab}	66.7 ± 2.4 ^{A,a}	5.8 ± 2.5 ^{A,ab}	3.8 ± 1.5 ^{B,b}	3.9 ± 0.5 ^{A,b}	4.3 ± 1.0 ^{A,c}
AD20	72.3 ± 4.8 ^{A,a}	69.8 ± 4.4 ^{A,a}	4.8 ± 2.3 ^{B,b}	6.3 ± 2.0 ^{A,a}	4.2 ± 0.7 ^{B,b}	5.1 ± 0.6 ^{A,b}
AD35	58.6 ± 2.3 ^{A,b}	53.5 ± 4.5 ^{A,b}	6.7 ± 2.2 ^{A,a}	3.5 ± 1.3 ^{B,b}	6.0 ± 1.1 ^{A,a}	5.9 ± 1.0 ^{A,a}

Uppercase letters in the same line indicate significant differences between sound (SoD) and caries-affected dentin (CAD); lowercase letters in each column indicate significant differences between adhesives with 5 wt% (AD5), 20 wt% (AD20), or 35 wt% (AD35) acidic monomer ($p<0.05$). *Statistical comparisons are restricted within each storage time.

Table 3. Medians (minima-maxima) for total count of exposed collagen found for each group tested

Adhesive	24 h		6 months		Average fold increase*	
	SoD	CAD	SoD	CAD	SoD	CAD
AD5	3 (0-20) [A,ab]	0 (0-116) [A,a]	76 (0-147) [A,a]	94 (37-176) [A,a]	9.5	4.3
AD20	0 (0-2) [B,b]	33 (4-93) [A,a]	5 (0-36) [A,b]	5 (3-139) [A,a]	28.8	1.2
AD35	69 (12-125) [A,a]	7 (0-69) [A,a]	46 (3-274) [A,a]	18 (0-108) [A,a]	1.1	1.4

SoD: sound dentin; CAD: caries-affected dentin. *24 h vs. 6 months.

For each storage time, uppercase letters in the same line indicate significant differences between sound (SoD) and caries-affected dentin (CAD); lowercase letters in each column indicate significant differences between adhesives with 5 wt% (AD5), 20 wt% (AD20), or 35 wt% (AD35) acidic monomer ($p<0.05$).

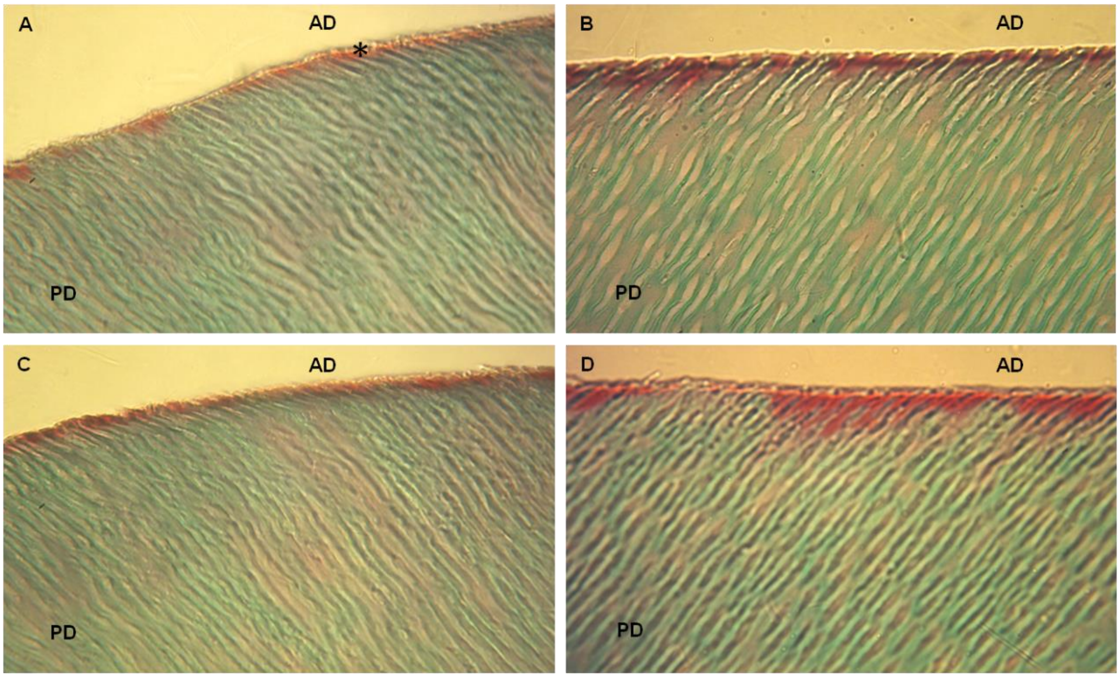

Figure 1. Representative light micrographs of bonded interfaces of AD20 (AD) stained with Goldner's Masson trichrome (original magnification: 400×). (A) SoD at 24 h; (B) CAD at 24 h; (C) SoD after 6 months; and (D) CAD after 6 months. Exposed collagen stained in red and partially demineralized dentin stained in green (PD). Adhesive-hybridized collagen stained in orange (asterisk). Intertubular demineralized dentin layer in CAD is thicker than in SoD, and a more evident red line along the base of the hybrid layer can be seen.

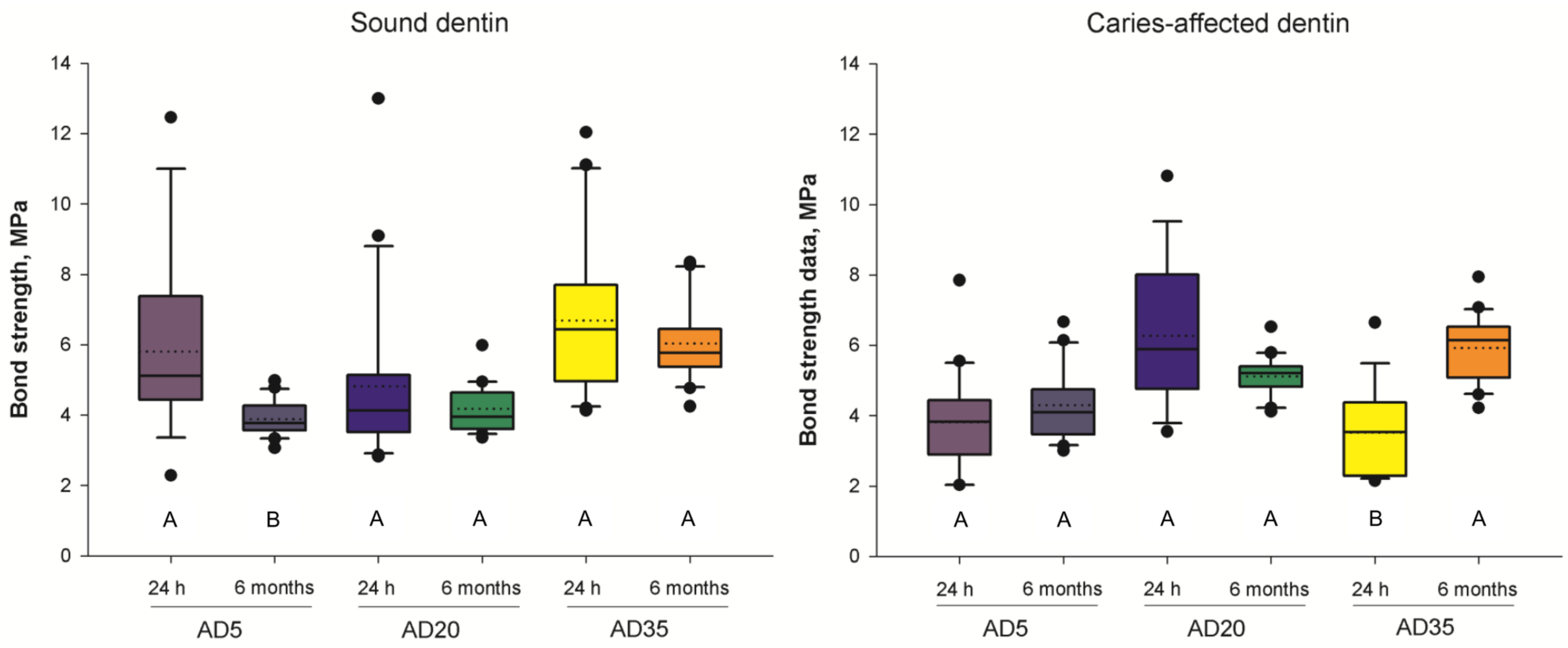

Figure 2. Box-plot comparing the bond strengths at 24 h and 6 months for each adhesive applied to sound and caries-affected dentin (solid and dashed lines in the center of each bar indicate medians and means). For each substrate, distinct letters indicate differences between 24 h and 6 months for each adhesive ($p<0.05$).

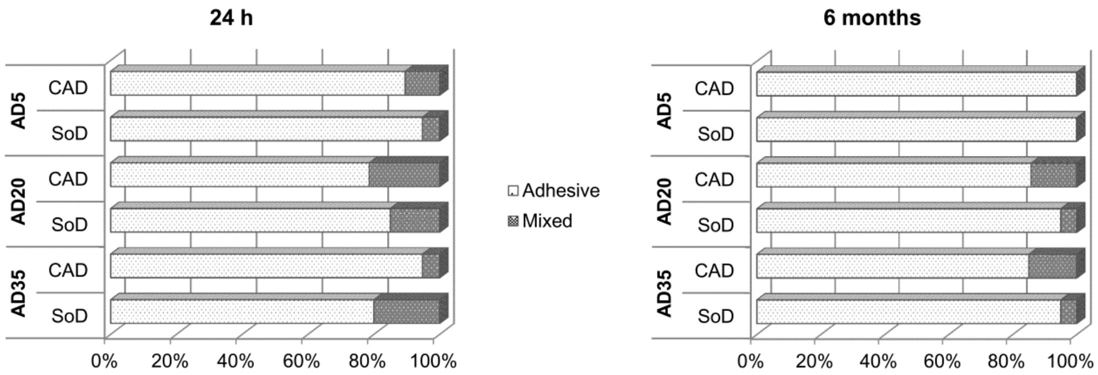

Figure 3. Distribution of the immediate and 6 months failure modes for all groups. Adhesive: failures between composite and dentin; mixed: failures partially adhesive and partially cohesive within dentin.

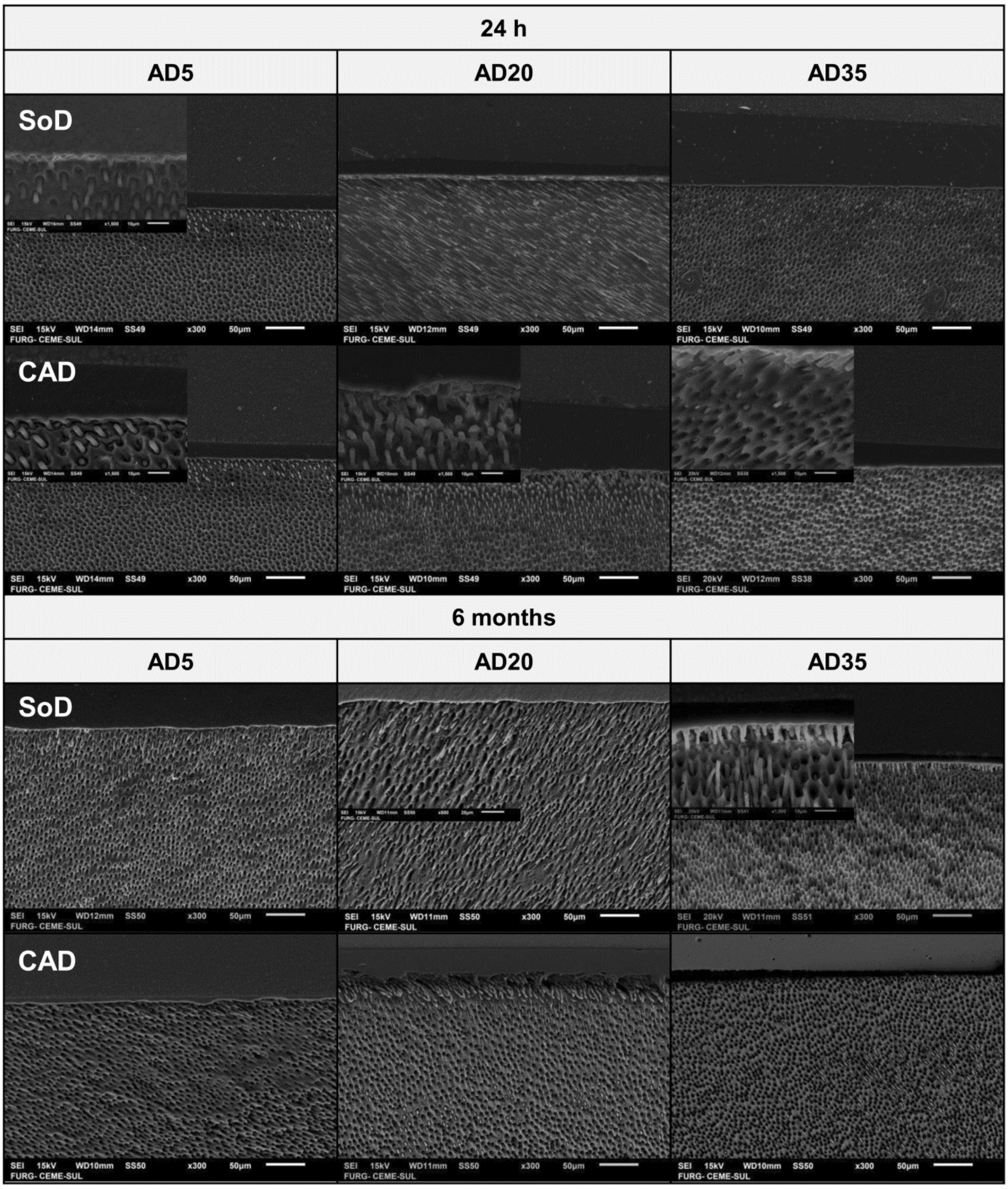

Figure 4. SEM micrographs of the bonded interfaces between the experimental adhesives and SoD or CAD (magnification: ×300 or ×1500 for the insert images). Both dentin substrates were impregnated with the adhesives. SoD always presented a planar dentin surface to interact with the adhesive, whereas the surface of CAD, in some cases, was more irregular. The hybrid layer was generally thicker in CAD than in SoD.

5 Considerações finais

A resistência de união do adesivo universal testado se mostrou comparável à de outros sistemas adesivos contemporâneos, embora dependente do substrato avaliado. Adesivos Universais parecem ter potencial de aplicabilidade em diferentes especialidades da odontologia .

A dentina afetada por cárie motrou-se um substrato mais desafiador para união do que a dentina sadia, independentemente da metodologia usada para adesão. Quando se faz a união em dentina afetada por cárie, a literatura *in vitro* aponta o uso preferencial de adesivos convencionais.

Os sistemas adesivos experimentais autocondicionantes de passo único e mistura prévia sintetizados nesse estudo foram efetivos como agentes de união à dentina; nem sempre a dentina afetada por cárie mostrou aspecto menos favorável do que a dentina sadia. No entanto, a dentina afetada por cárie mostrou-se dependente de fatores como a acidez do adesivo e o período de estocagem em água. Uma maior acidez do adesivo parece contribuir positivamente para uma maior durabilidade da união, independentemente da condição da dentina.

Referências

3MESPE. "Scotchbond™ Universal Adhesive." Retrieved November 25, 2014, from http://http://multimedia.3m.com/mws/media/754753O/scotchbond-universal-adhesive.pdf?fn=scotchbond_uni_brochure.pdf.

ABDALLA, A. I. Effect of long-term water aging on microtensile bond strength of self-etch adhesives to dentin. **American Journal Dentistry,** v.23, n.1, p.29-33, 2010.

ALEX, G. Is total-etch dead? Evidence suggests otherwise. **Compendium of Continuing Education in Dentistry,** v.33, n.1, p.12-14, 16-22, 24-15, 2012.

ARRAIS, C. A. G., GIANNINI, M., NAKAJIMA, M., TAGAMI, J. Effects of additional and extended acid etching on bonding to caries-affected dentine. **European Journal Oral Sciences,** v.112, n.5, p.458-464, 2004.

BANERJEE, A., KELLOW, S., MANNOCCI, F., COOK, R. J., WATSON, T. F. An in vitro evaluation of microtensile bond strengths of two adhesive bonding agents to residual dentine after caries removal using three excavation techniques. **Journal of Dentistry,** v.38, n.6, p.480-489, 2010.

BORUAH , L., CHANDRA , A., LOOMBA , K., UDAI SINGH, U., TIKKU, A. Influence of caries detection dye on bond strength of sound and carious affected dentin: An in-vitro study. **Journal of Conservative Dentistry** v.14, p.32-35, 2011.

BRESCHI, L., PERDIGAO, J., GOBBI, P., MAZZOTTI, G., FALCONI, M., LOPES, M. Immunocytochemical identification of type I collagen in acid-etched dentin. **Journal of Biomedical Materials Research Part A,** v.66, n.4, p.764-769, 2003.

BURROW, M. F., BOKAS, J., TANUMIHARJA, M., TYAS, M. J. Microtensile bond strengths to caries-affected dentine treated with Carisolv (R). **Australian Dental Journal,** v.48, n.2, p.110-114, 2003.

CEBALLOS, L., CAMEJO, D. G., FUENTES, M. V., OSORIO, R., TOLEDANO, M., CARVALHO, R. M., PASHLEY, D. H. Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine. **Journal of Dentistry,** v.31, n.7, p.469-477, 2003.

COSTA, T. R., FERREIRA, S. Q., KLEIN-JUNIOR, C. A., LOGUERCIO, A. D., REIS, A. Durability of surface treatments and intermediate agents used for repair of a polished composite. **Operative Dentistry,** v.35, n.2, p.231-237, 2010.

DACULSI, G., LEGEROS, R. Z., JEAN, A., KEREBEL, B. Possible physico-chemical processes in human dentin caries. **Journal of Dental Research,** v.66, n.8, p.1356-1359, 1987.

DE-ALMEIDA NEVES, A. A., COUTINHO, E., CARDOSO, M. V., DE MUNCK, J., VAN MEERBEEK, B.Current concepts and techniques for caries excavation and adhesion to residual dentin. **Journal Adhesive Dentistry,** v.13, p.7-22, 2011.

DE-MELO, M. A. S., GOES, D. D., DE-MORAES, M. D. R., SANTIAGO, S. L., RODRIGUES, L. K. A. Effect of chlorhexidine on the bond strength of a self-etch adhesive system to sound and demineralized dentin. **Brazilian Oral Research,** v.27, n.3, p.218-224, 2013.

DE MUNCK, J., VAN LANDUYT, K., PEUMANS, M., POITEVIN, A., LAMBRECHTS, P., BRAEM, M., VAN MEERBEEK, B. A critical review of durability of adhesion to tooth tissue: methods and results. **Journal of Dental Research,** v.84, p.118-132, 2005.

DE MUNCK, J., VARGAS, M., IRACKI, J., VAN LANDUYT, K., POITEVIN, A., LAMBRECHTS, P., VAN MEERBEEK, B. One-day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. **Operative Dentistry,** v.30, n.1, p.39-49, 2005.

DIENG-SARR, F., SHARROCK, P., DABSIE, F., GRÉGOIRE, G. Modifications of the organic and mineral fractions of dental tissues following conditioning by self-etching adhesives. **Journal of Dentistry,** v.39, n.2, p.141-147, 2011.

DOI, J., ITOTA, T., TORII, Y., NAKABO, S., YOSHIYAMA, M. Micro-tensile bond strength of self-etching primer adhesive systems to human coronal carious dentin. **Journal Oral Rehabilitation,** v.31, n.10, p.1023-1028, 2004.

EKAMBARAM, M., YIU, C. K., MATINLINNA, J. P. Bonding of resin adhesives to caries-affected dentin - A systematic review. **International Journal of Adhesion and Adhesives,** v.61, p.23-34, 2015.

EKAMBARAM, M., YIU, C. K., MATINLINNA, J. P., KING, N. M., TAY, F. R. Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine. **Journal of Dentistry,** v.42, n.6, p.709-3719, 2014.

ERGUCU, Z., CELIK, E. U., UNLU, N., TURKUN, M., OZER, F. Effect of Er,Cr:YSGG Laser on the Microtensile Bond Strength of Two Different Adhesives to the Sound and Caries-affected Dentin. **Operative Dentistry,** v.34, n.4, p.460-466, 2009.

ERHARDT, M. C., LOBO, M. M., GOULART, M., COELHO-DE-SOUZA, F. H., VALENTINO, T. A., PISANI-PROENCA, J., CONCEICAO, E. N., PIMENTA, L. A. Microtensile bond strength of etch-and-rinse and self-etch adhesives to artificially created carious dentin. **General Dentistry,** v.62, n.3, p.56-61, 2014.

ERHARDT, M. C., TOLEDANO, M., OSORIO, R., PIMENTA, L. A. Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure. **Dental Materials,** v.24, n.6, p.786-798, 2008.

ERHARDT, M. C. G., OSORIO, R., TOLEDANO, M. Dentin treatment with MMPs inhibitors does not alter bond strengths to caries-affected dentin. **Journal of Dentistry,** v.36, n.12, p.1068-1073, 2008.

ERHARDT, M. C. G., RODRIGUES, J. A., VALENTINO, T. A., RITTER, A. V., PIMENTA, L. A. F. In vitro µTBS of one-bottle adhesive systems: Sound versus artificially-created caries-affected dentin. **Journal of Biomedical Materials Research Part B: Applied Biomaterials,** v.86, n.1, p.181-187, 2008.

FONTES, S. T., LIMA, G. S., OGLIARI, F. A., PIVA, E., MORAES, R. R. Hybridization morphology and dentin bond stability of self-etch primers with different ethanol/water ratios. **Odontology,** v.100, n.2, p.181-186, 2012.

FUSAYAMA, T., NAKAMURA, M., KUROSAKI, N., IWAKU, M. Non-pressure adhesion of a new adhesive restorative resin. **Journal of Dental Research,** v.58, n.4, p.1364-1370, 1979.

GIACHETTI, L., SCAMINACI RUSSO, D., BERTINI, F., PIERLEONI, F., NIERI, M. Effect of operator skill in relation to microleakage of total-etch and self-etch bonding systems. **Journal of Dentistry,** v.35, n.4, p.289-293, 2007.

GIRIYAPPA, R. H., CHANDRA, B. S. Comparative evaluation of self-etching primers with fourth and fifth generation dentin-bonding systems on carious and normal dentin substrates: An in vitro shear bond strength analysis. **Journal Conservative Dentistry,** v.11, n.4, p.154-158, 2008.

HAAK, R., WICHT, M. J., NOACK, M. J. Does chemomechanical caries removal affect dentine adhesion? **European of Journal Oral Sciences,** v.108, n.5, p.449-455, 2000.

HAMAMA, H. H., YIU, C. K., BURROW, M. F. Effect of chemomechanical caries removal on bonding of self-etching adhesives to caries-affected dentin. **Journal Adhesive Dentistry,** v.16, n.6, p.507-516, 2014.

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

HASHIMOTO, M., OHNO, H., SANO, H., KAGA, M., OGUCHI, H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. **Biomaterials,** v.24, n.21, p.3795-3803, 2003.

HASHIMOTO, M., TAY, F. R., OHNO, H., SANO, H., KAGA, M., YIU, C., KUMAGAI, H., KUDOU, Y., KUBOTA, M., OGUCHI, H. SEM and TEM analysis of water degradation of human dentinal collagen. **Journal Biomedical Materials Research Part B: Applied Biomaterials,** v.66, n.1, p.287-298, 2003.

HICKEL, R., BRUSHAVER, K., ILIE, N. Repair of restorations--criteria for decision making and clinical recommendations**,** v.29, n.1, p.28-50, 2013.

HIGGINS, J. P. T., ALTMAN, D. G., STERNE, J. A. C. Assessing risk of bias in included studies. In: Higgins JPT, Green S, editors. **Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011),** v.8, p.www.cochrane-handbook.org., 2011.

HIGGINS, J. P. T., GREEN, S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 **Cochrane Library,** p.www.cochrane-handbook.org., 2011.

HSU, K. W., MARSHALL, S. J., PINZON, L. M., WATANABE, L., SAIZ, E., MARSHALL, G. W. SEM evaluation of resin-carious dentin interfaces formed by two dentin adhesive systems. **Dental Materials Journal,** v.24, n.7, p.880-887, 2008.

HUANG, X. Q., LI, L., HUANG, C., DU, X. J. Effect of ethanol-wet bonding with hydrophobic adhesive on caries-affected dentine. **European Journal Oral of Sciences,** v.119, n.4, p.310-315, 2011.

INOUE, G., TSUCHIYA, S., NIKAIDO, T., FOXTON, R. M., TAGAMI, J. Morphological and mechanical characterization of the acid-base resistant zone at the adhesive-dentin interface of intact and caries-affected dentin. **Operative Dentistry,** v.31, n.4, p.466-472, 2006.

ITO, S., SAITO, T., TAY, F. R., CARVALHO, R. M., YOSHIYAMA, M., PASHLEY, D. H. Water content and apparent stiffness of non-caries versus caries-affected human dentin. **Journal of Biomedical Materials Research Part B:Applied Biomaterials,** v.72B, n.1, p.109-116, 2005.

JOVES, G. J., INOUE, G., NAKASHIMA, S., SADR, A., NIKAIDO, T., TAGAMI, J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. **Dental Materials Journal,** v.32, n.1, p.138-143, 2013.

KAADEN, C., POWERS, J. M., FRIEDL, K. H., SCHMALZ, G. Bond strength of self-etching adhesives to dental hard tissues. **Clinical Oral Investigations,** v.6, n.3, p.155-160, 2002.

KANCA, J., 3RD. One step bond strength to enamel and dentin. **American Journal of Dentistry,** v.10, n.1, p.5-8, 1997.

KIMOCHI, T., YOSHIYAMA, M., URAYAMA, A., MATSUO, T. Adhesion of a new commercial self-etching/self-priming bonding resin to human caries-infected dentin. **Dental Materials,** v.18, n.4, p.437-443, 1999.

KITA, T., ISHII, K., YOSHIKAWA, K., YASUO, K., YAMAMOTO, K., AWAZU, K. In vitro study on selective removal of bovine demineralized dentin using nanosecond pulsed laser at wavelengths around 5.8 μm for realizing less invasive treatment of dental caries.**Journal of Lasers Medical Sciences,** v.30, n.3, p.961-967, 2015.

KOMORI, P. C. P., PASHLEY, D. H., TJADERHANE, L., BRESCHI, L., MAZZONI, A., DE GOES, M. F., WANG, L., CARRILHO, M. R. Effect of 2% Chlorhexidine Digluconate on the Bond Strength to Normal Versus caries-affected dentin. **Operative Dentistry,** v.34, n.2, p.157-165, 2009.

KOYUTURK, A. E., OZMEN, B., CORTCU, M., TOKAY, U., TOSUN, G., ERHAN SARI, M. Effects of Er:YAG laser on bond strength of self-etching adhesives to caries-affected dentin. **Microscopy Research and Technique,** v.77, n.4, p.282-288, 2014.

KOYUTURK, A. E., SENGUN, A., OZER, F., SENER, Y., GOKALP, A. Shear bond strengths of self-etching adhesives to caries-affected dentin on the gingival wall. **Dental Materials Journal,** v.25, n.1, p.59-65, 2006.

KUNAWAROTE, S., NAKAJIMA, M., FOXTON, R. M., TAGAMI, J. Effect of pretreatment with mildly acidic hypochlorous acid on adhesion to caries-affected dentin using a self-etch adhesive. **European Journal of Oral Sciences,** v.119, n.1, p.86-92, 2011.

LEAL, F. B., MADRUGA, F. C., PROCHNOW, E. P., LIMA, G. S., OGLIARI, F. A., PIVA, E., MORAES, R. R. Effect of acidic monomer concentration on the dentin bond stability of self-etch adhesives. **International Journal of Adhesion and Adhesives,** v.31, n.6, p.571-574, 2011.

LENZI, T. L., BRAGA, M. M., RAGGIO, D. P. Shortening the etching time for etch-and-rinse adhesives increases the bond stability to simulated caries-affected primary dentin. **Journal Adhesive Dentistry,** v.16, n.3, p.235-241, 2014.

LIMA, G. S., OGLIARI, F. A., DA SILVA, E. O., ELY, C., DEMARCO, F. F., CARRENO, N. L., PETZHOLD, C. L., PIVA, E. Influence of water concentration in an experimental self-etching primer on the bond strength to dentin. **Journal Adhesive Dentistry,** v.10, n.3, p.167-172, 2008.

LIMA, G. S., OGLIARI, F. A., MORAES, R. R., MATTOS, E. S., SILVA, A. F., CARREÑO, N. L. V., PETZHOLD, C. L., PIVA, E. Water Content in Self-Etching Primers Affects Their Aggressiveness and Strength of Bonding to Ground Enamel. **The Journal of Adhesion,** v.86, n.9, p.939-952, 2010.

MACEDO, C.L., MÜNCHOW, E.A., LIMA, G.S., ZANCHI, C.H., OGLIARI, F.A., PIVA, E. Incorporation of inorganic fillers into experimental resin adhesives: Effects on physical properties and bond strength to dentin. **International Journal of Adhesion and Adhesives,** v.62, p.78-84, 2015.

MACEDO, G. V., YAMAUCHI, M., BEDRAN-RUSSO, A. K. Effects of Chemical Cross-linkers on Caries-affected Dentin Bonding. **Journal of Dental Research,** v.88, n.12, p.1096-1100, 2009.

MALTZ, M., ALVES, L. S., JARDIM, J. J., MOURA MDOS, S., DE OLIVEIRA, E. F. Incomplete caries removal in deep lesions: a 10-year prospective study. **American Journal Dentistry,** v.24, n.4, p.211-214, 2011.

MALTZ, M., OLIVEIRA, E. F., FONTANELLA, V., CARMINATTI, G. Deep caries lesions after incomplete dentine caries removal: 40-month follow-up study. **Caries Research,** v.41, n.6, p.493-496, 2007.

MARSHALL, G. W., HABELITZ, S., GALLAGHER, R., BALOOCH, M., BALOOCH, G., MARSHALL, S. J. Nanomechanical properties of hydrated carious human dentin. **Journal of Dental Research,** v.80, n.8, p.1768-1771, 2001.

MASKE, T. T., ISOLAN, C. P., VAN DE SANDE, F. H., PEIXOTO, A. C., FARIA, E. S. A. L., CENCI, M. S., MORAES, R. R. A biofilm cariogenic challenge model for dentin demineralization and dentin bonding analysis. **Clinical Oral Investigations,** v.19, n.5, p.1047-1053, 2015.

MASSARA, M. L., ALVES, J. B., BRANDAO, P. R. Atraumatic restorative treatment: clinical, ultrastructural and chemical analysis. **Caries Research,** v.36, n.6, p.430-436, 2002.

MATINLINNA, J. P., LASSILA, L. V., OZCAN, M., YLI-URPO, A., VALLITTU, P. K. An introduction to silanes and their clinical applications in dentistry. **International Journal of Prosthodontics,** v.17, n.2, p.155-164, 2004.

MOBARAK, E. H. Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. **Operative Dentistry,** v.36, n.6, p.649-660, 2011.

MOBARAK, E. H., EL-BADRAWY, W. H. Microshear Bond Strength of Self-etching Adhesives to Caries-affected Dentin Identified Using the Dye Permeability Test. **Journal of Adhesive Dentistry,** v.14, n.3, p.245-250, 2012.

MOHER, D., LIBERATI, A., TETZLAFF, J., ALTMAN, D. G., GROUP, P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. **PLoS Medicine,** v.6, n.7, p.e1000097, 2009.

MONTAGNER, A. F., SARKIS-ONOFRE, R., PEREIRA-CENCI, T., CENCI, M. S. MMP Inhibitors on Dentin Stability: A Systematic Review and Meta-analysis. **Journal of Dental Research,** v.93, n.8, p.733-743, 2014.

MORAES, A. P., SARKIS-ONOFRE, R., MORAES, R. R., CENCI, M. S., SOARES, C. J., PEREIRA-CENCI, T. Can Silanization Increase the Retention of Glass-fiber posts? A Systematic Review and Meta-analysis of In Vitro Studies. **Operative Dentistry,** 2015.

MORAES, R.R., GUIMARAES, G.Z., OLIVEIRA, A.S., FAOT, F., CAVA, S.S. Impact of acidic monomer type and concentration on the adhesive performance of dental zirconia primers. **International Journal of Adhesion and Adhesives**, v.39: p. 49-53, 2012.

MORAES, R. R., FARIA-E-SILVA, A. L., OGLIARI, F. A., CORRER-SOBRINHO, L., DEMARCO, F. F., PIVA, E. Impact of immediate and delayed light activation on self-polymerization of dual-cured dental resin luting agents. **Acta Biomaterialia,** v.5, n.6, p.2095-2100, 2009.

MÜNCHOW, E. A., BOSSARDI, M., PRIEBE, T. C., VALENTE, L. L., ZANCHI, C. H., OGLIARI, F. A., PIVA, E. Microtensile versus microshear bond strength between dental adhesives and the dentin substrate. **International Journal of Adhesion and Adhesives,** v.46, p.95-99, 2013.

MUNCHOW, E. A., DA SILVA, A. F., DA SILVEIRA LIMA, G., WULFF, T., BARBOSA, M., OGLIARI, F. A., PIVA, E. Polypropylene glycol phosphate methacrylate as an alternative acid-functional monomer on self-etching adhesives. **Journal of Dentistry,** v.43, n.1, p.94-102, 2015.

MÜNCHOW, E. A., DE BARROS, G. D., ALVES, L. S., VALENTE, L. L., CAVA, S. S., PIVA, E., OGLIARI, F. A. Effect of elastomeric monomers as polymeric matrix of experimental adhesive systems: degree of conversion and bond strength characterization. **Applied Adhesion Science,** v.2, n.3, 2014.

MÜNCHOW, E. A., VALENTE, L. L., BOSSARDI, M., PRIEBE, T. C., ZANCHI, C. H., PIVA, E. Influence of surface moisture condition on the bond strength to dentin of etch-and-rinse adhesive systems. **Brazilian Journal of Oral Sciences,** v.13, n.3, p.182-186, 2014.

MUNOZ, M. A., LUQUE, I., HASS, V., REIS, A., LOGUERCIO, A. D., BOMBARDA, N. H. C. Immediate bonding properties of universal adhesives to dentine. **Journal of Dentistry,** v.41, n.5, p.404-411, 2013.

MUNOZ, M. A., SEZINANDO, A., LUQUE-MARTINEZ, I., SZESZ, A. L., REIS, A., LOGUERCIO, A. D., BOMBARDA, N. H., PERDIGAO, J. Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives. **Journal of Dentistry,** v.42, n.5, p.595-602, 2014.

NAIK, S. V., SHASHIKIRAN, N. D., CHAITRA, N. L., SYED, G. A microtensile bond strength evaluation of a single-bottle adhesive to caries-affected dentin in conventional versus minimal invasive caries removal techniques: An in-vitro study. **Indian Journal of Dental Research,** v.5, n.3, p.127-131, 2014.

NAKAJIMA, M., KITASAKO, Y., OKUDA, M., FOXTON, R. M., TAGAMI, J. Elemental distributions and microtensile bond strength of the adhesive interface to normal and caries-affected dentin. **Journal of Biomedical Materials Research Part B: Applied Biomaterials,** v.72, n.2, p.268-275, 2005.

NAKAJIMA, M., KUNAWAROTE, S., PRASANSUTTIPORN, T., TAGAMI, J. Bonding to caries-affected dentin. **Japanese Dental Science Review,** v.47, n.2, p.102-114, 2011.

NAKAJIMA, M., OGATA, M., OKUDA, M., TAGAMI, J., SANO, H., PASHLEY, D. H. Bonding to caries-affected dentin using self-etching primers (vol 12, pg 309, 1999). **American Journal Dentistry,** v.13, n.2, p.72-72, 2000.

NAKAJIMA, M., SANO, H., BURROW, M. F., TAGAMI, J., YOSHIYAMA, M., EBISU, S., CIUCCHI, B., RUSSELL, C. M., PASHLEY, D. H. Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives. **Journal of Dental Research,** v.74, n.10, p.1679-1688, 1995.

NAKAJIMA, M., SANO, H., URABE, I., TAGAMI, J., PASHLEY, D. H. Bond strengths of single-bottle dentin adhesives to caries-affected dentin. **Operative Dentistry,** v.25, n.1, p.2-10, 2000.

NAKAJIMA, M., SANO, H., ZHENG, L., TAGAMI, J., PASHLEY, D. H. Effect of moist vs. dry bonding to normal vs. caries-affected dentin with Scotchbond Multi-Purpose Plus. **Journal of Dental Research,** v.78, n.7, p.1298-1303, 1999.

NAVARRA, C. O., CADENARO, M., ARMSTRONG, S. R., JESSOP, J., ANTONIOLLI, F., SERGO, V., DI LENARDA, R., BRESCHI, L. Degree of conversion of Filtek Silorane Adhesive System and Clearfil SE Bond within the hybrid and adhesive layer: an in situ Raman analysis. **Dental Materials,** v.25, n.9, p.1178-1185, 2009.

NEVES, A. A., COUTINHO, E., CARDOSO, M. V., DE MUNCK, J., VAN MEERBEEK, B. Micro-tensile bond strength and interfacial characterization of an adhesive bonded to dentin prepared by contemporary caries-excavation techniques. **Dental Materials,** v.27, n.6, p.552-562, 2011.

NIKAIDO, T., KUNZELMANN, K. H., OGATA, M., HARADA, N., YAMAGUCHI, S., COX, C. F., HICKEL, R., TAGAMI, J. The in vitro dentin bond strengths of two adhesive systems in class I cavities of human molars. **Journal Adhesive Dentistry,** v.4, n.1, p.31-39, 2002.

NISHITANI, Y., YOSHIYAMA, M., TAY, F. R., WADGAONKAR, B., WALLER, J., AGEE, K., PASHLEY, D. H. Tensile strength of mineralized/demineralized human normal and carious dentin. **Journal of Dental Research,** v.84, n.11, p.1075-1078, 2005.

OGAWA, K., YAMASHITA, Y., ICHIJO, T., FUSAYAMA, T. The ultrastructure and hardness of the transparent layer of human carious dentin. **Journal of Dental Research,** v.62, n.1, p.7-10, 1983.

OMAR, H., EL-BADRAWY, W., EL-MOWAFY, O., ATTA, O., SALEEM, B. Microtensile bond strength of resin composite bonded to caries-affected dentin with three adhesives. **Operative Dentistry,** v.32, n.1, p.24-30, 2007.

PASHLEY, D. H., CARVALHO, R. M. Dentine permeability and dentine adhesion. **Journal of Dentistry,** v.25, n.5, p.355-372, 1997.

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

PERDIGAO, J. Dentin bonding-variables related to the clinical situation and the substrate treatment. **Dental Materials,** v.26, n.2, p.e24-37, 2010.

PERDIGAO, J., SWIFT, E. J., JR., DENEHY, G. E., WEFEL, J. S., DONLY, K. J. In vitro bond strengths and SEM evaluation of dentin bonding systems to different dentin substrates. **Journal of Dental Research,** v.73, n.1, p.44-55, 1994.

PEREIRA, P. N., NUNES, M. F., MIGUEZ, P. A., SWIFT, E. J., JR. Bond strengths of a 1-step self-etching system to caries-affected and normal dentin. **Operative Dentistry,** v.31, n.6, p.677-681, 2006.

PEUMANS, M., DE MUNCK, J., VAN LANDUYT, K. L., POITEVIN, A., LAMBRECHTS, P., VAN MEERBEEK, B. Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching. **Dental Materials,** v.26, n.12, p.1176-1184, 2010.

PIVA, E., OGLIARI, F. A., MORAES, R. R., CORA, F., HENN, S., CORRER-SOBRINHO, L. Papain-based gel for biochemical caries removal: influence on microtensile bond strength to dentin. **Brazilian Oral Research,** v.22, n.4, p.364-370, 2008.

REIS, A. F., BEDRAN-RUSSO, A. K., GIANNINI, M., PEREIRA, P. N. Interfacial ultramorphology of single-step adhesives: nanoleakage as a function of time. **Journal Oral Rehabilitation,** v.34, n.3, p.213-221, 2007.

RUGGERI, A., JR., PRATI, C., MAZZONI, A., NUCCI, C., DI LENARDA, R., MAZZOTTI, G., BRESCHI, L. Effects of citric acid and EDTA conditioning on exposed root dentin: An immunohistochemical analysis of collagen and proteoglycans. **Archives of Oral Biology,** v.52, n.1, p.1-8, 2007.

SARKIS-ONOFRE, R., SKUPIEN, J. A., CENCI, M. S., MORAES, R. R., PEREIRA-CENCI, T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. **Operative Dentistry,** v.39, n.1, p.E31-44, 2014.

SATTABANASUK, V., BURROW, M. F., SHIMADA, Y., TAGAMI, J. Resin adhesion to caries-affected dentine after different removal methods. **Australian Dental Journal,** v.51, n.2, p.162-169, 2006.

SAY, E. C., NAKAJIMA, M., SENAWONGSE, P., SOYMAN, M., OZER, F., TAGAMI, J. Bonding to sound vs caries-affected dentin using photo- and dual-cure adhesives. **Operative Dentistry,** v.30, n.1, p.90-98, 2005.

SCHEFFEL, D. L., RICCI, H. A., DE SOUZA COSTA, C. A., PASHLEY, D. H., HEBLING, J. Effect of reducing acid etching time on bond strength to noncarious and caries-affected primary and permanent dentin. **Pediatric Dentistry,** v.35, n.7, p.199-204, 2013.

SCHMIDLIN, P. R., SIEBENMANN, J., KOCHER, P., SEEMANN, R., ATTIN, T., BINDL, A. Effects of de- and remineralization of dentin on bond strengths yielded by one-, three-, and four-step adhesives. **Journal Adhesive Dentistry,** v.10, n.2, p.119-126, 2008.

SCHOLTANUS, J. D., PURWANTA, K., DOGAN, N., KLEVERLAAN, C. J., FEILZER, A. J. Microtensile Bond Strength of Three Simplified Adhesive Systems to Caries-affected Dentin. **Journal of Adhesive Dentistry,** v.12, n.4, p.273-278, 2010.

SELWITZ, R. H., ISMAIL, A. I., PITTS, N. B. Dental caries. **Lancet,** v.369, n.9555, p.51-59, 2007.

SENGUN, A., KOYUTURK, A. E., SENER, Y., OZER, F. Effect of desensitizers on the bond strength of a self-etching adhesive system to caries-affected dentin on the gingival wall. **Operative Dentistry,** v.30, n.4, p.430-435, 2005.

SENGUN, A., UNLU, N., OZER, F., OZTURK, B. Bond strength of five current adhesives to caries-affected dentin. **Journal Oral Rehabilitation,** v.29, n.8, p.777-781, 2002.

SINGH, U. P., TIKKU, A., CHANDRA, A., LOOMBA, K., BORUAH, L. C. Influence of caries detection dye on bond strength of sound and carious affected dentin: An in-vitro study. **Journal Conservative Dentistry,** v.14, n.1, p.32-35, 2011.

SKUPIEN, J. A., SARKIS-ONOFRE, R., CENCI, M. S., MORAES, R. R., PEREIRA-CENCI, T. A systematic review of factors associated with the retention of glass fiber posts. **Brazilian Oral Research,** v.29, n.1, p.1-8, 2015.

SONODA, H., BANERJEE, A., SHERIFF, M., TAGAMI, J., WATSON, T. F. An in vitro investigation of microtensile bond strengths of two dentine adhesives to caries-affected dentine. **Journal of Dentistry,** v.33, n.4, p.335-342, 2005.

SPENCER, P., SWAFFORD, J. R. Unprotected protein at the dentin-adhesive interface. **Quintessence International,** v.30, n.7, p.501-507, 1999.

SPENCER, P., WANG, Y. Adhesive phase separation at the dentin interface under wet bonding conditions. **Journal of Biomedical Materials Research,** v.62, n.3, p.447-456, 2002.

SPENCER, P., WANG, Y., KATZ, J. L., MISRA, A. Physicochemical interactions at the dentin/adhesive interface using FTIR chemical imaging. **Journal Biomedical Optics,** v.10, n.3, p.031104, 2005.

SPENCER, P., WANG, Y., WALKER, M. P., WIELICZKA, D. M., SWAFFORD, J. R. Interfacial chemistry of the dentin/adhesive bond. **Journal of Dental Research,** v.79, n.7, p.1458-1463, 2000.

SUPPA, P., RUGGERI, A., JR., TAY, F. R., PRATI, C., BIASOTTO, M., FALCONI, M., PASHLEY, D. H., BRESCHI, L. Reduced antigenicity of type I collagen and proteoglycans in sclerotic dentin. **Journal of Dental Research,** v.85, n.2, p.133-137, 2006.

SWIFT, E. J., JR., LINDEN, J. J., WEFEL, J. S. Effects of the XR-bonding system on in vitro caries. **American Journal Dentistry,** v.4, n.4, p.157-161, 1991.

TAK, O., USUMEZ, A. Diffusion of HEMA through Human Carious and Non-Carious Dentin In Vitro. **Journal of Prosthodontics-Implant Esthetic and Reconstructive Dentistry,** v.22, n.3, p.184-189, 2013.

TANIGUCHI, G., NAKAJIMA, M., HOSAKA, K., IWAMOTO, N., IKEDA, M., FOXTON, R. M., TAGAMI, J. Improving the effect of NaOCl pretreatment on bonding to caries-affected dentin using self-etch adhesives. **Journal of Dentistry,** v.37, n.10, p.769-775, 2009.

TAY, F. R., PASHLEY, D. H. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. **Dental Materials,** v.17, n.4, p.296-308, 2001.

TAY, F. R., PASHLEY, D. H. Have dentin adhesives become too hydrophilic? **Journal Canadian Dental Association,** v.69, n.11, p.726-731, 2003.

TAY, F. R., PASHLEY, D. H., HIRAISHI, N., IMAZATO, S., RUEGGEBERG, F. A., SALZ, U., ZIMMERMANN, J., KING, N. M. Tubular occlusion prevents water-treeing and through-and-through fluid movement in a single-bottle, one-step self-etch adhesive model. **Journal of Dental Research,** v.84, n.10, p.891-896, 2005.

TAY, F. R., PASHLEY, D. H., SUH, B. I., CARVALHO, R. M., ITTHAGARUN, A. Single-step adhesives are permeable membranes. **Journal of Dentistry,** v.30, n.7-8, p.371-382, 2002.

TIAN, T., TSOI, J. K., MATINLINNA, J. P., BURROW, M. F. Aspects of bonding between resin luting cements and glass ceramic materials. **Dental Materials,** v.30, n.7, p.e147-e162, 2014.

TUNCER, D., YAZICI, A. R., OZGUNALTAY, G., DAYANGAC, B. Clinical evaluation of different adhesives used in the restoration of non-carious cervical lesions: 24-month results. **Australian Dental Journal,** v.58, n.1, p.94-100, 2013.

VALENTE, L. L., MÜNCHOW, E. A., SILVA, M. F., MANSO, I. S., MORAES, R. R. Experimental methacrylate-based primers to improve the repair bond strength of dental composites - a preliminary study. **Applied Adhesion Science,** v.2, n.6, 2014.

VAN DE SANDE, F. H., AZEVEDO, M. S., LUND, R. G., HUYSMANS, M. C., CENCI, M. S. An in vitro biofilm model for enamel demineralization and antimicrobial dose-response studies. **Biofouling,** v.27, n.9, p.1057-1063, 2011.

VAN LANDUYT, K. L., SNAUWAERT, J., PEUMANS, M., DE MUNCK, J., LAMBRECHTS, P., VAN MEERBEEK, B. The role of HEMA in one-step self-etch adhesives. **Dental Materials Journal,** v.24, n.10, p.1412-1419, 2008.

VAN MEERBEEK, B., YOSHIHARA, K., YOSHIDA, Y., MINE, A., DE MUNCK, J., VAN LANDUYT, K. L. State of the art of self-etch adhesives. **Dental Materials,** v.27, n.1, p.17-28, 2011.

VANAJASAN, P. P., DHAKSHINAMOORTHY, M., RAO, C. S. Factors affecting the bond strength of self-etch adhesives: A meta-analysis of literature. **Journal Conservative Dentistry,** v.14, n.1, p.62-67, 2011.

VASCONCELOS, A. C., BERTI-COUTO, S. A., AZAMBUJA, A. A., SALUM, F. G., FIGUEIREDO, M. A., DA SILVA, V. D., CHERUBINI, K. Comparison of effects of clodronate and zoledronic acid on the repair of maxilla surgical wounds - histomorphometric, receptor activator of nuclear factor-kB ligand, osteoprotegerin, von Willebrand factor, and caspase-3 evaluation. **Journal Oral Patholhology & Medicine,** v.41, n.9, p.702-712, 2012.

WAMBIER, D. S., DOS SANTOS, F. A., GUEDES-PINTO, A. C., JAEGER, R. G., SIMIONATO, M. R. Ultrastructural and microbiological analysis of the dentin layers affected by caries lesions in primary molars treated by minimal intervention. **Pediatric Dentistry,** v.29, n.3, p.228-234, 2007.

WANG, Y., SPENCER, P., WALKER, M. P. Chemical profile of adhesive/caries-affected dentin interfaces using Raman microspectroscopy. **Journal of Biomedical Materials Research Part A,** v.81A, n.2, p.279-286, 2007.

WEI, S., SADR, A., SHIMADA, Y., TAGAMI, J. Effect of caries-affected dentin hardness on the shear bond strength of current adhesives. **Journal Adhesive Dentistry,** v.10, n.6, p.431-440, 2008.

WONG, L., SISSONS, C. H. Human dental plaque microcosm biofilms: effect of nutrient variation on calcium phosphate deposition and growth. **Archives of Oral Biology,** v.52, n.3, p.280-289, 2007.

XIE, J. X., FLAITZ, C. M., HICKS, M. J., POWERS, J. M. Bond strength of composite to sound and artificial carious dentin. **American Journal Dentistry,** v.9, n.1, p.31-33, 1996.

XUAN, W., HOU, B. X., LU, Y. L. Bond strength of different adhesives to normal and caries-affected dentins. **Chinese Medical Journal (Engl),** v.123, n.3, p.332-336, 2010.

YAZICI, A. R., AKCA, T., OZGUNALTAY, G., DAYANGAC, B. Bond strength of a self-etching adhesive system to caries-affected dentin. **Opererative Dentistry,** v.29, n.2, p.176-181, 2004.

YILDIZ, E., SIRINKARAARSLAN, E., YEGIN, Z., CEBE, M. A., TOSUN, G. Effect of caries removal techniques on the bond strength of adhesives to caries-affected primary dentin in vitro. **European Journal Paediatric Dentistry,** v.14, n.3, p.209-214, 2013.

YOSHIDA, Y., VAN MEERBEEK, B., NAKAYAMA, Y., YOSHIOKA, M., SNAUWAERT, J., ABE, Y., LAMBRECHTS, P., VANHERLE, G., OKAZAKI, M. Adhesion to and decalcification of hydroxyapatite by carboxylic acids. **Journal of Dental of Research,** v.80, n.6, p.1565-1569, 2001.

YOSHIOKA, M., YOSHIDA, Y., INOUE, S., LAMBRECHTS, P., VANHERLE, G., NOMURA, Y., OKAZAKI, M., SHINTANI, H., VAN MEERBEEK, B. Adhesion/decalcification mechanisms of acid interactions with human hard tissues. **Journal of Biomedical Materials Research,** v.59, n.1, p.56-62, 2002.

YOSHIYAMA, M., DOI, J., NISHITANI, Y., ITOTA, T., TAY, F. R., CARVALHO, R. M., PASHLEY, D. H. Bonding ability of adhesive resins to caries-affected and caries-infected dentin. **Journal Applied Oral Science,** v.12, n.3, p.171-176, 2004.

YOSHIYAMA, M., TAY, F. R., DOI, J., NISHITANI, Y., YAMADA, T., ITOU, K., CARVALHO, R. M., NAKAJIMA, M., PASHLEY, D. H. Bonding of self-etch and total-etch adhesives to carious dentin. **Journal of Dental Research,** v.81, n.8, p.556-560, 2002.

YOSHIYAMA, M., TAY, F. R., TORII, Y., NISHITANI, Y., DOI, J., ITOU, K., CIUCCHI, B., PASHLEY, D. H. Resin adhesion to carious dentin. **American Journal Dentistry,** v.16, n.1, p.47-52, 2003.

YOSHIYAMA, M., URAYAMA, A., KIMOCHI, T., MATSUO, T., PASHLEY, D. H. Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin. **Operative Dentistry,** v.25, n.3, p.163-169, 2000.

ZANCHI, C. H., D'AVILA, O. P., RODRIGUES, S. A., BURNETT, L. H., DEMARCO, F. F., PINTO, M. B. Effect of Additional Acid Etching on Bond Strength and Structural Reliability of Adhesive Systems Applied to Caries-affected Dentin. **Journal of Adhesive Dentistry,** v.12, n.2, p.109-115, 2010.

ZANCHI, C. H., LUND, R. G., PERRONE, L. R., RIBEIRO, G. A., DEL PINO, F. A. B., PINTO, M. B., DEMARCO, F. F. Microtensile bond strength of two-step etch-and-rinse adhesive systems on sound and artificial caries-affected dentin. **American Journal Dentistry,** v.23, n.3, p.152-156, 2010.

ZHANG, Y., WANG, Y. The effect of hydroxyapatite presence on the degree of conversion and polymerization rate in a model self-etching adhesive. **Dental Materials,** v.28, n.3, p.237-244, 2012.

Apêndices

Apêndice A- Nota da Tese

União adesiva à dentina sadia e dentina afetada por cárie

Adhesive bonding to sound dentin and caries-affected dentin

A cárie dentária é uma doença que progride de forma lenta na maioria dos indivíduos, raramente é autolimitante e, na ausência de tratamento, progride até destruir totalmente a estrutura dentária. O sucesso dos procedimentos restauradores estéticos depende, entre outros fatores, da eficácia dos sistemas de união utilizados. Em uma odontologia mais conservadora já esta estabelecida a remoção parcial do tecido cariado, preservando assim mais estrutura dentária. O objetivo da presente tese de Doutorado foi o desenvolvimento de um sistema de união que fosse de fácil aplicação tanto em dentes livres de cárie, quanto em situações em que há cárie residual. Os resultados comprovaram que os sistemas de união experimentais testados mostraram união satisfatória também ao tecido com cárie residual, porém mais estudos ainda são necessários. É de fundamental importância o desenvolvimento de materiais odontológicos que sejam simples e ao mesmo tempo eficazes para as situações clínicas vivenciadas pelos cirurgiões dentistas, sempre considerando que a preservação de estrutura dentária seja uma prioridade frente a qualquer tratamento.

Campo da pesquisa: Odontologia Restauradora; materiais odontológicos.

Candidato: Cristina Pereira Isolan, mestre em Reabilitação Oral pela Universidade Veiga de Almeida. UVA-RS.

Data da defesa e horário: 25/02/2016

Local: Auditório do Programa de Pós-graduação em Odontologia da Universidade Federal de Pelotas. 5º andar da Faculdade de Odontologia de Pelotas. Rua Gonçalves Chaves, 457.

Membros da banca: Prof[a.] Dr[a.] Giana da Silveira Lima. Doutora em Odontologia (Dentística) pela Universidade Federal de Pelotas; Prof. Dr. Maximiliano Sérgio Cenci. Doutor em Odontologia (Cariologia) pela Universidade Estadual de Campinas; Prof[a.] Dr[a] Francine Cardozo Madruga. Doutora em Odontologia (Materiais Odontológicos) pela Universidade Federal de Pelotas; Prof. Dr. Eliseu Aldrighi Münchow. Doutor em Odontologia (Dentística) pela Universidade Federal de Pelotas; Prof. Dr. Fábio Garcia Lima. Doutor em Odontologia (Dentística) pela Universidade Federal de Pelotas (suplente); Prof. Dr. Rafael Guerra Lund. Doutor em Odontologia (Dentística) pela Universidade Federal de Pelotas (suplente).

Orientador: Prof. Dr.Rafael Ratto de Moraes. Doutor em Materiais Dentários pela Universidade Estadual de Campinas.

Informação de contato: Cristina Pereira Isolan, cristinaisolan1@hotmail.com. Rua Dom Pedro II, 756.

Apêndice B – Súmula do Currículo

Cristina Pereira Isolan nasceu em 1976, em Pelotas, Rio Grande do Sul. Completou o ensino fundamental e médio em Escola privada na mesma cidade. No ano de 1996, ingressou na Faculdade de Odontologia da Universidade Paranaense (UNIPAR), tendo sido graduada cirugiã-dentista em 1999. No ano seguinte ingressou no Aperfeiçoamento em Odontogeriatria na Universidade Luterana do Brasil (ULBRA). Na sequência atuou em consultório privado, até o inicio do Doutorado. No ano 2001 iniciou Especialização em Prótese Dentária na Pontifícia Universidade Católica do Rio Grande do Sul (PUC-RS). No ano 2004 iniciou Mestrado no Programa de Pós-graduação em Odontologia da Universidade Veiga de Almeida (UVA-RJ), área de concentração Reabilitação Oral, onde trabalhou na avaliação epidemiológica de idosos. Dissertação defendida e aprovada em 2006. Em 2012 iniciou Doutorado na Universidade Federal de Pelotas (UFPel) na área de Materias Odontológicos. Durante o período de doutorado foi bolsista do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e desenvolveu trabalhos na área de adesão.

Publicações:

Bonding of adhesive luting agents to caries-affected dentin induced by a microcosm biofilm model. Peixoto A., Bicalho A., Isolan C., Maske T., Moraes R., Cenci M., Soares C., Faria-E-Silva A. Operative Dentistry. 2015.

Effect of diamond bur grit size on composite repair. Valente, L. L., Silva, M. F., Fonseca, A. S., Munchow, E. A., Isolan, C.P, Moraes, R. R. Journal of Adhesive Dentistry. 2015.

Effect of waiting time for placing resin composite restorations after bleaching on enamel bond strength. Pimentel, A. H., Valente, L. L., Isolan, C.P., Munchow, E. A., Piva, E., Moraes, R. R. Applied Adhesion Science. 2015.

Relative photon absorption determination and the influence of photoinitiator system and water content on C=C conversion, water sorption/solubility of experimental self-

etch adhesives. Sodré, C.S., Albuquerque, P.P.A.C., Isolan, C.P., Moraes, R.R., Schneider, L.F. International Journal of Adhesion and Adhesives. 2015.

A biofilm cariogenic challenge model for dentin demineralization and dentin bonding analysis. Maske T.T., Isolan C.P., Van de Sande F.H., Peixoto A.C., Faria-E-Silva A.L., Cenci M.S., Moraes R.R. Clinical Oral Investigations. 2014.

Bond strength of universal bonding agent and other contemporary dental adhesives applied to enamel, dentin, composite, and porcelain. Isolan C.P., Valente L.L., Münchow E.A., Basso G.R., Pimentel A.H., Schwantz J.K., Silva A.V., Moraes R.R. Applied Adhesion Science. 2014.

Anexos

ANEXO A: Parecer do Comitê de ética

MINISTÉRIO DA EDUCAÇÃO

UNIVERSIDADE FEDERAL DE PELOTAS

FACULDADE DE ODONTOLOGIA

COMITÊ DE ÉTICA E PESQUISA

PELOTAS, 03 de junho de 2013

PARECER Nº 25 /2013

O projeto de pesquisa intitulado **"Variáveis de formulação e união de adesivos autocondicionantes a dentina afetada por cárie".** está constituído de forma adequada, cumprindo, nas suas plenitudes preceitos éticos estabelecidos por este Comitê e pela legislação vigente, recebendo, portanto, PARECER APROVADO.

Prof. Dr. Renato Fabrício de Andrade Waldemarin

Coordenador do CEP- FOP/UFPel