



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José dos Campos
Instituto de Ciência e Tecnologia

MAURICIO YUGO DE SOUZA

**UTILIZAÇÃO DA TÉCNICA DA DENTINA ÚMIDA POR
ETANOL NA ADESÃO DENTINÁRIA: influência *in vitro* da *smear*
layer, avaliação clínica com diferentes protocolos adesivos e revisão
sistemática**

2019

MAURICIO YUGO DE SOUZA

**UTILIZAÇÃO DA TÉCNICA DA DENTINA ÚMIDA POR ETANOL NA
ADESÃO DENTINÁRIA: influência *in vitro* da *smear layer*, avaliação clínica
com diferentes protocolos adesivos e revisão sistemática**

Tese apresentada ao Instituto de Ciência e Tecnologia, Universidade Estadual Paulista (Unesp), Campus de São José dos Campos, como parte dos requisitos para obtenção do título de DOUTOR, pelo Programa de Pós-Graduação em ODONTOLOGIA RESTAURADORA.

Área: Dentística. Linha de pesquisa: Avaliação Clínica e Laboratorial de alterações da Estrutura Dental, de Materiais e de Técnicas de prevenção e Tratamento em Dentística.

Orientador: Prof. Associado Eduardo Bresciani

São José dos Campos

2019

BANCA EXAMINADORA

Prof. Associado Eduardo Bresciani (Orientador)

Instituto de Ciência e Tecnologia
Universidade Estadual Paulista (Unesp)
Campus de São José dos Campos

Profa. Associada Rebeca Di Nicoló

Instituto de Ciência e Tecnologia
Universidade Estadual Paulista (Unesp)
Campus de São José dos Campos

Profa. Associada Taciana Marco Ferraz Caneppele

Instituto de Ciência e Tecnologia
Universidade Estadual Paulista (Unesp)
Campus de São José dos Campos

Profa. Doutora Luciana Fávaro Francisconi dos Rios

Faculdade de Odontologia
Universidade de São Paulo (USP)
Campus de São Paulo

Prof. Associado Flávio Henrique Baggio Aguiar

Faculdade de Odontologia
Universidade Estadual de Campinas (Unicamp)
Campus de Piracicaba

São José dos Campos, 02 de Agosto de 2019.

AGRADECIMENTOS

Agradeço a todos os que direta ou indiretamente contribuíram durante todos estes anos para minha formação profissional e pessoal.

Agradeço à minha mãe Márcia e aos meus irmãos Marcello e Marcel, pelo total apoio e confiança que depositaram em mim – independente da escolha que eu tivesse, para que eu conseguisse finalizar o meu curso de Doutorado, principalmente neste último ano, de muita correria! Muito obrigado, amo vocês!!

Agradeço especialmente ao meu pai Mário (em memória), pois sempre me apoiou e fez de tudo para me proporcionar as condições necessárias para que eu chegasse até aqui. Não conseguiu acompanhar o trajeto no período do Doutorado, fisicamente, porém sei que independente de onde estiver nunca nos abandonará! Te dedico mais essa conquista! TE AMO!

Aos professores da disciplina de Dentística (Alessandra, César, Clóvis, Maria Filomena, Sérgio e Taciana) e funcionárias (Fernanda, Josiana e Liliane) do Departamento de Odontologia Restauradora da UNESP Campus de São José dos Campos, pela atenção e companheirismo.

Aos alunos de iniciação científica em que tive o prazer de conviver e ajudaram na realização dos trabalhos (Jéssica e Luan). Obrigado!

À Professora Rebeca Di Nicoló, que foi a primeira pessoa em que tive contato nesta instituição. Minha orientadora de mestrado que mesmo com tantas tarefas

administrativas, sempre se esforçou e conseguiu estar presente. Admiro muito a simplicidade e alegria em que enfrenta o dia-a-dia!

Em especial ao meu orientador Professor Eduardo Bresciani, que me desde o Mestrado, como co-orientador esteve presente e me incentivou em todos os momentos, tanto na vida profissional quanto na pessoal. Sempre acolhe com grande carinho e compreensão. A admiração que tenho pelo senhor só aumentou com o decorrer dos dias. É uma das pessoas em que me espelho! MUITO obrigado por me permitir de ter essa oportunidade na ‘família Bresciani’!

Aos colegas da pós-graduação pelo companheirismo diário.

Em especial aos queridos amigos – quarteto - que levarei para a vida toda! Vocês foram essenciais todos os dias, mesmo longe, vocês se fazem presente!

Ana Luiza, Mariane e Rafael sempre estavam dispostos, presentes e nunca mediram esforços para ajudar a superar os dias difíceis. Vocês têm a certeza de que nunca me esquecerei das nossas conversas, risadas, almoços, cafés, viagens e a principal, a conexão que criamos!

“A causa da derrota, não está nos obstáculos, ou no rigor das circunstâncias, está na falta de determinação e desistência da própria pessoa.” Buda

SUMÁRIO

RESUMO.....	7
ABSTRACT.....	9
1 INTRODUÇÃO.....	11
2 ARTIGO(S)	16
2.1 Artigo – Souza MY, Andrade JL, Bresciani E. Avaliação da nanodureza, módulo de elasticidade e nanoinfiltração da interface adesiva após a técnica da dentina úmida por etanol / <i>Assessment of nanohardness, elastic modulus, and nanoleakage of the adhesive interface using the ethanol-wet-bonding technique</i>	16
2.2 Artigo – Souza MY, Jurema ALB, Caneppele TMF, Bresciani E. Acompanhamento de seis meses de restaurações realizadas com a técnica da dentina úmida em etanol: estudo clínico randomizado / <i>Six-month performance of restorations produced with the ethanol-wet-bonding technique: A Randomized Trial</i>	34
2.3 Artigo – Souza MY, Jurema ALB, Caneppele TMF, Bresciani E. 18 meses da performance da técnica da dentina úmida por etanol com diferentes protocolos adesivos: estudo clínico randomizado controlado / <i>18-month performance of ethanol-wet-bonding technique with different adhesive protocols: a randomized controlled clinical trial</i>	55
2.4 Artigo – Souza MY, Andrade JL, Bresciani E. A técnica da dentina úmida por etanol influencia as propriedades adesivas de restaurações? / <i>Does the ethanol-wet-bonding influence the adhesive properties of dental restorations? A systematic review and metanalysis</i>	78
3 CONSIDERAÇÕES GERAIS.....	107
REFERÊNCIAS.....	112
APENDICE.....	117
ANEXOS.....	118

Souza MY. Utilização da técnica da dentina úmida por etanol na adesão dentinária: influência *in vitro* da *smear layer*, avaliação clínica com diferentes protocolos adesivos e revisão sistemática [tese]. São José dos Campos (SP): Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia; 2019.

RESUMO

Este estudo foi composto por um estudo *in vitro*, um estudo clínico randomizado, controlado e duplo cego e uma revisão sistemática, com objetivo de compreender e avaliar a aplicabilidade da técnica da dentina úmida por etanol (EWBT) em procedimentos restauradores. Estudo laboratorial: 48 incisivos bovinos foram divididos em 2 grupos, com a utilização ou não de etanol anterior ao sistema adesivo universal (Single Bond Universal) no modo autocondicionante. Em seguida blocos de resina composta (Filtek Z350 xt) foram confeccionados. A interface adesiva foi analisada, antes e após envelhecimento por 6 meses em água, com relação à nanodureza e módulo de elasticidade e em microscopia eletrônica de varredura (MEV) após a realização de nanoinfiltração com nitrato de prata. Os dados de nanodureza e módulo de elasticidade foram analisados estatisticamente com ANOVA e teste de Tukey ($\alpha=5\%$). Houve diferença estatística para as áreas avaliadas ($p<0.001$) e também para a aplicação do etanol ($p<0.001$). A dentina apresentou valores maiores de nanodureza quando comparado com a camada híbrida. EWBT foi capaz de manter as propriedades da interface adesiva mesmo após envelhecimento. Estudo clínico randomizado: Os voluntários receberam restaurações em cavidades do tipo classe V, provenientes de lesões cervicais não cariosas, com extensão e profundidade de no mínimo 1 mm. As restaurações foram realizadas aleatoriamente, de acordo com a divisão dos grupos, sendo o grupo controle sem pré-tratamento dentinário (SE) e três grupos com a intervenção, sendo associado com adesivo de 3 passos (E); hidrófobo (EB) ou com adesivo de 2 passos (EU). As restaurações foram avaliadas, por examinadores devidamente calibrados no início do estudo, após 7 dias, 6 e 18 meses, utilizando o critério USPHS modificado. Os dados foram analisados com testes chi-quadrado e Kaplan-Meier. O grupo com adesivo hidrófobo apresentou maior taxa de falha para retenção quando comparada aos demais grupos, tanto em 6 quanto em 18 meses. Revisão sistemática: Foram pesquisadas as principais bases de dados eletrônicas com a estratégia de busca definida de acordo com a pergunta de pesquisa e estratégia PICO, sendo P: dentes com lesões cervicais não cariosas; I: protocolo adesivo utilizando EWBT; C: protocolo adesivo convencional. Após análise do título e resumo dos artigos pré-selecionados, seguindo a questão PICO, 19

artigos laboratoriais e 3 estudos clínicos relacionados ao tema tiveram os dados extraídos. O risco de viés dos artigos selecionados foi estimado, sendo que para a maioria dos estudos laboratoriais e clínicos foi considerado baixo. Para os estudos laboratoriais houve diferença, sendo que o grupo controle apresentou melhores resultados quando comparado à EWBT. Com relação aos estudos clínicos não houve diferença entre os grupos controle e EWBT.

Palavras-chave: Adesivos dentinários. Dentina. Ensaio clínico. Etanol. Revisão sistemática.

Souza MY. Use of ethanol-wet-bonding technique in dentin adhesion: smear layer in vitro influence, clinical trial with different adhesives protocols and systematic review [doctorate thesis]. São José dos Campos (SP): São Paulo State University (Unesp), Institute of Science and Technology; 2019.

ABSTRACT

This study was composed by an in vitro study, a randomized, controlled and double blind clinical trial, and a systematic review, aiming to understand and evaluate the applicability of ethanol-wet-bonding technique (EWBT) in restorative procedures. Laboratory study: 48 bovine incisors were divided into 2 groups, according to EWBT use prior to the universal adhesive system (Single Bond Universal) in the self-etch mode. Blocks of composite resin (Filtek Z350 xt) were made. The adhesive interface was analyzed, on baseline and 6 months of water aging, by scanning electron microscopy (SEM) after nanoleakage with silver nitrate, besides the nanohardness and elastic modulus. The data of nanohardness and elastic modulus were statistically analyzed with ANOVA and Tukey test ($\alpha=5\%$). There was a statistically significant difference for the areas evaluated, in which dentin presented higher values, for nanohardness, than the hybrid layer ($p<0.001$) and for the use of ethanol ($p<0.001$). The use of ethanol was able to maintain the properties of the adhesive layer even after aging. Randomized clinical trial: The volunteers received restorations in class V cavities from non-carious cervical lesions (NCCL), with cavity extension and depth of at least 1 mm. The restorations were randomly performed according to group division: no ethanol dentin pretreatment (SE), with ethanol dentin pretreatment in association with 3-step (E) or 2-step (EU) or adhesive, or hydrophobic adhesive (EB). The restorations were evaluated by calibrated examiners at baseline, after 7 days, 6 and 18 months using the modified USPHS criteria. All data were statistically analyzed at Chi square and survival test ($\alpha=5\%$). The hydrophobic adhesive group presented higher failure rate for retention when compared to the other groups, both at 6 and 18 months follow-up. Systematic review: Main electronic databases were used for search and the strategy was defined according to the research question and PICO strategy, where P: teeth with NCCL; I: adhesive protocol using EWBT; C: conventional adhesive protocol; O: long-term clinical outcome after EWBT. After analyzing the title and abstract of the pre-selected articles, following the PICO question, 19 laboratory studies and 3 clinical trials related to EWBT. The data from selected studies were extracted and then, the risk of bias was estimated. For laboratory studies it was considered high and for clinical trials was considered low risk of bias. After statistical analysis, for laboratory studies the control

group presented better results when compared to EWBT. Regarding the clinical studies, there was no difference between control and EWBT groups.

Keywords: Clinical trial. Dentin. Dentin-bonding agents. Ethanol. Review.

1 INTRODUÇÃO

Com a constante evolução das pesquisas em relação aos sistemas adesivos e a estrutura dentária, é possível o aprimoramento de técnicas restauradoras com o objetivo de preservar a estrutura dentária sadia (Tassery et al., 2013), além do aumento da sobrevida da restauração.

Lesões cervicais não cariosas (LCNCs) caracterizam-se pela perda irreversível de tecido dentário, sendo que podem apresentar diferentes formatos, extensões e profundidades. Essas lesões são comumente observadas na prática clínica e são provenientes da erosão, abrasão e/ou abfração dos dentes. Inicialmente localiza-se no esmalte com lenta progressão na dentina, que gradualmente resulta em esclerose dentinária, devido a estímulos crônicos e de baixa intensidade (Levitch et al., 1994; Tay et al., 2004; Eliguzeloglu et al., 2011; Shetty et al., 2013; Borges et al., 2014). O tratamento restaurador nas LCNCs faz-se necessário não só para aliviar a hipersensibilidade, mas também para limitar a continuidade de perda de estrutura dental e melhorar a estética (Levitch et al., 1994). No entanto, a longevidade do tratamento restaurador nesse tipo de cavidade, apresenta alto índice de falha, como a perda da restauração, cáries secundárias e/ou descoloração marginal (Bartlett, Shah, 2006; Eliguzeloglu et al., 2011; Borges et al., 2014). A perda da restauração pode estar diretamente ligada à forma não retentiva da cavidade e localização das margens em dentina e cimento, as quais são áreas desfavoráveis para a adesão (Heymann et al., 1988; Tay et al., 2004; Eliguzeloglu et al., 2008). Por este motivo, as LCNCs representam a condição ideal para se avaliar clinicamente a efetividade de sistemas adesivos. Outros fatores como, geometria da cavidade, presença de dentina esclerosada, contração de polimerização e fator-C, também podem influenciar na longevidade das restaurações (Eliguzeloglu et al., 2011; Borges et

al., 2014).

Em relação aos adesivos, os primeiros sistemas formulados possuíam características hidrófobas, dessa forma dificultando a interação do material com o substrato dentinário. Esta dificuldade acontecia, pois o tecido dentinário é intrinsecamente úmido, além de ser composto por maior quantidade de conteúdo orgânico como, fluido dentinário, prolongamento dos odontoblastos e fibras colágenas, quando comparado ao esmalte dental. Ao longo do tempo, foram desenvolvidos sistemas adesivos com componentes, como o HEMA, o qual fornece característica mais hidrófila, permitindo uma união facilitada ao substrato dentinário (Pashley et al., 2011; Van Meerbeek et al., 2011).

Além das características inerentes aos sistemas adesivos, também há o objetivo de simplificar a técnica de restaurações diretas. Para isso, foi proposto o uso dos sistemas adesivos autocondicionantes, que eliminam a necessidade do condicionamento ácido total prévio. Esse tipo de material, devido ao menor número de passos clínicos e facilidade de aplicação, teve um aumento na popularidade de seu uso (Van Meerbeek et al., 2011; Ahn et al., 2015). O mecanismo de ação desses adesivos se dá pela desmineralização e infiltração dos monômeros simultaneamente no substrato. Isto resulta, de acordo com alguns estudos, em menor ou nenhuma sensibilidade pós-operatória, além de formar uma camada híbrida mais homogênea. Esta característica da camada híbrida influencia na longevidade da restauração, uma vez que o risco de falhas na penetração é baixo ou inexistente (Cardoso et al., 2008; Van Meerbeek et al., 2011; Ahn et al., 2015).

Acredita-se que para os sistemas adesivos convencionais, independente do modo de aplicação, para que haja uma união estável entre o material e o substrato dentinário, é necessário que a dentina permaneça úmida, a fim de permitir a penetração dos sistemas adesivos entre as fibras colágenas, originando a camada híbrida e consequentemente união (Spencer et al., 2012). Por outro

lado, sistemas adesivos estão em constante evolução e recentemente são representados pelos sistemas adesivos universais, os quais são considerados adaptáveis, uma vez que, com base em estudos clínicos e laboratoriais, devido ao maior grau de hidrofília dos adesivos universais, são capazes de reidratar fibras colágenas, possibilitando sua aplicação sobre substrato dentinário seco (Perdigão et al., 2014b; Lenzi et al., 2016; Tsujimoto et al., 2018). Além disso, os sistemas adesivos universais podem ser utilizados após condicionamento ácido do substrato ou no modo autocondicionante. Entretanto, devido à diferença de composição entre as estruturas do esmalte e dentina, há respostas distintas em relação ao condicionamento ácido, necessário para obtenção da adesão de materiais. No esmalte esta adesão é facilitada devido a maior quantidade de matéria inorgânica, os cristais de hidroxiapatita, que permitem a formação de tags resinosos. Enquanto que na dentina, devido à maior quantidade de matéria orgânica a adesão é mais complexa (Perdigão, Loguércio, 2014a). Devido a essas características, estudos que testam a união de sistemas adesivos são realizados no substrato dentinário, uma vez que esta união ainda é o maior desafio na odontologia restauradora.

A fim de minimizar o processo de degradação que a interface adesiva sofre ao longo do tempo, principalmente do colágeno dentinário e melhorar a penetração dos monômeros hidrofóbicos dos sistemas adesivos, alguns procedimentos podem ser realizados, como por exemplo, o pré-tratamento da superfície dentinária com etanol (EWBT) (Tjäderhane, 2015).

O etanol tem maior capacidade solvente e menor capacidade de ligação de hidrogênio do que a água, sendo utilizado para desidratação química da rede de colágeno desmineralizada. A finalidade da EWBT é criar uma matriz de colágena relativamente hidrófoba, através da diminuição da quantidade de água do substrato, permitindo que haja infiltração dos monômeros resinosos e conseqüentemente formação da camada híbrida e dificultando a hidrólise da

interface adesiva. No entanto, a técnica do etanol é sensível uma vez que o etanol apresenta alta volatilidade. Adicionalmente, devido à umidade intrínseca da dentina, dada pela presença de fluido dentinário no interior dos túbulos e por ligações estáveis entre fibras colágenas e moléculas de água, não é possível a completa substituição da água neste substrato (Pashley et al., 2011). Dessa forma, mesmo após o pré-tratamento com etanol a dentina apresenta água em sua constituição, fato que dificulta a união com adesivos hidrófobos (Nagpal et al., 2015; Tjäderhane, 2015).

Nesta técnica (EWBT), há formação de uma camada híbrida, menos hidrófila e mais resistente à degradação hidrolítica causada por enzimas endógenas como as metaloproteinases (MMPs), proporcionando possivelmente maior longevidade, devido a uma proteção eficaz do colágeno (Breschi et al., 2008). Apesar de a EWBT apresentar resultados clínicos adequados quando da utilização de adesivos hidrófobos (Araújo et al., 2013), ainda não há estudos que avaliaram clinicamente a utilização da EWBT com sistemas adesivos hidrófilos, questionamento suportado pela não remoção completa de água da dentina após aplicação do etanol, como relatado anteriormente. Com base nos temas abordados, nota-se que ainda há uma dificuldade em relação à obtenção da união dos sistemas adesivos com o tecido dentinário, o que afeta a longevidade das restaurações. Portanto é necessário avaliar a possibilidade do pré-tratamento do substrato dentinário para a obtenção de resultados de excelência no tratamento restaurador, uma vez que há poucos estudos clínicos avaliando a eficácia da EWBT.

Além disso, os trabalhos que utilizaram EWBT *in vitro* e que de certa forma relatam melhora em longo prazo da força adesiva, se apoiam na melhor impregnação dos sistemas adesivos em dentina não recoberta por smear layer (Nishitani et al., 2006; Hosaka et al., 2009; Kim et al., 2010; Liu et al., 2011; Scheffel et al., 2015). Foi observado que o mesmo protocolo em técnicas de

adesão em que a smear layer não é removida, estas situações são beneficiadas pela EWBT (Souza et al., 2018). Frente a estes resultados é necessário entender se há interação na impregnação dos adesivos universais mesmo com *smear layer* em posição e quais as alterações qualitativas e quantitativas que ocorrem na interface adesiva, após EWBT.

Por fim, a revisão sistemática terá como finalidade melhorar a compreensão do efeito do etanol 100% no tecido dentinário e de manter os pesquisadores atualizados em relação aos trabalhos de adesão, uma vez que se trata da ferramenta de maior validade científica dentro da pirâmide de evidência científica.

Desta maneira, realizando-se os três diferentes estudos, espera-se compreender a eficácia da EWBT após o emprego de diferentes protocolos adesivos, além do entendimento quando aplicada em substrato contendo smear layer.

2 ARTIGO(S)

2.1 Artigo – Souza MY, Andrade JL, Bresciani E. Avaliação da nanodureza, módulo de elasticidade e nanoinfiltração da interface adesiva após a técnica da dentina úmida por etanol / *Assessment of nanohardness, elastic modulus, and nanoleakage of the adhesive interface using the ethanol-wet-bonding technique**

RESUMO

Este estudo avaliou o efeito da técnica da dentina úmida por etanol (EWBT) na infiltração de sistema adesivo, por meio de nanodureza, módulo de elasticidade e nanoinfiltração, e sua estabilidade após 6 meses. Quarenta incisivos bovinos apresentando *smear layer* padronizada foram divididos em 2 grupos, de acordo com a EWBT antes do sistema adesivo universal no modo autocondicionante (Single Bond Universal - 3M ESPE). Blocos de resina foram construídos na superfície tratada (Filtek Z350xt - 3M ESPE). Metade dos espécimes (n=20) foram avaliados no início e a outra após 6 meses de armazenamento em água (n = 20). Os espécimes foram seccionados em fatias de 2 mm de espessura e a área adesiva (resina composta, camada híbrida e dentina) foi analisada pelos testes de nanodureza e módulo de elasticidade. Além disso, fatias do mesmo dente foram analisadas qualitativamente em MEV após nanoinfiltração por nitrato de prata. Os dados obtidos foram analisados por meio do teste ANOVA de dois fatores, teste de Mann-Whitney e teste de Tukey ($\alpha=5\%$). Para nanodureza e módulo de elasticidade, houve diferença para as áreas avaliadas ($p<0,001$), uma vez que a dentina apresentou valores maiores quando comparada à camada híbrida. Os grupos EWBT apresentaram maiores valores de nanodureza em todos os períodos avaliados ($p<0,001$). Houve diferença estatística para o envelhecimento ($p<0,001$), com valores maiores para a avaliação inicial. A resina não foi afetada pelas variáveis estudadas ($p>0,05$). Para nanoinfiltração, os grupos EWBT apresentaram valores médios mais baixos, especialmente após 6 meses, indicando maior capacidade de selamento ao longo do tempo. A utilização da EWBT levou a valores mais altos de nanodureza e maior capacidade de vedação da interface adesiva ao usar adesivo universal no modo autocondicionante após 6 meses de armazenamento de água.

Palavras-chave: Etanol. Dentina. Sistemas adesivos. Adesivos autocondicionantes. Camada híbrida.

*Artigo elaborado de acordo com as normas do Periódico *International Journal of Adhesion and Adhesives* (Print version ISSN 0143-7496). Submetido em: 03/06/2019.

ABSTRACT

This study evaluated the effect of ethanol-wet-bonding technique (EWBT) on dentin adhesive infiltration, by means of nanohardness, elastic modulus and nanoleakage, and its stability after 6-month. Forty bovine incisors presenting standardized smear layer were divided into 2 groups, according to EWBT prior to the universal adhesive system in the self-etch mode (Single Bond Universal – 3M ESPE). Resin blocks were built onto the treated surface (Filtek Z350xt - 3M ESPE). Half of specimens (n=20) were evaluated at baseline and the other (n=20) after 6-month in water storage. Specimens were sectioned into 2-mm thick slices and the adhesive area (composite resin, hybrid layer, and dentin) was analyzed by nanohardness and elastic modulus. Also, slices from the same tooth were qualitatively analyzed by SEM after nanoleakage by silver nitrate. The data obtained were analyzed using two-way ANOVA, Mann-Whitney, and Tukey's test ($\alpha=5\%$). For nanohardness and elastic modulus, there was difference for evaluated areas ($p<0.001$), once dentin presented higher values when compared to hybrid layer. The EWBT groups presented greater nanohardness values at all evaluated periods ($p<0.001$). There was statistical difference for aging ($p<0.001$), with greater values for baseline. Resin was not affected by the studied variables ($p>0.05$). For nanoleakage, EWBT groups presented overall lower median values, especially at the 6-month recall, indicating greater sealing ability over time. EWBT application led to higher values of nanohardness and higher sealing capacity of the adhesive interface when using multimode self-etch adhesive after 6-month water storage.

Keywords: Ethanol Dentin. Self-etch adhesive. Adhesive system. Hybrid layer.

1. Introduction

The ethanol-wet-bonding technique (EWBT) was proposed to minimize the degradation process that the adhesive interface undergoes over time and to overcome the incomplete penetration of hydrophobic monomers. [1-3] Two EWBT approaches were described in the literature. The progressive substitution, in which ethanol saturation is obtained using increasing concentrations of ethanol, [4,5] and the simplified protocol with a single step through the active application of 100% ethanol for 1 min. [6]

The EWBT creates a relatively hydrophobic collagen matrix by reducing the amount of water, retarding the adhesive interface hydrolysis, and it allows improved hydrophobic monomers infiltration to consequently form a more stable hybrid layer. [5,7] The hybrid layer formed is less hydrophilic and more resistant to hydrolytic degradation caused by endogenous enzymes such as metalloproteinases (MMPs), providing greater longevity due to an effective collagen protection. [8]

Although the technique is intended to displace water, there is no complete replacement of water by ethanol due to a stable bond between the collagen fibrils and water. [9] This fact might compromise a stable adhesion between dentin substrate and hydrophobic adhesives. [1,3] The remaining water after ethanol application might interfere with the adhesive procedures if hydrophobic adhesive is applied, supporting the application of hydrophilic systems. [10] Based on that fact, since only the excess of water is removed, [9] an alternative to increase the interaction between dentin substrate after EWBT and adhesives may be the application of an universal adhesive that is hydrophilic. [11]

The association between universal adhesives and EWBT has been tested in laboratory, [12] mainly due to the increased popularity of universal adhesives [13] and the possibility of being applied on dentin under the self-etch

mode. This association resulted in higher microtensile values even after thermomechanical aging, in a recent *in vitro* study. [12] The results of this recent *in vitro* study suggest that there were stable bonds after EWBT and hydrophilic self-etching adhesives, even in the presence of the smear layer. [12] There is however no study that evaluated the interaction between EWBT and the presence of smear layer on the formation of a proper hybrid layer, considering the potential infiltration of monomers.

Thus the aim of this *in vitro* study was to determine the influence of EWBT in the impregnation of self-etch adhesive to dentin, with or without aging, by means of nanohardness, elastic modulus and nanoleakage tests. The first null hypothesis was that there was no difference between the areas evaluated (hybrid layer, and dentin). The second null hypothesis was that EWBT was not able to change the characteristics of the adhesive interface such as nanoleakage, nanohardness and elastic modulus. The third null hypothesis was the assessed properties are not influenced by the aging. The fourth null hypothesis is that ethanol and aging do not influence the nanoleakage results.

2. Material and Methods

2.2 Specimens preparation

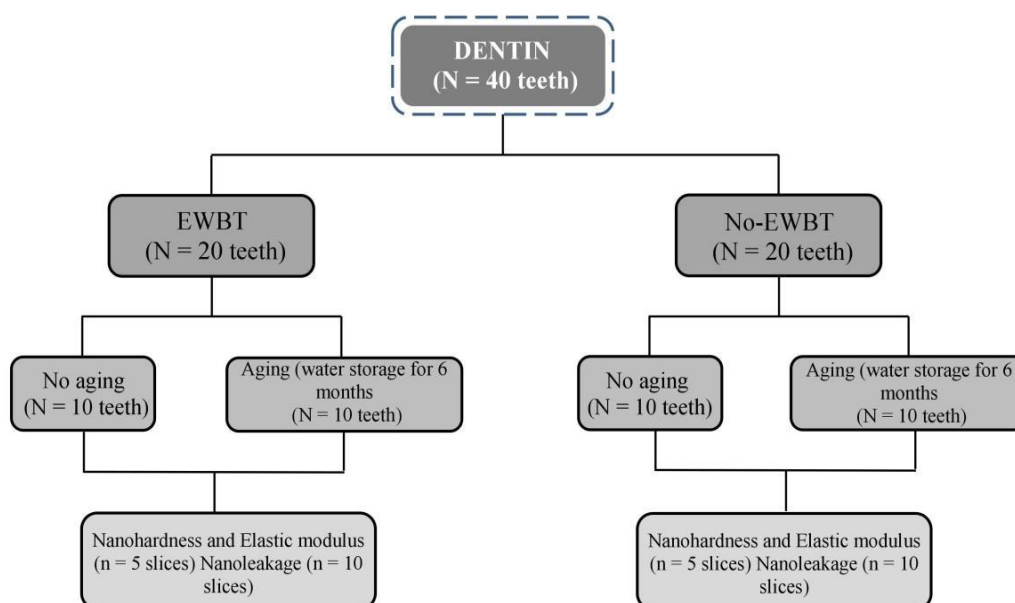
Forty bovine incisors were used and had the root cut close to the cementum-enamel junction. The buccal surface was grounded using 600-grit sandpaper (SiC, Fepa-P), resulting in a minimum dentin area of 6 x 5 mm. Specimen was embedded in self-curing acrylic resin (Jet Classic, São Paulo, SP, Brazil) with the dentin surface facing out. Smear layer was standardized, using an automated polishing device (Autopol, Panambra, São Paulo, SP, Brazil) with 1200, 2400 and 4000-grit sandpaper (SiC Fepa-P), under constant water irrigation at 300 rpm for 30 s for each grit.

Specimens were randomly divided into two groups (n=20 each)

according to the pretreatment or not with 100% ethanol. Previously to adhesive system application on self-etch mode (Single Bond Universal – 3M Deutschland GmbH, Neuss, Germany), the group that received EWBT, 100% ethanol was actively applied with a microbrush on dentin surface with a microbrush for 60 s, [1,12] followed by a light air-blasting to remove excess from the surface, keeping the dentin moist. For both groups, the application of adhesive system was active for 20 s, in accordance to the manufacturer's specifications. The adhesive system was lightcured for 20 s with a LED device (Radii-Cal – SDI, Victoria, Australia), with irradiance at 900 mW/cm². The universal adhesive components used on this study are: bisphenol-A-glycidyl methacrylate (Bis-GMA), 2-hydroxyethyl methacrylate (HEMA), 10-methacryloyloxydecyl dihydrogen phosphate (MDP), decamethylene dimethacrylate, ethyl methacrylate, propenoic acid, copolymer of acrylic and itaconic acid, dimethylaminobenzoate, methyl ethyl ketone, ethanol, water, camphorquinone, and silane-treated silica.

Resin blocks (Filtek Z350 XT, shade A2E, 3M ESPE) were built onto the treated surfaces, using a silicon mold, in two 2 mm increments, light-cured for 20 s each. After this, half of specimens in each group (n=10) were stored in distilled water at 37°C for 48 h to allow adequate post-curing time [14] before the analysis on baseline and the other half (n=10) was stored in the same conditions for 6 month. The water was renewed weekly. (Figure 1)

Figure 1 – Experimental design of the study



2.3 Preparing the samples

Samples were sectioned into 2 mm thick slices using a diamond saw. The slices were embedded in self-curing acrylic resin (Jet Classic, São Paulo, SP, Brazil), and the surface were polished using an automated polishing device (Autopol, Panambra, São Paulo, SP, Brazil) with 1200, 2400 and 4000-grit sandpaper (SiC Fepa-P), under constant water irrigation at 300 rpm for 30 s for each grit. Different slices from the same tooth were analyzed for the nanohardness/elastic modulus or for the nanoleakage.

2.4 Nanohardness (MPa) and elastic modulus (GPa) tests

In each specimen (n=10 slices for each group at each assessed time), 3 measurements were made in each area: resin composite, hybrid layer, and sound dentin. The indentations had a distance of 10 μ m between them, and were carried out with controlled temperature in 21 °C and load of 1000 μ N for 5 s (Anton Paar TriTec NHT2, Anton Paar, Peseux, Switzerland).

2.5 Nanoleakage test and Scanning Electron Microscopy (SEM)

For nanoleakage, the samples were immersed in ammoniacal silver nitrate that was prepared according to the protocol previously described. [15,16] The samples were placed in the ammoniacal silver nitrate in total darkness for 18 h, rinsed thoroughly in distilled water and immersed in photodeveloping solution for 6 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface.

Penetration of silver nitrate into the hybrid layer and adhesive–dentin interface were evaluated through analysis of 2000x magnification SEM micrographs and graded as: no leakage = score 0, slight leakage = score 1 (< 25% of the evaluated area) and moderate leakage = score 2 (25% ≤ 50% of the evaluated area) and distinct leakage = score 3 (> 50% of the evaluated area). [16] For SEM analysis two samples of each group were selected.

2.6 Statistical analysis

The data obtained for nanohardness and elastic modulus were submitted for two-way ANOVA and Tukey test ($\alpha=5\%$). Nanoleakage data were submitted to Mann-Whitney test ($\alpha=5\%$).

3. Results

Resin composite presented superior nanohardness and elastic modulus than dentin and the hybrid layer ($p<0.001$). None of the variables (use of ethanol and aging) influenced this specific evaluated area (resin composite). Resin was thus removed from the comparison with the other areas and served as a control due to the stability of data considering the parameters evaluated. Also considering nanohardness and elastic modulus, dentin presented higher values when compared to the hybrid layer ($p<0.001$).

Overall, the use of ethanol influenced nanohardness readings ($p<0.001$). Baseline and after 6 months isolated readings also presented different results

with the use of ethanol for both the hybrid layer and dentin ($p < 0.001$). The nanohardness of non-aged and aged ethanol groups were similar after 6 months in the hybrid layer, and there was a reduction after aging for the same groups in dentin, being the aged value similar to the non-aged non-ethanol group. (Table 1)

Considering aging, dentin and the hybrid layer were different in nanohardness and elastic modulus ($p < 0.001$) regardless the use or not of ethanol, once baseline presented higher values when compared to 6-month water storage. (Table 1)

Table 1 – Mean and standard deviation for nanohardness and elastic modulus for each area (resin; hybrid layer e dentin) at baseline and after 6 month evaluation

<i>Resin composite</i>	<i>Baseline^A</i>		<i>After 6 month^A</i>	
	<i>Without EWBT</i>	<i>EWBT</i>	<i>Without EWBT</i>	<i>EWBT</i>
<i>Nanohardness (Mpa)</i>	<i>1297.48 ± 326.54</i>	<i>1264.91 ± 504.44</i>	<i>1293.63 ± 434.33</i>	<i>1118.73 ± 142.36</i>
<i>Elastic modulus (GPa)</i>	<i>15.73 ± 2.11</i>	<i>17.64 ± 2.76</i>	<i>16.19 ± 1.68</i>	<i>16.51 ± 0.84</i>

<i>Hybrid Layer</i>	<i>Baseline^A</i>		<i>After 6 months^B</i>	
	<i>Without EWBT</i>	<i>EWBT</i>	<i>Without EWBT</i>	<i>EWBT</i>
<i>Nanohardness (Mpa)</i>	<i>256.19 ± 67.83^a</i>	<i>406.07 ± 219.72^b</i>	<i>196.06 ± 117.57^c</i>	<i>324.55 ± 175.65^b</i>
<i>Elastic modulus (GPa)</i>	<i>13.50 ± 5.27</i>	<i>14.18 ± 6.65</i>	<i>8.85 ± 4.66</i>	<i>9.37 ± 3.15</i>

<i>Dentin</i>	<i>Baseline^A</i>		<i>After 6 months^B</i>	
	<i>Without EWBT</i>	<i>EWBT</i>	<i>Without EWBT</i>	<i>EWBT</i>
<i>Nanohardness (Mpa)</i>	<i>414.55 ± 202.71^a</i>	<i>675.01 ± 160.37^b</i>	<i>229.48 ± 75.32^c</i>	<i>421.22 ± 189.47^a</i>
<i>Elastic modulus (GPa)</i>	<i>20.93 ± 3.30</i>	<i>22.76 ± 4.06</i>	<i>13.42 ± 4.61</i>	<i>18.73 ± 3.64</i>

Different uppercase letters mean significant differences among the groups before and after aging ($p < 0.001$), for both nanohardness and elastic modulus. Different lowercase mean significant differences in relation to EWBT at baseline or after aging ($p < 0.001$).

For the nanoleakage, there were no differences between baseline and after aging evaluations, regardless of the use or not of ethanol ($p=0.096$). Overall, differences were detected for the use of ethanol ($p=0.021$). Those differences were detected in the 6-month evaluation period ($p=0.040$) and not in the baseline ($p=0.111$). Differences are related to lesser infiltration scores overtime while using the EWBT. (Table 2)

Table 2 – Nanoleakage evaluation for baseline and after 6 month water storage

Nanoleakage scores	Baseline*		6 months*	
	EWBT ^a	Without EWBT ^a	EWBT ^a	Without EWBT ^b
0	7	4	4	-
1	-	-	2	4
2	-	-	1	2
3	-	2	1	3
Evaluated samples	7	6	8	9
Non-evaluated samples (lost during processing)	3	4	2	1
Median (minimum and maximum values)	0 (0 – 0)	0 (0 – 3)	0.5 (0 – 3)	2.0 (1 – 3)

Asterisks represent similarity between baseline and 6 months results ($p=0.096$).

Different lower case letters mean significant differences between groups receiving or not ethanol, at baseline or at the 6 months recall ($p<0.05$).

4. Discussion

The mechanical properties of a properly formed hybrid layer might present improved mechanical characteristics when compared to demineralized non-properly infiltrated collagen fibers (not properly formed hybrid layer), supporting the present study. Moreover, the knowledge about the nanohardness and elastic modulus properties of the adhesive interface is relevant, once it is also directly related to how dental tissue and restorative materials respond to masticatory efforts. [17] In addition, for resin composite restorations, a good seal is also related to the ability of the adhesive system to withstand the immediate or late mechanical stresses due to resin polymerization contraction or masticatory efforts. [1]

The evaluated areas resulted in different mechanical characteristics, and thus the first null hypothesis was rejected (Table 1). Resin composite served as a control area as the nanohardness and elastic modulus values were not changed considering the EWBT or the aging process, as expected. Dentin nanohardness was greater than the hybrid layer observations, possibly due to dentin substrate components that are related to its physical-mechanical properties. The reported difference between dentin and hybrid layer may be explained by the hardness of the dentin that is related to the apatite crystals, which are responsible to the compression resistance. [17] Reports in the literature stated that the hybrid layer (11.7 ± 7.2) had a lower elastic modulus, therefore softer and more elastic, when compared to the sound dentin (19.1 ± 8.3). [18] This behavior may be related to the demineralization process and to the adhesive system composition. Considering the elastic modulus, dentin presented higher values when compared to hybrid layer, independently to EWBT or the aging process. Collagen fibers, together with dentin tubules number and direction, are responsible for the elasticity of the tissue, directly affecting dentin elastic modulus. [17,21] The differences observed in the present study are related to

the fact dentin within the hybrid layer was demineralized and the penetrated adhesive system contributed to the observed characteristics. This is supported by a study that indicated the mode of application of adhesives might influence the elastic modulus of dentin, which is greater for etch-and-rinse when compared to self-etch protocols. [22]

In relation to EWBT, the use of the approach was able to increase the initial nanohardness values of both the hybrid layer and the dentin. Greater values for ethanol groups were observed even after the aging process. The second null hypothesis was then rejected. The present results might be explained by the fact that EWBT is capable of increasing the interfibrillar spaces, allowing greater penetration and greater amount of resin monomers comparing to the conventional technique, and consequently protecting the collagen fibrils and increasing mechanical properties of the formed hybrid layer. [19,20] In addition, this result corroborates with a study in the literature that reported that smear layer did not decreased the bond strength of the hydrophilic adhesive on self-etch mode after EWBT and even after aging. [12] Besides that, for dentin, the higher values after EWBT may be related to the ethanol ability to dehydrate organic molecules. [12,7]

Another factor that may contribute to maintaining the hybrid layer characteristics while using the universal adhesive system on the self-etching mode to dentin is the maintenance of hydroxyapatite around the collagen fibrils, which might protect the adhesive interface from degradation. [19,23,24] EWBT was proposed to improve the impregnation of the hydrophobic monomers in the dentin substrate, [1-3] and to decrease water amount of the interface, [9] fact that would improve the hybrid layer characteristics at baseline and overtime. As the adhesive restorations longevity depends on a stable adhesion between restorative materials and the hard tissues and this pretreatment decrease water amount from adhesive interface, [9] it is believed that the hydrolysis of hybrid

layer should be reduced.

Differences in adhesives formulations might also influence the results, [22] and the presence of smear layer may difficult the infiltration of the resin components from the adhesive. In self-etch adhesives, the 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) is a major hydrophobic component. This component is an acidic monomer stable to hydrolysis and durable in intra-oral conditions. [25] However, MDP-primed dentin is degradable with water aging, once 10-MDP molecular structures include hydrophilic functional groups that are susceptible to cycling conditions. [25] These facts corroborate with this study since higher nanohardness and elastic modulus values were obtained on baseline when compared to 6 months regardless EWBT. The third null hypothesis was then rejected. Aging was able to decrease the nanohardness and elastic modulus values, which may indicates degradation by hydrolysis, since the sorption of water results in reduction of bonding forces between the polymer chains during polymerization, decreasing the mechanical properties of the adhesive interface. [26,27] In addition, it is reported that self-etching adhesives may act as semi-permeable membranes, even after polymerization, therefore more susceptible to aging. [26,28]

For nanoleakage, lower overall scores were observed for the use of EWBT, and especially at the 6-month assessment. No influence of aging was detected for nanoleakage. The fourth null hypothesis was thus partially rejected. This characteristic can be explained by the decrease of the amount of water in the dentin substrate by ethanol, once contemporary adhesives do not completely displace water that can lead to dilution of monomers and water sorption. [27] In addition, EWBT increases the stiffness of collagen fibrils and also reduces the matrix volume and the fibrils diameter, which favors the interfibrillar spaces increasing and the greater impregnation of the adhesive systems and hydrophobic monomers (BisGMA). [29,30] These characteristics might explain

the reduced nanoleakage of EWBT group after aging. One might question the fact the hydrophilic monomers are capable to bond with water and to consequently form silver nitrate deposits, [20] and this characteristic might hinder the results. Although this assumption seems to be pertinent, as both groups used the same adhesive in the same mode, the assumption was discarded.

The nanoleakage results are in agreement with the literature that reports that higher values of dentin adhesion after EWBT were found, when using a universal adhesive system in the self-etching mode, even after thermomechanical aging. [12] This report enforces the resin impregnation and collagen characteristics, as previously discussed. Longer aging periods would probably lead to more nanoleakage as the aging process has been already detected in the mechanical properties analyses.

The combination of EWBT with hydrophilic adhesive systems seems to be a possible indication considering the present and previous studies.

Considering the limitations of the present study and based on the methodology employed, further researches should evaluate the EWBT effect on the adhesive system impregnation by evaluating adhesive layer separately from the hybrid layer. Other substrate properties after EWBT associated with hydrophilic adhesives and smear layer presence might be investigated. Clinical trials should be performed for indicating this technique in a clinical scenario.

6. Conclusion

The simplified EWBT presented higher values of nanohardness and elastic modulus and higher sealing capacity of the adhesive interface even after a universal multi-mode adhesive application on self-etching mode and after aging for 6 months. This result suggests that the use of universal adhesives with EWBT is an alternative to create a stable bond and possible clinical indication.

6. Funding

This work was supported by FAPESP [grant number: 2017/13640-9 - second author's scholarship] and CNPq (Brazilian National Council for Scientific and Technological Development).

Declarations of interest: none

7. References

- [1] Nagpal R, Manuja N, Pandit IK (2015) *Effect of ethanol wet bonding technique on the durability of resin- dentin bond with contemporary adhesive systems. J Clin Pediatr Dent 39:133-142*
- [2] Talungchit S, Jessop JL, Cobb DS, Qian F, Geraldeli S, Pashley DH, Armstrong SR (2014) *Ethanol-wet bonding and chlorhexidine improve resin-dentin bond durability: quantitative analysis using raman spectroscopy. J Adhes Dent 16:441-450. doi:10.3290/j.jad.a32695*
- [3] Tjaderhane L (2015) *Dentin bonding: can we make it last? Oper Dent 40:4-18. doi:10.2341/14-095-BL*
- [4] Sadek FT, Castellan CS, Braga RR, Mai S, Tjaderhane L, Pashley DH, Tay FR (2010) *One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. Dent Mater 26:380-386. doi:10.1016/j.dental.2009.12.009*
- [5] Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR (2010) *Ethanol wet-bonding challenges current anti-degradation strategy. J Dent Res 89:1499-1504. doi:10.1177/0022034510385240*
- [6] Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, Watson TF, Osorio R (2010) *Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Dent Mater 26:368-379. doi:10.1016/j.dental.2009.12.008*

- [7] Ahn J, Jung KH, Son SA, Hur B, Kwon YH, Park JK (2015) Effect of additional etching and ethanol-wet bonding on the dentin bond strength of one-step self-etch adhesives. *Restor Dent Endod* 40:68-74. doi:10.5395/rde.2015.40.1.68
- [8] Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E (2008) Dental adhesion review: aging and stability of the bonded interface. *Dent Mater* 24:90-101. doi:10.1016/j.dental.2007.02.009
- [9] Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, Donnelly A, Garcia-Godoy F (2007) From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am J Dent* 20:7-20
- [10] 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. doi:10.1016/j.dental.2010.10.016
- [11] Rosa WL, Piva E, Silva AF (2015) Bond strength of universal adhesives: A systematic review and meta-analysis. *J Dent* 43:765-776. doi:10.1016/j.jdent.2015.04.003
- [12] Souza MY, R DIN, Bresciani E (2018) Influence of ethanol-wet dentin, adhesive mode of application, and aging on bond strength of universal adhesive. *Braz Oral Res* 32:e102. doi:10.1590/1807-3107bor-2018.vol32.0102
- [13] Miyazaki M, Tsujimoto A, Tsubota K, Takamizawa T, Kurokawa H, Platt JA (2014) Important compositional characteristics in the clinical use of adhesive systems. *J Oral Sci* 56:1-9
- [14] Singh V, Misra A, Marangos O, Park J, Ye Q, Kieweg SL, Spencer P (2010) Viscoelastic and fatigue properties of model methacrylate-based dentin adhesives. *J Biomed Mater Res B Appl Biomater* 95:283-290. doi:10.1002/jbm.b.31712
- [15] Tay FR, Pashley DH, Yoshiyama M (2002) Two modes of nanoleakage

expression in single-step adhesives. *J Dent Res* 81:472-476. doi:10.1177/154405910208100708

[16] Yuan Y, Shimada Y, Ichinose S, Tagami J (2007) Qualitative analysis of adhesive interface nanoleakage using FE-SEM/EDS. *Dent Mater* 23:561-569. doi:10.1016/j.dental.2006.03.015

[17] Senawongse P, Pongprueksa P, Tagami J (2010) The effect of the elastic modulus of low-viscosity resins on the microleakage of Class V resin composite restorations under occlusal loading. *Dent Mater J* 29:324-329

[18] Nakazawa Y, Seino E, Ushiki T, Ogata T, Hirai Y, Kawada E, Oda Y (1999) Microhardness evaluations of resin-dentin bonding areas by nano-indentation. *Bull Tokyo Dent Coll* 40:47-54

[19] Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, Tay FR, Pashley DH (2009) Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. *J Dent Res* 88:146-151. doi:10.1177/0022034508328910

[20] Reis A, Klein-Junior CA, Accorinte Mde L, Grande RH, dos Santos CB, Loguercio AD (2009) Effects of adhesive temperature on the early and 6-month dentin bonding. *J Dent* 37:791-798. doi:10.1016/j.jdent.2009.06.007

[21] Pongprueksa P, Senawongse P, Vongphan N (2014) Effect of dentinal tubule orientation on the modulus of elasticity of resin-infiltrated demineralized dentin. *Dent Mater J* 33:54-58

[22] Yasuda G, Inage H, Kawamoto R, Shimamura Y, Takubo C, Tamura Y, Koga K, Miyazaki M (2008) Changes in elastic modulus of adhesive and adhesive-infiltrated dentin during storage in water. *J Oral Sci* 50:481-486

[23] Mine A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Van Ende A, Matsumoto M, Yoshida Y, Kuboki T, Yatani H, Van Meerbeek B (2014) Dentin-smear remains at self-etch adhesive interface. *Dent Mater* 30:1147-1153. doi:10.1016/j.dental.2014.07.006

- [24] Perdigao J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, Loguercio AD (2014) A new universal simplified adhesive: 18-month clinical evaluation. *Oper Dent* 39:113-127. doi:10.2341/13-045-C
- [25] Zhou J, Wurihan, Shibata Y, Tanaka R, Zhang Z, Zheng K, Li Q, Ikeda S, Gao P, Miyazaki T (2019) Quantitative/qualitative analysis of adhesive-dentin interface in the presence of 10-methacryloyloxydecyl dihydrogen phosphate. *J Mech Behav Biomed Mater* 92:71-78. doi:10.1016/j.jmbbm.2018.12.038
- [26] Catelan A, Pollard T, Bedran-Russo A, Santos PD, Ambrosano G, Aguiar F (2014) Light-curing time and aging effects on the nanomechanical properties of methacrylate- and silorane-based restorations. *Oper Dent* 39:389-397. doi:10.2341/12-504-L
- [27] Mortazavi V, Samimi P, Rafizadeh M, Kazemi S (2012) A randomized clinical trial evaluating the success rate of ethanol wet bonding technique and two adhesives. *Dent Res J (Isfahan)* 9:588-594
- [28] Reis A, Loguercio AD (2009) A 36-month clinical evaluation of ethanol/water and acetone-based etch-and-rinse adhesives in non-carious cervical lesions. *Oper Dent* 34:384-391. doi:10.2341/08-117
- [29] Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, Tay KC (2007) Bonding BisGMA to dentin--a proof of concept for hydrophobic dentin bonding. *J Dent Res* 86:1034-1039. doi:10.1177/154405910708601103
- [30] Kim J, Gu L, Breschi L, Tjaderhane L, Choi KK, Pashley DH, Tay FR (2010) Implication of ethanol wet-bonding in hybrid layer remineralization. *J Dent Res* 89:575-580. doi:10.1177/0022034510363380.

2.2 Artigo – Souza MY, Jurema ALB, Caneppele TMF, Bresciani E. Acompanhamento de seis meses de restaurações realizadas com a técnica da dentina úmida em etanol: estudo clínico randomizado / *Six-month performance of restorations produced with the ethanol-wet-bonding technique: A Randomized Trial**

RESUMO

Este estudo clínico controlado randomizado duplo-cego avaliou a eficácia do pré-tratamento da dentina com etanol a 100% (EWBT – ethanol-wet-bonding technique) e diferentes protocolos adesivos em lesões cervicais não cariosas (LCNC) após 6 meses. Pacientes apresentando pelo menos uma LCNC foram incluídos. 148 LCNC foram aleatoriamente divididas em 4 grupos: SE (Sem EWBT [SE] + adesivo de três passos (Scotchbond Multi Purpose, 3M ESPE [MP]), E (EWBT + MP); EB (EWBT + [Bond - terceiro passo do MP], e EU (EWBT + adesivo universal (Single Bond Universal, 3M ESPE). Condicionamento ácido (Condac 37%, FGM) e resina composta nanohíbrida (Z350xt, 3M ESPE) foram utilizados. Os examinadores treinados e calibrados (Kappa=0,61) avaliaram as restaurações inicialmente (7 dias) e após 6 meses de avaliação, utilizando os critérios USPHS modificado. Os dados foram submetidos ao teste qui-quadrado ($\alpha=0,05$). Diferenças na taxa de retenção entre os grupos foi observada para os tratamentos ($p=0,003$). EB apresentou a menor taxa de sucesso em comparação com os outros grupos ($p<0,02$). Não foram detectadas diferenças significativas entre NE, E e EU ($p>0,49$). As taxas de retenção foram de 97,23% 97,30%, 78,95% e 100% para NE, E, EB e EU, respectivamente. Em relação à sensibilidade pós-operatória, uma redução significativa foi encontrada para os grupos E ($p=0,027$) e EU ($p<0,01$) após 6 meses. Após 6 meses, EWBT associado ao sistema adesivo hidrofóbico apresentou a maior taxa de falha.

Palavras-chave: Dentina. Etanol. Adesivos. Abrasão dentária.

*Artigo elaborado de acordo com as normas do Periódico *Brazilian Oral Research* (Print version 1807-3107).
Publicado em: 01/07/2019.

ABSTRACT

This double-blind randomized controlled clinical trial evaluated the effectiveness of dentin pretreatment with 100% ethanol (EWBT – ethanol wet bonding technique) and different adhesive protocols in noncarious cervical lesions (NCCL) after 6 months. Patients presenting at least one NCCL were included. NCCLs (n=148) were randomly assigned to 4 groups: NE (Non-EWBT + three-step etch-and-rinse (Scotchbond Multi Purpose, 3M ESPE [MP]), E (EWBT + MP); EB (EWBT + [Bond – third step of MP]), and EU (EWBT + universal adhesive (Single Bond Universal, 3M ESPE). Conventional acid-etching (Condac 37%, FGM) and nanohybrid resin composite (Z350, 3M ESPE) were used. Trained and calibrated examiners (Kappa = 0.61) evaluated the restorations at baseline (7 days) and 6-month recall using the USPHS modified criteria. Data were subjected to Chi square ($\alpha = 0.05$). Differences in the success rate were found for the treatments ($p = 0.003$). EB presented the lowest success rate compared with the other groups ($p < 0.02$). No significant differences were detected among NE, E, and EU ($p > 0.49$). The survival rates were 97.23%, 97.30%, 78.95%, and 100% for NE, E, EB, and EU, respectively. Regarding postoperative sensitivity, a significant reduction was found for groups E ($p = 0.027$) and EU ($p < 0.01$) after 6 months. After 6 months, EWBT associated to the hydrophobic adhesive system had the highest failure rate.

Keywords: Dentin. Ethanol. Adhesives. Tooth abrasion.

Introduction

Dental tissues undergo different responses to acid-etching procedures¹ and consequently to different adhesion patterns. For enamel, adhesion is facilitated because of the predominance of hydroxyapatite crystals and the consequent adequate formation of resin microtags.^{1, 2} However, dentin consists of predominantly organic components, mainly collagen fibers, extension of odontoblasts, and dentinal fluid, which leads to a more variable and challenging bonding procedure.²⁻⁴

A meta-analysis⁵ indicated that the best adhesion results to dentin were observed with the use of 2-step self-etch (moderate pH) and 3-step etch-and-rinse adhesives. The authors also concluded that additional studies are required to improve dental adhesion. Although new dental adhesives have been introduced, including adhesives named “universal” or “multi-mode,” resin restorations may still fail after a short lifespan because of degradation of the adhesive interface.⁶ Universal adhesives are adaptable, as they can be applied to wet or dry dentin and with both self-etch and etch-and-rinse protocols.³ In addition to improvements in the adhesive, some additional measures proposed to minimize interface degradation include dentin pretreatment,^{7, 8} addition of different chlorhexidine concentrations to adhesives,⁷ formulation of experimental adhesives,⁹ use of matrix metalloprotease (MMPs) inhibitors,¹⁰ and the ethanol wet bonding technique (EWBT).¹¹

The EWBT might extend the longevity of resin–dentin bonding if improved resin monomer infiltration and the consequent improved formation of the hybrid layer occurs.¹² Ethanol has a higher solvent ability and lower hydrogen bonding capacity than water. In the EWBT, it is used to dehydrate demineralized collagen fibrils chemically, creating a relatively hydrophobic dentin collagen matrix, which decreases the interface hydrolysis associated with water removal from the substrate.¹² Two protocols have been reported for

applying the EWBT: progressive water substitution, in which dentin ethanol saturation is obtained using increasing ethanol concentrations (takes about 3 to 4 min¹²); and a simplified protocol, where dehydration occurs with a single 1-minute application of 100% ethanol.¹³

Because of the high volatility of ethanol, the technique is sensitive; moreover ethanol does not completely displace water from dentin, a fact that may hinder the infiltration of hydrophobic adhesives.¹¹ In vitro data suggest that the formed hybrid layer is less hydrophilic and more resistant to long-term hydrolytic degradation caused by water. The EWBT might improve longevity by effectively protecting the collagen.¹⁴ Few clinical studies have evaluated the EWBT as a dentin pretreatment.^{15, 38} The use of ethanol has been evaluated with a modified progressive technique³⁸ and by using two coats of hydrophobic bonding resin,¹⁵ with no significant differences because of the technique in these two clinical studies. In another study, a formulated experimental hydrophobic adhesive containing ethanol in its hydrophobic primer composition resulted in acceptable performance.¹⁶ The primer solution in that study was prepared by diluting an experimental hydrophobic adhesive in absolute ethanol up to 50%weight.¹⁶ To the best of the authors' knowledge, only one report on the clinical use of the EWBT with a commercially available hydrophobic adhesive has been published.¹⁵ Moreover, only one study evaluated the cytotoxicity of the EWBT on human pulp cells; the authors concluded that it does not increase pulpal damage when compared with water-wet bonding after 48 hours.¹⁷

Non-carious cervical lesions (NCCLs) represent cervical tooth wear not associated with dental caries. These lesions may have different extensions, shapes (wedge, flat, concave, or acute angle) and depths.¹⁸ The restoration of NCCLs remains a challenge for clinicians, since retention loss can vary from 0% to 50%.¹⁹ For this reason, this type of cavity is the most appropriate for testing tooth adhesion in clinical trials. Factors responsible for restoration loss

include the difficulty of adhesion between substrate and materials, the degree of dentin sclerosis, and the adequate establishment of the hybrid layer.¹⁹ In addition, both mechanical and non-mechanical factors decrease the longevity of restorations for NCCLs, including microshear forces during mastication.¹⁹

Based on the presented information and regarding the problems associated with dentin adhesion, possible dentin pretreatments that favor dentin adhesion should be assessed with well-designed clinical studies.⁵ A recent systematic review emphasized the need for clinical trials to determine the best dentin pretreatment.⁵ Therefore, the purpose of this clinical study was to evaluate the effectiveness of dentin pretreatment with 100% ethanol on the restoration of NCCLs using different adhesive protocols. The null hypothesis was that the ethanol wet bonding technique in association with different adhesive protocols does not influence the success of NCCLs restorations overtime.

Methodology

Ethics

The local Institutional Review Board approved the present study (protocol number: 2.022.383). The study was also registered in the clinical trials registry database REBEC (<http://www.ensaiosclinicos.gov.br>) under protocol RBR-5hncr3.

Sample size calculation

For this study, the sample size was calculated using an online statistical website - Sealed Envelope Ltda (www.sealedenvelop.com). For power calculation, equivalence trial function under binary outcome was selected, and the following parameters were chosen: $\alpha = 5\%$, power at 80%, success of control and experimental group at 96%, and limit of equivalence at 15%.²⁰

Sample size was estimated at 30 restorations per group. Considering the possible loss of experimental units, a further five restorations were planned for each group, a total of 35 restorations per group and 140 restorations in total.

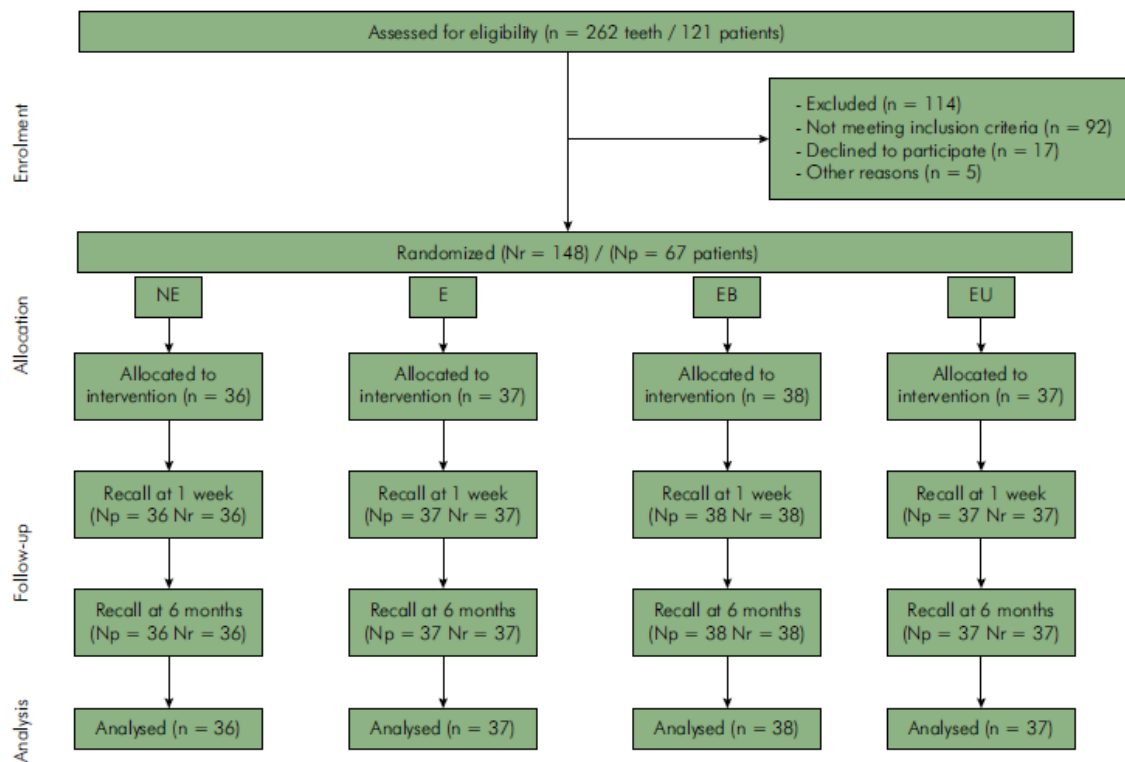
Inclusion and exclusion criteria

The inclusion criteria for patient selection were as follows: minimum age of 18 years; presence of at least one NCCL cavity in permanent canines or premolars; lesion depth and cervical width of 1 mm at minimum; presence of the antagonist and adjacent teeth; vital pulp and absence of painful symptoms; NCCL with no previous restorative treatment; and good oral health and absence of periodontal disease.

The exclusion criteria for selection were as follows: teeth with cervical caries lesions and patients with systemic diseases (e.g. gastroesophageal reflux) or allergies to the materials used; presence of deleterious habits or bruxism; and use of removable prostheses with clamps on the target teeth.

For this study, 67 participants met the inclusion criteria, signed the informed consent and were thus included (Figure 1).

Figure 1 - Flowchart including patient selection, restorations performed, and evaluation phase.



Study design

This randomized controlled clinical trial followed CONSORT guidelines.²¹ As soon as the patient arrived at the clinic, the teeth were chosen for this clinical trial following the inclusion and exclusion criteria and the informed consent form was signed. Prior to the restorative procedure, a person not involved in this clinical trial generated a randomization sequence of treatments for each patient, considering the four protocols. For patients receiving more than one treatment, in case more than one tooth was included in the study, treatments were allocated following a crescent tooth number (international tooth numbering system). Each patient received at least one and a maximum of four restorations; no patient received duplicate treatments. Thus, the treatments were allocated randomly for the included NCCLs.

Restorative procedure

All participants underwent professional pumice prophylaxis using a polishing brush. Isolation was then done with a lip retractor, cotton rolls, gingival displacement cord, and a saliva ejector. A bevel was not placed before restoring the lesion.

All cavities were conditioned with 37% phosphoric acid (Condac, FGM, Brazil) for 15 s, followed by rinsing for 15 s and drying with absorbent paper. Lesions allocated under the EWBT protocol received 100% ethanol, actively applied for 60 s with a microbrush. The adhesive systems were then applied, following the groups:

Control Group – NE: Non-EWBT [NE] + three-step etch-and-rinse (Scotchbond Multi Purpose, 3M ESPE [MP]); primer was applied to etched substrate and gently dried for 5 s + bond was actively applied for 20 s, followed by a gentle blow of air for 5 s and light polymerizing for 10 s.

E: EWBT [E] + three-step etch-and-rinse (Scotchbond Multi Purpose, 3M ESPE); 100% ethanol was actively applied for 60 s + primer was applied to etched substrate and gently dried for 5 s + bond was actively applied for 20 s, followed by a gentle blow of air for 5 s and light polymerizing for 10 s.

EB: EWBT [E] + Bond (Scotchbond Multi Purpose, 3M ESPE) [B] (third step – Hydrophobic adhesive); 100% ethanol was actively applied for 60 s + bond was actively applied for 20 s, followed by a gentle blow of air for 5 s and light polymerizing for 10 s.

EU: EWBT [E] + Universal adhesive (Scotchbond Universal, 3M ESPE) [U] (Hydrophilic); 100% ethanol was actively applied for 60 s + universal adhesive was actively applied for 20 s, followed by a gentle blow of air for 5 s and light polymerizing for 10 s.

Filtek Z350 XT (3M ESPE, Sumaré, Brazil) was used with an incremental technique for the restorations. Each increment of 2 mm, maximum,

was polymerized for 20 s. The increments were initially placed on the incisal wall and then on the gingival wall. Most of restorations were completed with 2 increments. For all restorative procedures the light polymerizing was performed with an LED device with a power density of 900 mW/cm². Finishing was performed with fine finishing diamond rotary instruments (KG Sorensen, São Paulo, SP, Brazil). After 7 days, the restoration was polished with abrasive discs (Sof-Lex™ Pop-On, 3M ESPE, MN, USA).

Periods and evaluation criteria

The restorations were evaluated by two calibrated examiners (interexaminer agreement of 81%, Kappa 0.61) not involved with the restorative procedures. Evaluations were performed at baseline (7 days) and after 6 months according to USPHS modified criteria (Figure 2). The patients and examiners were blind to the study parameters.

Figure 2 - USPHS modified criteria.

SCORES	USPHS modified criteria
Retention	
Alfa (A)	No restorative material loss
Charlie (C)	Partial or complete loss of restorative material
Marginal discoloration	
Alfa (A)	No discoloration between tooth structure and restorative material
Bravo (B)	Marginal discoloration which can be polished away
Charlie (C)	Discoloration in interface restorative material and tooth, no able to polish
Marginal adaptation	
Alfa (A)	Closely adapted, no detectable margin
Bravo (B)	Detectable marginal discrepancy clinically acceptable
Charlie (C)	Marginal crevice, clinically unacceptable
Delta (D)	Mobile restoration, partially or totally fractured
Secondary caries	
Alfa (A)	No caries present
Charlie (C)	Caries present
Anatomic form	
Alfa (A)	Continuous, well contoured
Bravo (B)	Slight discontinuity or under contoured without dentin exposure, clinically acceptable
Charlie (C)	Discontinuous, sever under contoured, with dentin exposure, clinically unacceptable
Post-operative sensitivity	
Alfa (A)	No post-operative
Bravo (B)	Sensitive but with intensity decreasing
Charlie (C)	Constant sensitivity, without intensity decreasing
Surface texture	
Alfa (A)	Texture such as enamel
Bravo (B)	Texture such as resin composite
Charlie (C)	Surface with pores or cracks, with dental pick retention

Statistical analysis

All data were analyzed using the chi-square test to verify the distribution of the success rate and postoperative sensitivity among groups. The level of significance was set at 5%.

Results

The demographics of the study participants are shown in Table 1. The participants were evenly distributed into groups with regard to sex, age, restored tooth, and the presence of wear facets in relation to restoration success or failure. Failures were detected only for the retention parameter. Differences

in success rates were detected among all groups (chi-square; $p=0.003$). EB presented lower success rates when compared with all tested adhesive protocols: EB versus NE ($p=0.002$), EB versus E ($p=0.025$), and EB versus EU ($p=0.0023$). However, no significant differences were detected for other comparisons: NE versus E ($p=0.496$), NE versus EU ($p=1.000$), E versus EU ($p=0.497$). The survival rates were 97.23%, 97.30 %, 78.95 %, and 97.30 %, for NE, E, EB, and EU, respectively. (Table 2)

Overall, tooth sensitivity was less after 6 months in comparison with the baseline observations ($p<0.001$). A significant reduction in postoperative sensitivity after 6 months was detected for E ($p=0.027$) and EU ($p<0.001$). (Table 2). For other criteria, no significant differences ($p>0.05$) were found.

Table 1. Demographics of the studied population. Distribution into studied groups by sex, age, restored teeth, and the frequency of wear facets in relation to restoration success or failure.

			NE	E	EB	EU
Men			19/36 (52.77%)	23/37 (62.16%)	18/38 (47.37%)	15/37 (40.54%)
Women			17/36 (47.23%)	14/37 (37.83%)	20/38 (52.63%)	22/37 (59.45%)
Age (years)			55.75	53.21	52. 60	55.43
Maxillary Canine			4/148 (2.70%)	4/148 (2.70%)	7/148 (4.73%)	3/148 (2%)
Mandibular Canine			2/148 (1.35%)	2/148 (1.35%)	1/148 (0.67%)	0/148 (0%)
Maxillary Premolar			10/148 (6.75%)	13/148 (8.78%)	12/148 (8.10%)	10/148 (6.75%)
Mandibular Premolar			21/148 (14.19%)	18/148 (12.16%)	18/148 (12.16%)	24/148 (16.21%)
Tooth wear facet	Presence	Success	14	19	10	19
		Loss	3	1	6	0
	Absence	Success	19	15	15	18
		Loss	0	2	7	0

Table 2. Distribution of number of restorations and percentage (%) according to each evaluated criteria at 7 days and 6 months.

CRITERIA	SCORES	BASELINE - 7 DAYS				6 MONTHS			
		NE	EU	EB	E	NE	EU	EB	E
Retention	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	35 (97.23%) ^a	36 (97.30%) ^a	30 (78.95%) ^b	36 (97.30%) ^a
	Charlie	0	0	0	0	1 (2.77%)	1 (2.70%)	8 (21.05%)	1 (2.70%)
Marginal discoloration	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	32 (88.89%)	33 (89.19%)	30 (78.95%)	36 (97.30%)
	Bravo	0	0	0	0	3 (8.34%)	3 (8.11%)	0	0
	Charlie	0	0	0	0	0	0	0	0
Marginal adaptation	Alfa	35 (97.23%)	37 (100%)	37 (97.37%)	37 (100%)	32 (88.89%)	32 (86.49%)	28 (73.68%)	34 (89.19%)
	Bravo	1 (2.77%)	0	1 (2.63%)	0	3 (8.34%)	4 (10.81%)	2 (5.27%)	4 (10.81%)
	Charlie	0	0	0	0	0	0	0	0
Secondary caries	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	35 (97.23%)	36 (100%)	30 (78.95%)	36 (97.30%)
	Charlie	0	0	0	0	0	0	0	0
Anatomic form	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	35 (97.23%)	35 (94.60%)	30 (78.95%)	35 (94.60%)
	Bravo	0	0	0	0	0	1 (2.70%)	0	1 (5.40%)
	Charlie	0	0	0	0	0	0	0	0
Postoperative sensitivity	Alfa	27 (75%)	30 (81.08%)	28 (23.68%)	30 (81.08%)	32 (88.89%)	35 (94.60%)*	28 (73.68%)	35 (94.60%)*
	Bravo	8 (22.22%)	7 (18.92%)	9 (76.32%)	7 (18.92%)	3 (8.34%)	1 (2.70%)	2 (5.27%)	1 (5.40%)
	Charlie	1 (2.77%)	0	0	0	0	0	0	0
Surface texture	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	31 (86.11%)	31 (83.79%)	26 (68.42%)	31 (83.79%)
	Bravo	0	0	0	0	4 (11.12%)	5 (13.51%)	4 (10.53%)	5 (13.51%)
	Charlie	0	0	0	0	0	0	0	0

Different superscript letters indicate differences on retention rates at the 6-month recall ($p < 0.05$)

Asterisks indicate groups presenting significant reduction of post-operative sensitivity at the 6-month recall in relation to the baseline ($p < 0.05$)

Discussion

Complete removal of water from the dentin is not possible with the adhesive systems, leading to the dilution of monomers and water sorption of the adhesive layer during aging, with consequent hydrolysis.^{15, 22} In order to minimize the hybrid layer and unprotected collagen hydrolysis overtime, the EWBT approach has been advocated for several reasons. The EWBT might remove and replace free and loosely bound water around the orifice of the dentin tubules and within the collagen microfibrils.^{22, 23} However, the water is not completely removed from the dentin substrate.¹¹ Questions regarding the effect of the amount of remaining water on the bond strength with hydrophobic adhesives have been raised, and that issue has been evaluated in an in vitro study.²⁴ The results were acceptable when hydrophilic adhesives were used with EBWT, possibly due to the combination of the remaining water in dentin and the water from the adhesive composition. The implementation of a new layer of water in a dehydrated dentin surface is questionable, but that association has been reported to be effective in in vitro conditions, warranting clinical investigations.

The EWBT approach also prevents phase separation of the adhesive,²⁵ possibly improving long-term bond strength.²⁶ In addition, the use of the EWBT might decrease the diameter of collagen fibrils and the matrix volume, consequently increasing the interfibrillar spaces and leading to greater impregnation of the adhesive system and hydrophobic monomers.²³ The results from micro-Raman²⁷ and two-photon laser confocal microscopy²⁸ analyses indicated that the EWBT favors the relatively homogeneous distribution of methacrylates (BisGMA) in the interfibrillar spaces. This allows the formation of a hybrid layer with improved mechanical and bonding properties, since the greater the amount of hydrophobic infiltrated resin monomers, the greater the resin-dentin bonding strength.²⁹ Improved hydrophobic monomer impregnation

protects and encapsulates collagen fibers more efficiently²⁷; and it decreases both water sorption and the enzyme-catalyzed hydrolytic cleavage of collagen,^{12, 23} increasing collagen rigidity, which contributes to the formation of a more effective hybrid layer.²⁶ In spite of the reported benefits of using the EWBT, the inclusion of an extra step in the adhesive protocol is a disadvantage, as it adds to the clinical time and may increase technique sensitivity. The main challenge of dental adhesion, which is related to the dentin substrate, is still not solved. Thus, testing alternative protocols, even with additional steps is justified.

The null hypothesis that the ethanol-wet-bonding technique does not influence NCCL restoration success over time was partially accepted. The combination of hydrophilic adhesive systems and the EWBT was compared to the control group (NE), while the use of a commercially available hydrophobic adhesive associated with the EWBT resulted in lower success rates when compared with the other tested groups.

The three-step etch-and-rinse adhesive system was considered the gold standard in a systematic review⁵, supporting its present use as the control group. Organic solvents within hydrophilic adhesive systems, such as acetone and ethanol, are preferred to water, as they encourage infiltration of resin monomers into collagen fibrils, resulting in better adhesive bonding.³⁰ According to Mair and Padipatvuthikul,³¹ organic solvents displace water molecules within the dentin matrix and, because of their high evaporation capacity, facilitate the diffusion of monomers into demineralized dentin. However, the presence of residual solvent in the adhesive interface might result in disturbances in the polymerization reaction, which would reduce adhesion. The better the evaporation capacity, the lower the amount of residual solvent at the adhesive interface.³² Because the Single Bond Universal adhesive system also contains ethanol as a solvent, the EWBT may have facilitated the impregnation of resin monomers, resulting in stable bonding.

Although the increased permeability of monomers may increase toxicity potential and compromise the longevity of restorations,³¹ this was not observed in the present study during the evaluated period. Moreover, the EWBT may increase the absorption and degree of conversion of the resin monomers²⁵ and produce a hydrophobic collagen matrix with improved sealing, even if a hydrophilic adhesive is used. In addition, the EWBT reduces the sorption of water and collagen hydrolysis, making the hybrid layer more stable.³³ All those reported advantages might explain the positive results obtained with the hydrophilic adhesive used in the present study. Longer evaluation periods may reveal a possible benefit of associating the EWBT with the hydrophobic adhesive systems tested.

Improved bonding between hydrophobic adhesives and dentin has been reported with EWBT, possibly because of improved water removal from the collagen matrix and proper collagen encapsulation, forming a less hydrophilic hybrid layer.^{16, 34} However, clinical trials evaluating the EWBT did not show significant differences after 12 or 18 months, with retention rates for the EWBT at 91.67% and 93.55%.^{35, 38} Technique differences in those studies are related to the use of a formulated hydrophobic primer (hydrophobic bond diluted in ethanol in a concentration of 10% or 50 w% ethanol) or the ethanol not being actively applied.^{12, 15, 35, 36, 38} Although no differences were detected when compared with the control groups, one study reported that the 91.7% success for the EWBT in comparison with the 100% success rate for the control could be related to the sensitivity of the EWBT. This sensitivity in association with the viscosity of commercially available hydrophobic adhesives might explain the present data. Combining the EWBT with hydrophobic adhesive led to reduced success rates when compared with those of the other groups (EB versus NE [$p=0.002$], EB versus E [$p=0.025$], and EB versus EU [$p=0.023$]). The viscosity of the hydrophobic adhesive may have restricted monomer infiltration

into the demineralized dentin, as studies using diluted hydrophobic monomers for application resulted in improved clinical success rates.^{15, 35} The present study used commercially available hydrophobic monomers with the EWBT. Clinicians do not have access to diluted hydrophobic monomers, and the in vitro or in vivo results reported previously might not be reproducible as they depend on experimental materials.

For the universal adhesive, besides possible water removal from the dentin matrix and infiltration of resin monomers (Bis-GMA/TEGDMA), the interaction of 10-MDP functional monomer with calcium hydroxyapatite of the dentin³⁷ might also support the obtained results. That interaction helps decrease the dissolution rate of the Ca-salt formed and helps maintain the durability of the bonded interface.³⁷ Longer-term evaluations are required to validate this statement. The effect of EWBT on the self-etching mode of universal adhesives might be explained by the ethanol application modifying the organic matrix of the smear layer,²⁴ allowing crystals to be available for bonding with the adhesive system and possibly assisting the interdiffusion of the adhesive monomers in the smear layer and the collagen fibrils.

In addition, the combination of the EWBT and universal adhesives in the self-etch mode should be evaluated for possible interactions between the smear layer and the EWBT approach. Furthermore, clinical studies using self-etching adhesives with longer evaluation periods are needed. Limitations of this present clinical trial include that the restorations were not independent, the evaluation period was short, and some characteristics may have influenced the restorative treatment success, such as the thickness layer of the adhesives, adhesive viscosity, and wettability, in addition to the variable occlusion of the participants. For the participant occlusion, the distribution of failed and successful restorations was not related to the presence or absence of wear facets, suggesting that characteristic might have not influenced the results.

The value of short-term (6-month) recall information is unclear. Defining a better adhesive protocol with a dentin pretreatment might have affected the results. The importance of the present clinical trial relates to the fact that only one study evaluating the EWBT assessed the results using a commercially available product and mainly to the fact that the results were not favorable for the original technique at the very short-term presently reported.

Conclusions

The association between EWBT with commercially available hydrophobic adhesive system should be limited due to reduced restoration longevity. All hydrophilic adhesive systems presented greater success rate at this evaluation period.

References

- 1.Suda S, Tsujimoto A, Barkmeier WW, Nojiri K, Nagura Y, Takamizawa T, et al. Comparison of enamel bond fatigue durability between universal adhesives and two-step self-etch adhesives: Effect of phosphoric acid pre-etching. Dent Mater J. 2018;37(2):244-55. Epub 2018 Mar 30.*
- 2.Perdigao J. Dentin bonding as a function of dentin structure. Dent Clin North Am. 2002;46(2):277-301, vi. Epub 2002 Apr.*
- 3.Perdigao J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, et al. A new universal simplified adhesive: 18-month clinical evaluation. Oper Dent. 2014;39(2):113-27. Epub 2014 Mar-Apr.*
- 4.Carrilho MR, Carvalho RM, Tay FR, Yiu C, Pashley DH. Durability of resin-dentin bonds related to water and oil storage. Am J Dent. 2005;18(6):315-9. Epub 2005 Dec.*
- 5.Peumans M, De Munck J, Mine A, Van Meerbeek B. Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. A*

systematic review. *Dent Mater.* 2014;30(10):1089-103. Epub 2014 Oct.

6.Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. *Dent Mater.* 2011;27(1):1-16. Epub 2011 Jan.

7.Vallabhdas AK, Kumar CNV, Kabbinala P, Nayak R, Rajakumari M, Shilpa T. Evaluation of Hybrid Layer and Bonding Interface after Water Storage with and without the Usage of 2% Chlorhexidine: A Scanning Electron Microscope Study. *J Contemp Dent Pract.* 2018;19(1):52-9. Epub 2018 Jan 1.

8.Aggarwal V, Bhasin SS. Application of Calcium Silicate Materials After Acid Etching May Preserve Resin-Dentin Bonds. *Oper Dent.* 2018;43(5):E243-E52. Epub 2018 Sep/Oct.

9.Provenzi C, Collares FM, Cuppini M, Samuel SMW, Alves AK, Bergmann CP, et al. Effect of nanostructured zirconium dioxide incorporation in an experimental adhesive resin. *Clin Oral Investig.* 2018;22(6):2209-18. Epub 2018 Jul.

10.Hashimoto M, Hirose N, Kitagawa H, Yamaguchi S, Imazato S. Improving the durability of resin-dentin bonds with an antibacterial monomer MDPB. *Dent Mater J.* 2018;37(4):620-7. Epub 2018 Jul 29.

11.Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am J Dent.* 2007;20(1):7-20. Epub 2007 Feb.

12.Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR. Ethanol wet-bonding challenges current anti-degradation strategy. *J Dent Res.* 2010;89(12):1499-504. Epub 2010 Dec.

13.Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, et al. Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Part II: Effects of mechanical cycling load on microtensile bond

strengths. *Dent Mater.* 2011;27(6):563-72. Epub 2011 Jun.

14.Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. *Dent Mater.* 2008;24(1):90-101. Epub 2008 Jan.

15.Mortazavi V, Samimi P, Rafizadeh M, Kazemi S. A randomized clinical trial evaluating the success rate of ethanol wet bonding technique and two adhesives. *Dent Res J (Isfahan).* 2012;9(5):588-94. Epub 2012 Sep.

16.Sadek FT, Castellan CS, Braga RR, Mai S, Tjaderhane L, Pashley DH, et al. One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. *Dent Mater.* 2010;26(4):380-6. Epub 2010 Apr.

17.Scheffel DL, Sacono NT, Ribeiro AP, Soares DG, Basso FG, Pashley D, et al. Immediate human pulp response to ethanol-wet bonding technique. *J Dent.* 2015;43(5):537-45. Epub 2015 May.

18.Eliguzeloglu E, Eraslan O, Omurlu H, Eskitascioglu G, Belli S. The effect of cavity shape and hybrid layer on the stress distribution of cervical composite restorations. *Eur J Dent.* 2011;5(2):180-5. Epub 2011 Apr.

19.Heintze SD, Ruffieux C, Rousson V. Clinical performance of cervical restorations--a meta-analysis. *Dent Mater.* 2010;26(10):993-1000. Epub 2010 Oct.

20.Reis A, Loguercio AD. A 36-month clinical evaluation of ethanol/water and acetone-based etch-and-rinse adhesives in non-carious cervical lesions. *Oper Dent.* 2009;34(4):384-91. Epub 2009 Jul-Aug.

21.Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340:c332. Epub 2010 Mar 23.

22.Osorio E, Toledano M, Aguilera FS, Tay FR, Osorio R. Ethanol wet-bonding technique sensitivity assessed by AFM. *J Dent Res.* 2010;89(11):1264-9. Epub 2010 Nov.

- 23.Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, et al. *Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. J Dent Res.* 2009;88(2):146-51. Epub 2009 Feb.
- 24.Souza MY, R DIN, Bresciani E. *Influence of ethanol-wet dentin, adhesive mode of application, and aging on bond strength of universal adhesive. Braz Oral Res.* 2018;32:e102. Epub 2018 Oct 11.
- 25.Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, et al. *Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res.* 2011;90(8):953-68. Epub 2011 Aug.
- 26.Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, et al. *Bonding BisGMA to dentin--a proof of concept for hydrophobic dentin bonding. J Dent Res.* 2007;86(11):1034-9. Epub 2007 Nov.
- 27.Shin TP, Yao X, Huenergardt R, Walker MP, Wang Y. *Morphological and chemical characterization of bonding hydrophobic adhesive to dentin using ethanol wet bonding technique. Dent Mater.* 2009;25(8):1050-7. Epub 2009 Aug.
- 28.Sauro S, Watson TF, Mannocci F, Tay FR, Pashley DH. *Prevention of water contamination of ethanol-saturated dentin and hydrophobic hybrid layers. J Adhes Dent.* 2009;11(4):271-8. Epub 2009 Aug.
- 29.Kim J, Gu L, Breschi L, Tjaderhane L, Choi KK, Pashley DH, et al. *Implication of ethanol wet-bonding in hybrid layer remineralization. J Dent Res.* 2010;89(6):575-80. Epub 2010 Jun.
- 30.Ahn J, Jung KH, Son SA, Hur B, Kwon YH, Park JK. *Effect of additional etching and ethanol-wet bonding on the dentin bond strength of one-step self-etch adhesives. Restor Dent Endod.* 2015;40(1):68-74. Epub 2015 Feb.
- 31.Mair L, Padipatvuthikul P. *Variables related to materials and preparing for bond strength testing irrespective of the test protocol. Dent Mater.* 2010;26(2):e17-23. Epub 2010 Feb.

- 32.Salvio LA, Hipolito VD, Martins AL, de Goes MF. Hybridization quality and bond strength of adhesive systems according to interaction with dentin. *Eur J Dent*. 2013;7(3):315-26. Epub 2013 Jul.
- 33.Tjaderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer-A review. *Dent Mater*. 2013;29(10):999-1011. Epub 2013 Oct.
- 34.Huang X, Li L, Huang C, Du X. Effect of ethanol-wet bonding with hydrophobic adhesive on caries-affected dentine. *Eur J Oral Sci*. 2011;119(4):310-5. Epub 2011 Aug.
- 35.Araujo JF, Barros TA, Braga EM, Loretto SC, Silva e Souza Pde A, Silva e Souza MH. One-year evaluation of a simplified ethanol-wet bonding technique: a randomized clinical trial. *Braz Dent J*. 2013;24(3):267-72. Epub 2013.
- 36.Nagpal R, Manuja N, Pandit IK. Effect of ethanol wet bonding technique on the durability of resin- dentin bond with contemporary adhesive systems. *J Clin Pediatr Dent*. 2015;39(2):133-42. Epub 2015 Winter.
- 37.Yokota Y, Fujita KN, Uchida R, Aida E, Aoki NT, Aida M, et al. Quantitative Evaluation of MDP-Ca Salt and DCPD after Application of an MDP-based One-step Self-etching Adhesive on Enamel and Dentin. *J Adhes Dent*. 2016;18(3):205-13. Epub 2016.
- 38.Barros TAF, Da Araújo JF, Braga EMF, Silva e Souza PdAR, Loretto SC, Souza Júnior MHSE. Ethanol-Wet Bonding Technique: 18-month Clinical Evaluation. *Int J Odontostomatol*. 2016;10(2):267-76. Epub 2016 2016-08..

2.3 Artigo – Souza MY, Jurema ALB, Caneppele TMF, Bresciani E. 18 meses da performance da técnica da dentina úmida por etanol com diferentes protocolos adesivos: estudo clínico randomizado controlado / 18-month performance of ethanol-wet-bonding technique with different adhesive protocols: a randomized controlled clinical trial*

RESUMO

OBJETIVOS: Este ensaio clínico avaliou a eficácia da técnica da dentina úmida por etanol (EWBT - E) e diferentes protocolos adesivos em lesões cervicais não cariosas (LCNC) após 18 meses. **MATERIAIS E MÉTODOS:** Um ensaio clínico randomizado, controlado, duplo-cego foi realizado. Pacientes apresentando pelo menos uma LCNC foram incluídos. 148 LCNC foram aleatoriamente alocadas para 4 grupos: NE (Non-EWBT [NE] + adesivo de três passos (Scotchbond Multi-Purpose, 3M ESPE), EMP (Etanol [E] adesivo de três passos (Scotchbond Multi-Purpose, 3M ESPE [MP]); EB (E + terceiro passo de MP [B]) e EU (E + adesivo universal - Single Bond Universal, 3M ESPE [U]). Todas as cavidades foram condicionadas com ácido fosfórico a 37% (Condac, FGM) e então restaurado com resina nanohíbrida (Filtek Z350xt, 3M ESPE). Avaliadores treinados ($\kappa=0,61$) avaliaram as restaurações no início do estudo - 7 dias e após 6, 12 e 18 meses seguindo o critério USPHS modificado. Os dados foram submetidos aos testes de qui-quadrado e Kaplan-Meier ($\alpha=0,05$). **RESULTADOS:** Diferenças no sucesso foram encontradas entre os tratamentos ($p<0,001$). EB apresentou a menor taxa de sucesso em comparação com os outros grupos ($p<0,001$). Nenhuma diferença significativa foi detectada para NE, EMP e EU ($p\geq 0,290$). Após 18 meses, as taxas de sobrevivência foram de 96,02%, 97,68%, 65,58% e 99,06%, para NE, EMP, EB e EU, respectivamente. **CONCLUSÕES:** Após 6, 12 e 18 meses, EWBT associado ao sistema adesivo hidrófobos apresentou a maior falha de retenção. EWBT com adesivos hidrófilos foram semelhantes ao grupo controle. **RELEVÂNCIA CLÍNICA:** EWBT apresentou resultados clínico promissores quando associado a adesivos hidrófilos. A associação com um adesivo hidrófobo comercialmente disponível apresentou as piores taxas de retenção de restaurações em LCNC.

Palavras-chave: Dentina. Etanol. Adesão. Adesivos. Ensaio clínico.

*Artigo elaborado de acordo com as normas do Periódico *Clinical Oral Investigations* (Print version ISSN 1432-698). Submetido em: 17/06/2019.

ABSTRACT

OBJECTIVES: This clinical trial evaluated the effectiveness of ethanol wet bonding technique (EWBT – E) and different adhesive protocols in non-carious cervical lesions (NCCLs) after 18 months. **MATERIALS AND METHODS:** A randomized, controlled, double-blind clinical trial was performed. Patients presenting at least one NCCL were included. NCCLs (n=148) were randomly assigned to 4 groups: NE (Non-EWBT [NE] + three-step etch-and-rinse (Scotchbond Multi-Purpose, 3M ESPE), EMP (E + three-step etch-and-rinse (Scotchbond Multi-Purpose, 3M ESPE [MP]); EB (E + third step of MP [B]) and EU (E + universal adhesive - Single Bond Universal, 3M ESPE [SU]). All cavities were conditioned with phosphoric acid 37% (Condac, FGM) and then restored with nanohybrid resin composite (Filtek Z350, 3M ESPE). Trained and calibrated examiners ($\kappa=0.61$) evaluated the restorations at baseline – 7 days, 6, 12 and 18 months recall using the USPHS modified criteria. Data were subjected to Chi-square and Kaplan-Meier tests ($\alpha=0.05$). **RESULTS:** Differences in success, were found for the treatments ($p<0.001$). EB presented the lowest success rate compared with the other groups ($p<0.001$). No significant differences were detected for NE, EMP and EU ($p\geq 0.290$). At the 18-month recall, the survival rates were 96.02%, 97.68%, 65.58%, and 99.06%, for NE, EMP, EB, and EU, respectively. **CONCLUSIONS:** After 6, 12 and 18 months, the EWBT associated with hydrophobic adhesive system presented the highest retention failure. EWBT with hydrophilic adhesives were similar to the control group. **CLINICAL RELEVANCE:** EWBT presents promising clinical results when associated with hydrophilic adhesives. The association with a commercially available hydrophobic adhesive presented the worst retention rates of NCCLs restorations.

Keywords: Dentin, Ethanol, Dental Bonding, Adhesives, Clinical Trial.

Introduction

Dentin presents a great amount of organic components, mainly collagen fibers and dentinal fluid, that are responsible for the different response to acid etching and consequently the adhesion pattern and longevity when compared with enamel [1, 2]. The best adhesion result to dentin, according to a meta-analysis, is provided by the 2-step self-etch or 3-step etch-and-rinse adhesives [3]. The impaired dentin adhesion and the material-based standard protocol endorse the constant development of adhesive systems in an attempt to clinically improve the adhesion to dentin.

Recent adhesive materials are represented by the marketed multi-mode/universal adhesives [4]. This material is considered adaptable, since it could be used on wet or dry dentin and under self-etch or etch-and-rinse protocols [5, 4], fact that supports its increased popularity [6].

Independently of the adhesive or mode of application used, the interface degradation still decreases the resin restorations lifespan [7]. Due to this, some strategies were proposed to improve the adhesion overtime, such as dentin pre-treatments [8-10], addition of different chlorhexidine concentrations on adhesives [11], formulation of experimental adhesives [12, 13], the use of matrix metalloprotease (MMPs) inhibitors [14], or the ethanol-wet-bonding technique (EWBT) [7]. Two protocols for applying the EWBT have been reported in the literature, the progressive water substitution and the simplified technique. The first employs increasing ethanol concentrations, and clinically this procedure takes about 3-4 min [15], while the last results in dentin dehydration with a single 1-minute application of 100% ethanol, being a more clinically acceptable time [16].

Considering that the adhesive interface hydrolysis might be one of the main reasons of failures overtime, the EWBT may be a better strategy during the adhesion process, once ethanol presents higher solvent ability and lower

hydrogen bonding capacity than water [17]. Those characteristics favor the water substitution, possibly leading to reduce hydrolysis. The EWBT approach creates a relatively hydrophobic collagen matrix by reducing the amount of water, improving the hydrophobic monomers infiltration and consequently forming a more hydrophilic hybrid layer [15,18], possibly retarding the adhesive interface hydrolysis. However, the technique is sensitive due to the high volatility of ethanol, and it also does not displace water completely, since there is a stable bond between the collagen fibrils with water [19], fact that may hinder the infiltration of hydrophobic adhesives [19]. Considering the biological aspects of the technique, the EWBT was considered safe since there was no pulpal damage on human cells in comparison to water-wet bonding approach after 48h [20].

For evaluating the EWBT clinically, studies have reported the use of ethanol under the modified progressive technique [21, 22] or using two 100% ethanol applications [23], with no differences between the techniques. Additionally, a study reporting the use of a formulated experimental hydrophobic primer, presenting 50 weight% in ethanol and co-monomer resin blend [24], has also been reported.

Besides the fact that the EWBT might improve longevity due to an effective collagen protection [25], few clinical studies evaluated EWBT as dentin pre-treatment [21-23]. In addition, laboratory results of the association between universal adhesives on self-etch mode and EWBT is reported to present improved bond strength even after thermomechanical aging [26]. The previous evaluation report of the present study revealed that the use of EWBT under the conceived approach (associated to hydrophobic adhesive) resulted in lower survival rates after a short-term evaluation (6 months), possibly due to the lack of using a diluted hydrophobic agent [27]. The authors pointed that longer evaluations are required to describe the success behavior of

the tested protocols with time. Based on the presented information and regarding the problems associated to non-carious cervical lesions (NCCLs) considering the retention loss can vary from 0% to 50% [28], possible dentin pre-treatments that favor dentin adhesion should be clinically assessed, under well designed clinical studies [6,11] for supporting evidence-based Dentistry and proper clinical practice.

According to a recent systematic review and meta-analysis [3], clinical trials are needed to determine the best dentin pre-treatment within the dentin adhesion subject. In addition, the results of the present study could provide data to support systematic reviews and meta-analysis. Thus, the aim of this clinical trial was to evaluate the effectiveness of dentin pre-treatment with 100% ethanol on NCCLs using different adhesive protocols. The null hypothesis was that EWBT in association to different adhesives protocols does not influence NCCLs restoration success overtime.

Material and methods

Ethics and Sample size

This present study was approved by local Institutional Review Board (protocol number: 2.022.383) and registered in a clinical trials database REBEC (<http://www.ensaiosclinicos.gov.br>) under protocol: RBR-5HNCR3. This randomized controlled clinical trial followed CONSORT guidelines [28].

The sample size was calculated using an online statistical website (Sealed Envelope Ltda - www.sealedenvelop.com). For power calculation, equivalence trial function under binary outcome was selected. The following parameters were chosen: $\alpha = 5\%$, power at 80%, success of control and experimental group at 96% and limit of equivalence at 15% [29]. Sample size was estimated with at least 30 restorations per group.

Inclusion and non-inclusion criteria

The patient must have presented all characteristics for selection and inclusion in the study: minimum age of 18 years; presence of at least one NCCL in permanent canines or premolars with vital pulp and absence of painful symptoms; lesion depth and cervical width of 1 mm at minimum; presence of the antagonist and adjacent teeth; NCCLs with no previous restorative treatment; good oral health and absence of periodontal disease.

The non-inclusion criteria were as follows: teeth with cervical caries lesions; patients with allergies to the materials used or systemic diseases presence (e.g. gastroesophageal reflux); deleterious habits or severe bruxism; use of removable prostheses with clamps on the target teeth or fixed orthodontic appliances.

Study design

For this study, 67 participants met the inclusion criteria; they signed the informed consent and were thus included. Prior to the restorative procedure, a person not involved to this clinical trial generated a randomization sequence of treatments for each tooth, considering the four protocols. Thus, the treatments were allocated randomly for the included NCCLs. For patients receiving more than one treatment, in case more than one tooth was included in the study, treatments were allocated following a crescent tooth number according to the international tooth numbering system, once no patient received duplicated treatments.

All volunteers were submitted to professional pumice prophylaxis using polishing brush. Isolation was done with lip retractor, cotton rolls, gingival retractor cord n° 000 (Ultrapak, Ultradent Products Inc), and saliva ejector. No bevel was performed on enamel prior to restorations and all cavities were conditioned with 37% phosphoric acid (Condac, FGM, Brazil) for 15 s,

followed by rinsing for 15 s and drying with absorbent paper. The adhesive systems were applied according manufacturer's instructions (Table 1).

Table 1 – Acronyms of groups according to the use of ethanol and adhesive

GROUP	Ethanol dentin pre-treatment	Adhesive System used	Mode of application
Control - NE	No – NE	three-step etch-and-rinse (Scotchbond Multi-Purpose, 3M ESPE) - MP	Primer was applied to etched substrate and dried for 5 s + bond was actively applied for 20 s, followed by a gentle blow of air for 5 s and lightcuring for 10 s
EMP	Yes – E	three-step etch-and-rinse (Scotchbond Multi-Purpose, 3M ESPE) - MP	100% ethanol was actively applied for 60 s with microbrush + primer was applied to etched substrate and dried for 5 s + bond was actively applied for 20 s, followed by a gentle blow of air for 5 s and lightcuring for 10 s
EB	Yes – E	Bond (Scotchbond Multi-Purpose, 3M ESPE; third step – Hydrophobic) - B	100% ethanol was actively applied for 60 s with microbrush + hydrophobic bond was actively applied for 20 s, followed by a gentle blow of air for 5 s and lightcuring for 10 s
EU	Yes – E	Universal adhesive (Scotchbond Universal, 3M ESPE - Hydrophilic) - U	100% ethanol was actively applied for 60 s with microbrush + universal adhesive was actively applied for 20 s, followed by a gentle blow of air for 5 s and lightcuring for 10 s

protocols. Adhesive materials and clinical sequence are also presented.

The restorations were made using Filtek Z350 XT (3M ESPE, Sumaré, Brazil) with an incremental technique, in which each increment was 2 mm on maximum. Light activation of restorations was done for 20 s. The increments were initially placed on the incisal wall and then on the gingival wall. Most of restorations were completed with 2 increments. For all restorative procedures, the polymerizing was performed with a LED device with a power density of 900 mW/cm², on minimum. Finishing was performed with fine finishing diamond rotary instruments (KG Sorensen, São Paulo, SP, Brazil). After 7 days, the restorations were polished with abrasive discs (Sof-Lex™ Pop-On, 3M ESPE,

MN, USA). Restorations were performed by a single trained operator.

Evaluation

The restorations were evaluated by two calibrated examiners (interexaminer agreement of 81%, Kappa 0.61) not involved with allocation and restorative procedures. Evaluations were performed at baseline (7 days), after 6, 12 and 18 months according to USPHS modified criteria. (Table 2) The patients and examiners were blind to the study parameters.

Table 2 – USPHS modified criteria used to evaluate the NCCLs restorations

SCORES	Criteria
Retention	
Alfa (A)	No restorative material loss
Charlie (C)	Partial or complete loss of restorative material
Marginal discoloration	
Alfa (A)	No discoloration between tooth structure and restorative material
Bravo (B)	Marginal discoloration which can be polished away
Charlie (C)	Discoloration in interface restorative material and tooth, no able to polish
Marginal adaptation	
Alfa (A)	Closely adapted, no detectable margin
Bravo (B)	Detectable marginal discrepancy clinically acceptable
Charlie (C)	Marginal crevice, clinically unacceptable
Delta (D)	Mobile restoration, partially or totally fractured
Secondary caries	
Alfa (A)	No caries present
Charlie (C)	Caries present
Anatomic form	
Alfa (A)	Continuous, well contoured
Bravo (B)	Slight discontinuity or under contoured without dentin exposure, clinically acceptable
Charlie (C)	Discontinuous, sever under contoured, with dentin exposure, clinically unacceptable
Post-operative sensitivity	
Alfa (A)	No post-operative
Bravo (B)	Sensitive but with intensity decreasing
Charlie (C)	Constant sensitivity, without intensity decreasing
Surface texture	
Alfa (A)	Texture such as enamel
Bravo (B)	Texture such as resin composite
Charlie (C)	Surface with pores or cracks, with dental pick retention

Statistical Analysis

All data were analyzed using the chi-square test to verify the distribution of the success rate among groups and among assessed periods. The Kaplan-Meier survival analysis was also performed. Data for the frequency analysis (success among groups and periods) considered the intention-to-treat data

treatment, in which data was imputed for non-show participants during the recalls. The level of significance for both tests was set at 5%.

Results

A flowchart of this clinical trial is shown in Figure 1 and the demographics of the study participants are shown in Table 3. The participants were distributed into groups with regard to sex, age, restored tooth, and the presence of wear facets in relation to restoration success or failure. Failures were detected only for the retention parameter. Differences in retention rate were detected among all groups ($p < 0.0001$). EB presented lower success rates when compared with all tested adhesive protocols: EB x NE ($p = 0.0008$), EB x EMP ($p = 0.0001$), and EB x EU ($p < 0.0001$). No significant differences were detected for other comparisons: NE x EMP ($p = 0.6204$), NE x EU ($p = 0.2905$), EMP x EU ($p = 0.5555$). The survival rates for 18-month assessments were 96.02%, 97.68%, 65.58%, and 99.06%, for NE, EMP, EB, and EU, respectively. (Table 4)

Figure 1 - Flowchart of the clinical trial. Number of restorations evaluated in each recall per group is also presented.

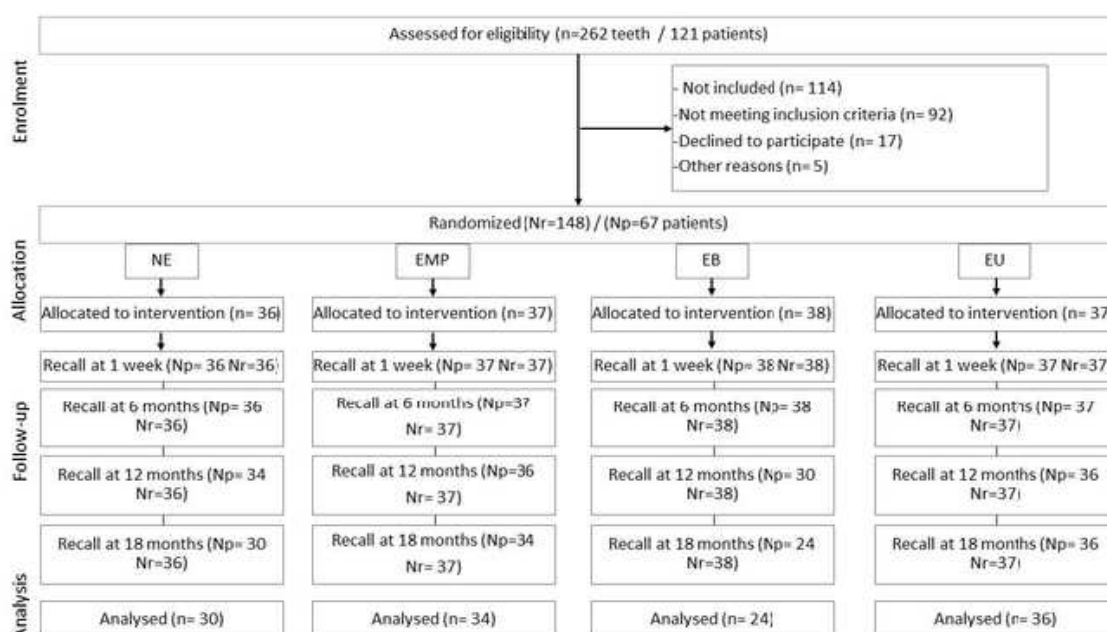


Table 3 - Distribution into studied groups by gender, age, restored tooth, and also the frequency of wear facets in relation to success or failure rates after 18 months.

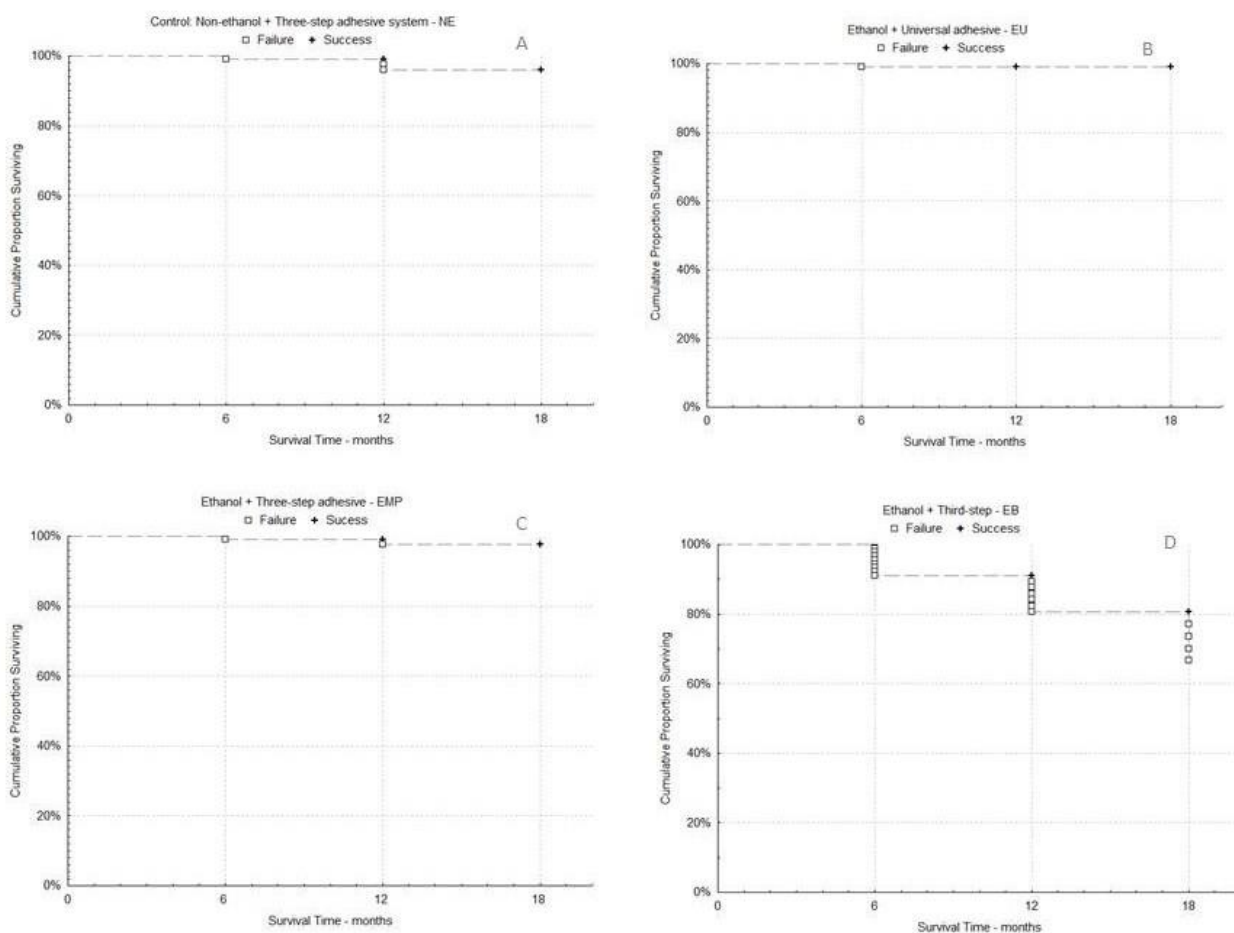
Population/Groups			NE	EMP	EB	EU
Tooth from male subjects			19/36 (52.77%)	23/37 (62.16%)	18/38 (47.37%)	15/37 (40.54%)
Tooth from female subjects			17/36 (47.23%)	14/37 (37.83%)	20/38 (52.63%)	22/37 (59.45%)
Mean age (years)			55.75	53.21	52.60	55.43
Upper Canine / teeth included			4/36 (11.05%)	4/37 (10.81%)	7/38 (18.42%)	3/37 (8.11%)
Lower Canine / teeth included			2/36 (5.01%)	2/37 (5.41%)	1/38 (2.63%)	0/37 (0%)
Upper Premolar / teeth included			10/36 (26.61%)	13/37(35.13%)	12/38(31.57%)	10/37 (27.03%)
Lower Premolar / teeth included			21/36(57.33%)	18/37(48.65%)	18/38(47.38%)	24/37(64.86%)
Tooth wear facet	Presence	Success after 18-mo	14	19	7	19
		Loss after 18-mo	3	1	9	0
	Absence	Success after 18-mo	19	15	12	17
		Loss after 18-mo	0	2	10	1

Table 4 – Distribution of number of evaluated restorations and percentage (%) according to each criteria at baseline, 6, 12 and 18 months.

CRITERIA	SCORES	BASELINE - 7 DAYS				6 MONTHS				12 MONTHS				18 MONTHS			
		NE	EU	EB	EMP	NE	EU	EB	EMP	NE	EU	EB	EMP	NE	EU	EB	EMP
Retention	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	35 (97.23%) ^a	36 (97.30%) ^a	30 (78.95%) ^b	36 (97.30%) ^a	32 (91.43%) ^a	36 (100%) ^a	24 (75.00%) ^b	34 (94.12%) ^a	30 (100%) ^a	36 (100%) ^a	19 (73.69%) ^b	34 (100%) ^a
	Charlie	-	-	-	-	1 (2.77%)	1 (2.70%)	8 (21.05%)	1 (2.70%)	2 (8.57%)	-	6 (25.00%)	2 (5.88%)	-	-	5 (26.31%)	-
Marginal discoloration	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	32 (88.89%)	33 (89.19%)	30 (78.95%)	36 (97.30%)	30 (88.23%)	33 (91.66%)	22 (73.33%)	35 (97.22%)	30 (100%)	32 (94.12%)	18 (88.89%)	32 (94.12%)
	Bravo	-	-	-	-	3 (8.34%)	3 (8.11%)	-	-	2 (11.76%)	3 (8.33%)	2 (26.66%)	1 (2.77%)	-	4 (5.88%)	-	4 (5.88%)
	Charlie	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marginal adaptation	Alfa	35 (97,23%)	37 (100%)	37 (97,37%)	37 (100%)	32 (88.89%)	32 (86.49%)	28 (73.68%)	34 (94.44%)	29 (90.62%)	34 (94.44%)	23 (95.83%)	33 (94.28%)	29 (96.67%)	34 (94.45%)	15 (83.34%)	32 (94.12%)
	Bravo	1 (2,77%)	-	1 (2,63%)	-	3 (8.34%)	4 (10.81%)	2 (5.27%)	2 (5.56%)	3 (9.38%)	2 (5.56%)	1 (4.17%)	2 (5.72%)	1 (3.33%)	2 (5.56%)	3 (16.66%)	2 (5.88%)
	Charlie	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Secondary caries	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	35 (97.23%)	36 (100%)	30 (78.95%)	36 (97.30%)	32 (91.43%)	36 (100%)	24 (80.00%)	35 (97.22%)	30 (100%)	36 (100%)	18 (88.89%)	34 (100%)
	Charlie	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anatomic form	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	35 (97.23%)	35 (94.60%)	30 (78.95%)	35 (94.60%)	32 (91.43%)	36 (100%)	24 (80.00%)	35 (97.22%)	30 (100%)	36 (100%)	18 (88.89%)	34 (100%)
	Bravo	-	-	-	-	-	1 (2.70%)	-	1 (5.40%)	-	-	-	-	-	-	-	-
	Charlie	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Post-operative sensitivity	Alfa	27 (75%)	30 (81.08%)	29 (68.97%)	30 (81.08%)	32 (88.89%)	35 (94.60%)*	28 (73.68%)	35 (94.60%)*	31 (96.87%)	32 (97.22%)	20 (83.33%)	33 (94.28%)	30 (100%)	35 (97.23%)	17 (94.45%)	34 (100%)
	Bravo	8 (22.22%)	7 (18.92%)	9 (31.03%)	7 (18.92%)	3 (8.34%)	1 (2.70%)	2 (5.27%)	1 (5.40%)	1 (3.13%)	4 (2.78%)	4 (16.66%)	2 (5.72%)	-	1 (2.77%)	1 (5.55%)	-
	Charlie	1 (2,77%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Surface texture	Alfa	36 (100%)	37 (100%)	38 (100%)	37 (100%)	31 (86.11%)	31 (83.79%)	26 (68.42%)	31 (83.79%)	28 (87.50%)	34 (94.44%)	21 (87.5%)	25 (71.43%)	26 (86.67%)	28 (77.77%)	16 (88.89%)	28 (82.35%)
	Bravo	-	-	-	-	4 (11.12%)	5 (13.51%)	4 (10.53%)	5 (13.51%)	4 (12.50%)	2 (5.56%) ^a	3 (12.50%)	10 (28.57%) ^b	4 (13.33%)	8 (22.23%)	2 (11.11%)	6 (17.65%)
	Charlie	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Overall, tooth sensitivity was less frequent after 18 months in comparison with the baseline observations ($p>0.002$). (Table 4) For other criteria, no significant differences were found ($p>0.1056$). Considering the intention-to-treat data treatment the frequency analysis (success among groups and periods) is shown on Figure 2.

Figure 2 – Frequency analysis for each group (success among groups and periods).



Discussion

Based on the clinical success observed, the null hypothesis that EWBT does not influence NCCLs success overtime was partially accepted. The use of a

commercially available hydrophobic adhesive in association with the EWBT resulted in lower success rates. On the other hand, hydrophilic groups associated with 100% ethanol presented similar success rates to the control group (NE).

Although a decrease in the collagen fibrils diameter and the matrix volume is expected after ethanol application, the present lack of differences between the use of EWBT associated with hydrophilic adhesives and the control group does not support the benefits of that decrease at the evaluated period. That reported decrease is known to improve collagen interfibrillar spaces, leading to greater impregnation of the adhesive system and hydrophobic monomers [31]. Besides all the influences of EWBT on dentin substrate, this approach might also increase the degree of conversion of the resin monomers [32], producing a condition with better sealing even after hydrophilic adhesive use. Based on the 18-month results of the present study, the association between EWBT and hydrophilic adhesives may be indicated, once no deterioration of the bonding properties was detected clinically. Greater amount of water in the adhesive interface in comparison with the use of hydrophobic adhesives might be questioned, once it may favor monomers dilution and water sorption that results in hydrolysis overtime [33, 34]. This concern, however, should not be considered in view of the present results, and further follow-ups are required.

Moreover, the literature suggests that after EWBT, there is a decrease of water sorption and enzyme-catalyzed hydrolytic cleavage of collagen [31, 15], and also an increase in collagen rigidity, which contributes to the formation of a more effective and stable hybrid layer. In micro-Raman [35] and two-photon laser confocal microscopy [36] analyses, it was verified homogeneous distribution of methacrylate monomers within the interfibrillar spaces, which improves the collagen fibers protection and encapsulation [35] and the hybrid layer mechanical properties, resulting in improved bond strength [37]. Although

EWBT pretreatment is referred to remove and replace free and loosely bound water around dentin tubule orifices and within the collagen microfibrils [31, 33, 38], the employment of EWBT and adhesive systems does not result in complete removal of water from dentin matrix [19], allowing monomers dilution, water sorption and hydrolysis of the adhesive interface [33, 34]. Improved success rates were not detected in the present study while associating the EWBT and hydrophilic adhesive systems in comparison with the control group. Groups receiving the hydrophilic adhesives in association or not with the EWBT presented survival rates greater than 90%. Longer evaluation periods are necessary to properly address the possible referred differences resulted from the reported laboratorial advantages on the EWBT. Future evaluations are granted due to the reported more stable formed hybrid layer, resulted from improved monomer impregnation and also due to the capacity of EWBT to prevent the phase separation of adhesives [32], and thus improving bond strength over time [39].

Ethanol is an organic solvent that displaces water molecules within dentin matrix and presents higher evaporation capacity that leads to facilitated monomers impregnation [40]. The association between dentin matrix treated by EWBT and the ethanol from the Scotchbond Universal Adhesive may have facilitated the impregnation of resin monomers to hybrid layer formation, resulting in a stable bonding. Further recall visits are granted to possibly detect the beforehand described positive influence on the hybrid layer formation and stability. Although the literature states that increased permeability of monomers may increase toxicity potential and compromise the longevity of restorations [40], no clinical adverse reports were observed in the present trial after 18 months.

The universal adhesive employed in association with the EWBT presents 10-MDP, component that favors the interaction of the adhesive components with

calcium from hydroxyapatite of the dentin [41]. This interaction decreases the dissolution rate of Ca-salt formed and helps maintaining the bonding interface durability overtime [42]. Besides that, the association between the applied ethanol and the ethanol from the adhesive composition may have aid proper infiltration of resin monomers into the dentin matrix resulting in difficult hydrolysis overtime. Although similar results were detected among the three groups receiving hydrophilic monomers, the chemical interaction may positively influence future results. Considering the possible benefits of Universal adhesives (easy handling, chemical bonding, and possible facilitated monomer infiltration), results longer recalls may indicate its use with the EWBT.

The EWBT was initially proposed in association with hydrophobic adhesives due to water removal from collagen matrix and proper collagen encapsulation, leading to a less hydrophilic hybrid layer [24, 43] that hinders hydrolysis. Although proper in vitro results for the EWBT in association with hydrophobic adhesives are reported [17, 44], clinical evaluation after 12 or 18 months recalls the EWBT did not lead to significant differences, with success rates greater than 91% [21-23]. Those results may be dependent on the use of a formulated hydrophobic primer, in which the bond was diluted to 10 or 50 w% in ethanol [21-23]. Those previous clinical data are similar to the use of hydrophilic systems in our study. On the opposite, the use of a commercially available hydrophobic adhesive led to lower success rates in comparison with all other groups. The viscosity of the hydrophobic adhesive may have influenced monomer infiltration into the demineralized dentin, and consequently, a less stable hybrid layer was formed since fewer collagen fibers were protected [27]. This fact might be related to the gradual losses observed for EB group at all evaluated periods in the present study. Lack of retention in the hydrophobic group was distributed within all evaluated periods, highlighting the fact that improper adhesive penetration might have happened in several degrees. In

consideration to adhesive impregnation, studies using diluted hydrophobic monomers prior to hydrophobic monomer application resulted in proper clinical success rates [22, 23]. Moreover, laboratorial data might not have taken into consideration the dentin fluid flux from the pulp, and such scenario in the clinical reality might also have influenced the worst clinical success observed, especially considering the hydrophobic group. The previously reported diluted hydrophobic primer (dilution of the bond) is not commercial available with no access to clinicians, thus being a barrier for its use within the approach. In addition, for EB was observed progressive failure rate at 6, 12 and 18 months. Based on the results of this trial, after 18 months of follow up, the EWBT combined with the hydrophobic adhesive presented lower retention rates when compared to the other groups. Moreover, the EWBT with hydrophilic adhesives presented similar results ($p>0.290$) to control group, based on this, EWBT may not be necessary to improve retention rate clinically. Considering that self-etch adhesives are preferred for dentin bonding [45], the research group is also assessing the combination of EWBT and universal adhesive on self-etch mode, for possible interactions between the smear layer and EWBT.

The importance of the present data relates to the fact that the results were not in favor to the original EWBT from 6 months to 18 months follow up. Future recalls are required to validate this statement. Furthermore, the treatment success may be directly affected by the thickness layer of adhesives, adhesive viscosity and wettability. For the malocclusion of patients, a balanced distribution of failed and succeed restorations regarding the presence/absence of wear facets was detected (Table 3), suggesting that characteristic might have not influenced the results.

Conclusions

EWBT in association with hydrophilic strategies presented adequate

retention rates at 6, 12 and 18 months. All hydrophilic groups regardless EWBT were similar to control. However, EWBT associated to hydrophobic adhesive (third step of the three-step etch-and-rinse adhesive) should have limited indication due to lower clinical performance observed at all evaluated periods.

Compliance with Ethical Standards

- *Conflict of Interest:* Author Mauricio Yugo de Souza declares that he has no conflict of interest. Author Ana Luiza Barbosa Jurema declares that she has no conflict of interest. Author Taciana Marco Ferraz Caneppele declares she has no conflict of interest. Author Eduardo Bresciani declares he has no conflict of interest.

- *Funding:* none.

- *Ethical approval:* All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

- *Informed consent* was obtained from all individual participants included in the study

- *This article does not contain any studies with animals performed by any of the authors.*

References

1. Suda S, Tsujimoto A, Barkmeier WW, Nojiri K, Nagura Y, Takamizawa T, Latta MA, Miyazaki M (2018) Comparison of enamel bond fatigue durability between universal adhesives and two-step self-etch adhesives: Effect of phosphoric acid pre-etching. *Dent Mater J* 37:244-255. doi:10.4012/dmj.2017-059
2. Perdigao J (2002) Dentin bonding as a function of dentin structure. *Dent Clin*

North Am 46:277-301, vi

3. Peumans M, De Munck J, Mine A, Van Meerbeek B (2014) Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. A systematic review. *Dent Mater* 30:1089-1103. doi:10.1016/j.dental.2014.07.007
4. Perdigao J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, Loguercio AD (2014) A new universal simplified adhesive: 18-month clinical evaluation. *Oper Dent* 39:113-127. doi:10.2341/13-045-C
5. Leite M, Costa CAS, Duarte RM, Andrade AKM, Soares DG (2018) Bond Strength and Cytotoxicity of a Universal Adhesive According to the Hybridization Strategies to Dentin. *Braz Dent J* 29:68-75. doi:10.1590/01036440201801698
6. Miyazaki M, Tsujimoto A, Tsubota K, Takamizawa T, Kurokawa H, Platt JA (2014) Important compositional characteristics in the clinical use of adhesive systems. *J Oral Sci* 56:1-9
7. 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. doi:10.1016/j.dental.2010.10.016
8. Vallabhdas AK, Kumar CNV, Kabbinala P, Nayak R, Rajakumari M, Shilpa T (2018) Evaluation of Hybrid Layer and Bonding Interface after Water Storage with and without the Usage of 2% Chlorhexidine: A Scanning Electron Microscope Study. *J Contemp Dent Pract* 19:52-59
9. Porto I, Nascimento TG, Oliveira JMS, Freitas PH, Haimeur A, Franca R (2018) Use of polyphenols as a strategy to prevent bond degradation in the dentin-resin interface. *Eur J Oral Sci* 126:146-158. doi:10.1111/eos.1240332
10. Aggarwal V, Bhasin SS (2018) Application of Calcium Silicate Materials After Acid Etching May Preserve Resin-Dentin Bonds. *Oper Dent*. doi:10.2341/17-306-L

11. Malaquias P, Gutierrez MF, Hass V, Stanislawczuk R, Bandeca MC, Arrais C, Farago PV, Reis A, Loguercio AD (2018) Two-year Effects of Chlorhexidine-containing Adhesives on the In Vitro Durability of Resin-dentin Interfaces and Modeling of Drug Release. *Oper Dent* 43:201-212. doi:10.2341/16-333-L
12. Provenzi C, Collares FM, Cuppini M, Samuel SMW, Alves AK, Bergmann CP, Leitune VCB (2018) Effect of nanostructured zirconium dioxide incorporation in an experimental adhesive resin. *Clin Oral Investig* 22:2209-2218. doi:10.1007/s00784-017-2311-z
13. Gou YP, Meghil MM, Pucci CR, Breschi L, Pashley DH, Cutler CW, Niu LN, Li JY, Tay FR (2018) Optimizing resin-dentin bond stability using a bioactive adhesive with concomitant antibacterial properties and anti-proteolytic activities. *Acta Biomater* 75:171-182. doi:10.1016/j.actbio.2018.06.008
14. Hashimoto M, Hirose N, Kitagawa H, Yamaguchi S, Imazato S (2018) Improving the durability of resin-dentin bonds with an antibacterial monomer MDPB. *Dent Mater J* 37:620-627. doi:10.4012/dmj.2017-209
15. Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR (2010) Ethanol wet-bonding challenges current anti-degradation strategy. *J Dent Res* 89:1499-1504. doi:10.1177/0022034510385240
16. Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, Watson TF, Osorio R (2011) Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Part II: Effects of mechanical cycling load on microtensile bond strengths. *Dent Mater* 27:563-572. doi:10.1016/j.dental.2011.02.010
17. Nagpal R, Manuja N, Pandit IK (2015) Effect of ethanol wet bonding technique on the durability of resin- dentin bond with contemporary adhesive systems. *J Clin Pediatr Dent* 39:133-142
18. Ahn J, Jung KH, Son SA, Hur B, Kwon YH, Park JK (2015) Effect of

additional etching and ethanol-wet bonding on the dentin bond strength of one-step self-etch adhesives. *Restor Dent Endod* 40:68-74. doi:10.5395/rde.2015.40.1.68

19. Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, Donnelly A, Garcia-Godoy F (2007) From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am J Dent* 20:7-20

20. Scheffel DL, Sacono NT, Ribeiro AP, Soares DG, Basso FG, Pashley D, Costa CA, Hebling J (2015) Immediate human pulp response to ethanol-wet bonding technique. *J Dent* 43:537-545. doi:10.1016/j.jdent.2015.02.014

21. Barros TADF, Da Araújo JF, Braga EMF, Silva e Souza PdAR, Loretto SC, Souza Júnior MHSE (2016) Ethanol-Wet Bonding Technique: 18-month Clinical Evaluation. *Int J Odontomast* 10:267-276

22. Araujo JF, Barros TA, Braga EM, Loretto SC, Silva e Souza Pde A, Silva e Souza MH (2013) One-year evaluation of a simplified ethanol-wet bonding technique: a randomized clinical trial. *Braz Dent J* 24:267-272. doi:10.1590/0103-6440201302128

23. Mortazavi V, Samimi P, Rafizadeh M, Kazemi S (2012) A randomized clinical trial evaluating the success rate of ethanol wet bonding technique and two adhesives. *Dent Res J (Isfahan)* 9:588-594

24. Sadek FT, Castellan CS, Braga RR, Mai S, Tjaderhane L, Pashley DH, Tay FR (2010) One-year stability of resin- dentin bonds created with a hydrophobic ethanol-wet bonding technique. *Dent Mater* 26:380-386. doi:10.1016/j.dental.2009.12.009

25. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E (2008) Dental adhesion review: aging and stability of the bonded interface. *Dent Mater* 24:90-101. doi:10.1016/j.dental.2007.02.009

26. Souza MY, Di Nicoló R, Bresciani E (2018) Influence of ethanol-wet dentin,

adhesive mode of application, and aging on bond strength of universal adhesive. *Braz Oral Res* 32:e102. doi:10.1590/1807-3107bor-2018.vol32.0102

27. Souza MY, Jurema ALB, Caneppele TMF, Bresciani E (2019) Six-month performance of restorations produced with the ethanol-wet-bonding technique: a randomized trial. *Braz Oral Res* [Epub ahead of print] 10.1590/1807-3107bor-2019.vol33.0052

28. Heintze SD, Ruffieux C, Rousson V (2010) Clinical performance of cervical restorations--a meta-analysis. *Dent Mater* 26:993-1000. doi:10.1016/j.dental.2010.06.003

29. Schulz KF, Altman DG, Moher D, Group C (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332. doi:10.1136/bmj.c332

30. Reis A, Loguercio AD (2009) A 36-month Clinical Evaluation of Ethanol/Water and Acetone-based Etch-and-Rinse Adhesives in Non-carious Cervical Lesions. *Oper Dent* 34:384-391. doi:10.2341/08-117

31. Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, Tay FR, Pashley DH (2009) Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. *J Dent Res* 88:146-151. doi:10.1177/0022034508328910

32. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR (2011) Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *J Dent Res* 90:953-968. doi:10.1177/0022034510391799

33. Osorio E, Toledano M, Aguilera FS, Tay FR, Osorio R (2010) Ethanol wet-bonding technique sensitivity assessed by AFM. *J Dent Res* 89:1264-1269. doi:10.1177/0022034510376403

34. Mortazavi V, Samimi P, Rafizadeh M, Kazemi S (2012) A randomized clinical trial evaluating the success rate of ethanol wet bonding technique and

two adhesives. *J Dent Res* 9:588

35. Shin TP, Yao X, Huenergardt R, Walker MP, Wang Y (2009) Morphological and chemical characterization of bonding hydrophobic adhesive to dentin using ethanol wet bonding technique. *Dent Mater* 25:1050-1057. doi:10.1016/j.dental.2009.03.006

36. Sauro S, Watson TF, Mannocci F, Tay FR, Pashley DH (2009) Prevention of water contamination of ethanol-saturated dentin and hydrophobic hybrid layers. *J Adhes Dent* 11:271-278

37. Kim J, Gu L, Breschi L, Tjäderhane L, Choi KK, Pashley DH, Tay FR (2010) Implication of ethanol wet-bonding in hybrid layer remineralization. *J Dent Res* 89:575-580

38. Tjaderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho M, Carvalho RM, Tay FR, Pashley DH (2013) Strategies to prevent hydrolytic degradation of the hybrid layer -A review. *Dent Mater* 29:999-1011. doi:10.1016/j.dental.2013.07.016

39. Tay F, Pashley DH, Kapur R, Carrilho M, Hur Y, Garrett L, Tay K (2007) Bonding BisGMA to dentin—a proof of concept for hydrophobic dentin bonding. *J Dent Res* 86:1034-1039

40. Mair L, Padipatvuthikul P (2010) Variables related to materials and preparing for bond strength testing irrespective of the test protocol. *Dent Mater* 26:e17-e23

41. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt K (2011) State of the art of self-etch adhesives. *Dent Mater* 27:17-28

42. Yokota Y, Fujita K, Uchida R, Aida E, Aoki N, Aida M, Nishiyama N (2016) Quantitative Evaluation of MDP-Ca Salt and DCPD after Application of an MDP-based One-step Self-etching Adhesive on Enamel and Dentin. *J Adhes Dent* 18:205-13. doi: 10.3290/j.jad.a36133

43. Huang X, Li L, Huang C, Du X (2011) Effect of ethanol-wet bonding with

hydrophobic adhesive on caries-affected dentine. Eur J Oral Sci 119:310-315. doi:10.1111/j.1600-0722.2011.00830.x

44. Kuhn E, Farhat P, Teitelbaum AP, Mena-Serrano A, Loguercio AD, Reis A, Pashley DH (2015) *Ethanol-wet bonding technique: Clinical versus laboratory findings. Dent Mater 31:1030-1037. doi:10.1016/j.dental.2015.05.01*

45. da Rosa WLdO, Piva E, da Silva AF (2015) *Bond strength of universal adhesives: A systematic review and meta-analysis. J Dent 43:765-776. doi:10.1016/j.jdent.2015.04.003*

2.4 Artigo – Souza MY, Andrade JL, Bresciani E. A técnica da dentina úmida por etanol influencia as propriedades adesivas de restaurações? / *Does the ethanol-wet-bonding influence the adhesive properties of dental restorations? A systematic review and metanalysis**

RESUMO

Objetivos: Uma revisão sistemática com metanálise foi realizada para avaliar a eficácia da técnica da dentina úmida em etanol (EWBT) no sucesso da restauração e na resistência de união à dentina. **Métodos:** Foi realizada uma busca abrangente, sem restrições, no banco de dados *MEDLINE* (via *PubMed*), *Scopus*, *Web of Science*, Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS), Biblioteca Brasileira de Odontologia (BBO), Biblioteca *Cochrane* e Literatura cinzenta na Europa (SIGLE), para identificar ensaios clínicos randomizados e estudos laboratoriais. A metanálise foi realizada utilizando modelos de efeitos fixos ou aleatórios para os estudos clínicos e laboratoriais, respectivamente. **Dados:** Após a remoção de duplicatas, 1254 artigos foram identificados. Após triagem de título e resumo, 3 estudos clínicos e 21 estudos laboratoriais permaneceram. Dois estudos laboratoriais foram excluídos, enquanto 19 estudos permaneceram para análise qualitativa. Nenhuma diferença entre pré-tratamentos, água ou EWBT foi observada na análise dos estudos clínicos. Foi observada uma diferença significativa na força de união favorecendo a técnica úmida por água, quando comparada com EWBT, para os estudos laboratoriais. **Conclusões:** Para estudos clínicos, não foi observada diferença entre as técnicas EWBT e água. Para estudos laboratoriais, menor força de união à dentina foi detectada, independentemente do modo de aplicação da EWBT. A associação com clorexidina ou o uso de adesivos autocondicionantes resultou em resultados semelhantes entre os grupos. Para os demais parâmetros avaliados, a abordagem úmida em água apresentou maiores resultados de união.

Palavras-chave: Dentina. Colagem dentária. Etanol. Sistemas adesivos. Revisão sistemática.

*Artigo elaborado de acordo com as normas do Periódico *Journal of Dentistry* (Print version ISSN 0300-5712).

ABSTRACT

Objectives: A systematic review with metanalysis was performed to evaluate the effectiveness of ethanol-wet-bonding technique (EWBT) on the restoration success and on the bond strength to dentin. Methods: A comprehensive search was performed without restrictions on MEDLINE (via PubMed), Scopus, Web of Science, Latin American and Caribbean Health Sciences Literature database (LILACS), Brazilian Library in Dentistry (BBO), Cochrane Library, and the System for information on Grey Literature in Europe (SIGLE), to identify randomized clinical trials and laboratory studies. Metanalysis was conducted using fixed or random effects models for the clinical and laboratorial studies, respectively. Data: After the removal of duplicates, 1254 articles were identified. After title and abstract screening, 3 clinical trials and 21 laboratory studies remained. Two laboratorial studies were further excluded, whereas 19 studies remained for qualitative analysis. No difference between pretreatments, water or EWBT, was observed for the clinical trials analysis. A significant difference in the bond strength favoring water-wet approach was observed when compared with the EWBT in laboratorial studies. Conclusions: For clinical trials, no difference between EWBT and water-wet approaches was observed. For laboratory studies, lower bond strength to dentin was detected, regardless the EWBT mode of application. The association with chlorhexidine or the use of self-etch adhesives resulted in similar results between groups. For the others parameters evaluated, the water wet approach presented greater bonding results.

Keywords: Dentin. Dental bonding. Ethanol. Adhesives. Systematic review.

1. Introduction

Adhesive Dentistry has advanced mainly on the strategies regarding the adhesive systems application. The first strategy is based on the smear layer removal, which is referred to as the etch-and-rinse approach^{1, 2}. A second strategy depends on modifying the smear layer, which aims to incorporate it into the adhesive layer. This strategy is referred as the self-etch approach³. In addition, other strategy available consists on selective enamel etching and self-etch on the dentin substrate. This approach may be used with self-etch or universal adhesives, being those last referred as multi-mode^{4, 5}.

Dental tissues present different amount of organic and inorganic components on the enamel and dentin substrates⁶, fact that influences the adhesion pattern of adhesives. Additionally, adhesive interface still undergoes degradation overtime and consequently the resin restorations may fail after a short lifespan¹. In an attempt to prevent the interface degradation and to increase the restorations lifespan, additional measures were proposed. Some of the approaches reported in the literature are the addition of chlorhexidine (CHX) into adhesives⁷, formulation of experimental adhesives⁸, use of matrix metalloprotease (MMPs) inhibitors⁹, and the ethanol wet bonding technique (EWBT)¹⁰.

Based on the laboratorial studies that evaluated the EWBT, it is reported that this pretreatment might extend the longevity of resin–dentin bonding, once

the resin monomer infiltration is improved and consequently a hybrid layer with better quality is formed^{11, 12}. After EWBT, it is reported that the excess of water from substrate is removed, and a hydrophobic dentin collagen matrix is created, which decreases the interface degradation¹¹. Two protocols have been reported for applying the EWB; the progressive technique, in which the ethanol saturation is obtained using increasing concentrations¹¹; and the simplified technique, in which the ethanol 100% is applied with a single application¹³.

Few clinical studies have evaluated the EWBT as a dentin pretreatment¹⁴⁻¹⁶, and divergent results were found. Moreover, many laboratory studies evaluated the effectiveness of EWBT on the bond strength to dentin, and while not considering the heterogeneity of methods, such as the mode of application of adhesives¹², association with CHX^{11, 17}, different mode of light polymerizing¹⁸, or caries-affected dentin^{17, 19}, some studies concluded there is an improvement of bond strength after EWBT.

Based on available literature, the effectiveness of EWBT is unclear. In addition, clinical decision-making procedures should be based on scientific information, once studies are continually published on new techniques and restorative materials. Evidence-based Dentistry is an approach to oral health care requiring the judicious integration of systematic assessments of clinically relevant scientific evidence. Systematic reviews and metanalysis are considered to be the highest level of evidence supporting evidence-based decision-making.

Therefore, the aim of this study was to determine whether there are evidence-based differences in the success rate of NCCLs restorations after EWBT and on the bond strength to dentin. In face of the controversial results published in the literature, the aim of this systematic review with metanalysis was to answer the following PICO question (population, intervention, comparison and outcome): Does the success rate of NCCLs restorations is increased after ethanol-wet-bonding technique when compared to water wet? Does the bond strength to dentin is increased after EWBT when compared to control group?

2. Material and Methods

Protocol and Registration

This study protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO - CRD42018103065) and followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for report²⁰.

Inclusion and Exclusion Criteria

The inclusion criteria were defined according to the research question and PICO strategy (Patient – teeth with NCCLs or dentin specimens; Intervention – restorations performed with EWBT; Comparison – adhesive protocols with etch-and-rinse or self-etch adhesives; Outcome – retention rate of NCCLs

restorations or bond strength to dentin), taking into account clinical trials and laboratory studies that evaluated the EWBT.

Studies that assessed both EWBT simplified or progressive were included in this review. Clinical trials that evaluated the success rate of NCCLs restorations were included. Laboratory studies with different adhesive protocols, EWBT, aging processes, presence of hydrophobic primer and different mechanical tests in which evaluated the bond strength with dentin were also included.

The inclusion and exclusion criteria were established by a consensus reached from all researchers after discussion, considering the research question and the objectives of the study.

Literature search strategy

Seven electronic databases (MEDLINE via PubMed, Scopus, Web of Science, Latin American and Caribbean Health Sciences Literature database (LILACS), Brazilian Library in Dentistry (BBO), Cochrane Library and the System for information on Grey Literature in Europe (SIGLE)) were searched from their inception to May 2019. Appropriate mesh terms were used and the search strategy adapted for each of the databases. The structured search strategy and data extraction were conducted by two independent examiners (Figure 1).

Figure 1 - Electronic database and search strategy for each database

Pubmed (May 2019 - 2099 articles) ((((((((((((Tooth Wear[MeSH Terms]) OR Tooth Wear[Title/Abstract]) OR Dental Wear[Title/Abstract]) OR Tooth Erosion[Title/Abstract]) OR Eroded[Title/Abstract]) OR Dental Erosion[Title/Abstract]) OR NCCL[Title/Abstract]) OR Class V[Title/Abstract]) OR Non carious cervical lesion*[Title/Abstract]) OR Cervical lesion*[Title/Abstract]) OR Noncarious cervical lesion*[Title/Abstract]) OR Tooth Cervix[MeSH Terms]) OR Cementoenamel Junction*[Title/Abstract]) OR CEJ[Title/Abstract])) AND ((((((((((((Dental Cements[MeSH Terms]) OR Dental Cements[Title/Abstract]) OR Dental Adhesive*[Title/Abstract]) OR Luting Agent*[Title/Abstract]) OR Dental Cement[Title/Abstract]) OR Dentin Bonding Agents[Title/Abstract]) OR Adhesives[MeSH Terms]) OR Adhesives[Title/Abstract]) OR Glue*[Title/Abstract]) OR Dental Bonding[MeSH Terms]) OR Dental Etching[MeSH Terms]) OR Ethanol[MeSH Terms]) OR Ethanol[Title/Abstract]) OR Ethyl Alcohol[Title/Abstract]) OR Absolute Alcohol[Title/Abstract]) OR dentin pretreatment[Title/Abstract]))
Scopus (Filter: Dentistry; May 2019 - 1353 articles) (TITLE-ABS-KEY ("Tooth Wear" OR "Dental Wear" OR "Tooth Erosion" OR eroded OR nccl OR "Class V" OR "Cervical lesions" OR "Noncarious cervical lesions" OR "Tooth Cervix" OR "Cementoenamel Junctions" OR cej)) AND (TITLE-ABS-KEY ("Luting Agent" OR "Dental Cement" OR "Dentin Bonding Agents" OR adhesives OR glue OR "Dental Bonding" OR "Dental Etching" OR ethanol OR "dentin pretreatment"))
Web of Science (May 2019 - 975 articles) TS=("Luting Agent" OR "Dental Cement" OR "Dentin Bonding Agents" OR adhesives OR glue OR "Dental Bonding" OR "Dental Etching" OR ethanol OR "dentin pretreatment"); TS=("Tooth Wear" OR "Dental Wear" OR "Tooth Erosion" OR eroded OR nccl OR "Class V" OR "Cervical lesions" OR "Noncarious cervical lesions" OR "Tooth Cervix" OR "Cementoenamel Junctions" OR cej)
Lilacs and BBO (May 2019 - 63 articles) ("Desgaste dos Dentes" OU "Desgaste Dental" OU "Erosão Dental" OU "Erosão" OU LCNC OU "Classe V" OU Lesão cervical não cariiosa* OU Lesão cervical* OU "Colo do Dente" OU Junção Cimento esmalte* OU JCE) E ("Cimentos Dentais" OU Adesivo Dental* OU Agente de Cimentação* OU "Cimento Dental" OU "Agentes de União Dentinários" OU Adesivos OU Cola* OU "União Dentária" OU "Condicionamento Dental" OU Etanol OU "Álcool Etílico" OU "Álcool Absoluto" OU "pré-tratamento dentinário"); ("Desgaste de los Dientes") O "Desgaste Dental" O "Erosión Dental" O "Erosión" O LCNC O "Clase V" O Lesión cervical no cariiosa* O Lesión cervical* O "Colo del diente" O Unión cimento esmalte* O JCE E ("Cementos Dentales") O Adhesivo Dental* O Agente de Cimentación* O "Cemento Dental" O "Agentes de Unión Dentales" O Adhesivos O Cola* O "Unión Dental" O "Acondicionamiento dental" O Etanol O "Alcohol Etílico" O "Alcohol Absoluto" O "pretratamiento dentinário".
Cochrane Library (May 2019 - 1495 articles) #1Tooth Cervix; #2 "Cementoenamel Junction*"; #3 CEJ #4 #1 or #2 or #3 #5 Tooth Wear; #6 "Tooth Wear"; #7 "Dental Wear"; #8 "Tooth Erosion"; #9 "Eroded"; #10 "Dental Erosion"; #11 "NCCL"; #12 "Class V"; #13 "Non carious cervical lesion*"; #14 "Cervical lesion*"; #15 "Noncarious lesions"; #16 #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 #17 #4 or #16 #18 Dental Cements; #19 "Dental Cements"; #20 "Dental Adhesive*"; #21 "Luting Agent*"; #22 "Orthodontic Adhesive*"; #23 "Dental Cement"; #24 "Dentin Bonding Agents"; #25 #18 or #19 or #20 or #21 or #22 or #23 or #24 #26 Adhesives; #27 Adhesives; #28 "Glue*"; #29 #26 or #27 or #28 #30 Dental Bonding; #31 Dental Etching; #32 Ethanol; #33 "Ethanol"; #34 "Ethyl Alcohol"; #35 "Absolute Alcohol"; #36 "dentin pretreatment"; #37 #32 or #33 or #34 or #35 or #36 #38 #25 or #29 or #30 or #31 or #37 #39 #17 and #38

Study Selection and Data Collection Process

Duplicates were then removed in a two-step procedure. Firstly, a specific tool from EndNote to remove duplicates was used. Due to differences in the indexation process of articles in the different databases, not all duplicates were removed. Thus, a second step was performed, the selected articles were alphabetically organized by title and duplicates could be identified and removed manually. Articles were further screened by title and abstract taking into account the eligibility criteria. Then, full-text from selected articles was obtained and two reviewers (M.Y.S and E.B.) classified those that met the inclusion criteria. Relevant information about the study design, participants, interventions, and outcomes were extracted using customized extraction forms by the authors independently. Concerning aging, data that represented longer recall for clinical trials or laboratory studies and no aging were used.

Assessment of risk of bias

Risk of bias for clinical trials was evaluated according to the following parameters: sequence generation, allocation, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias.

For laboratory studies the risk of bias was evaluated according to the following parameters: materials used according to the manufacturer's instructions, samples with similar dimensions, similar pattern on testing

machine (cross-head speed), blinding of the operator of the testing machine, the presence of a control group.

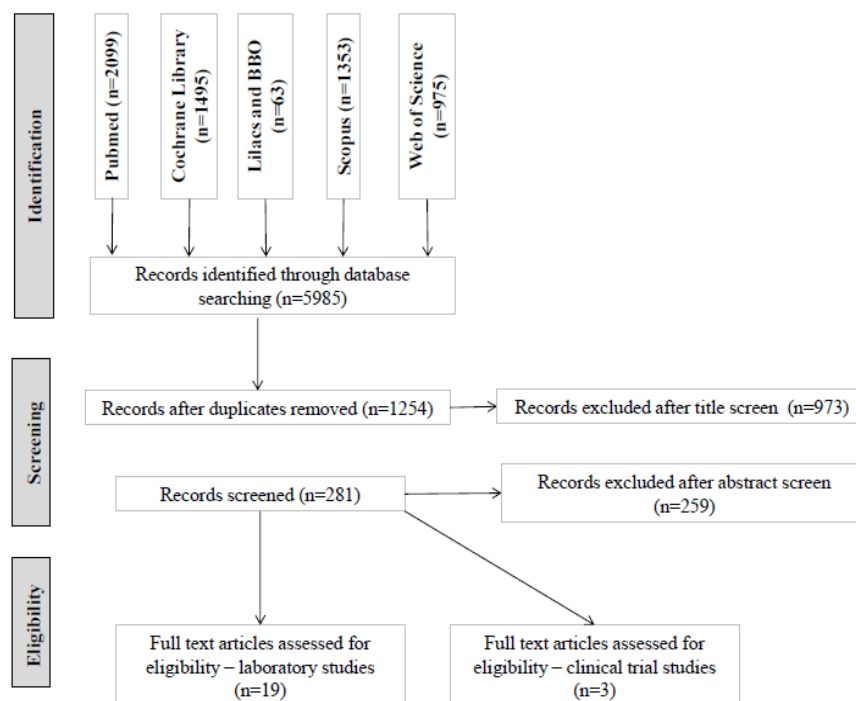
If the authors reported the parameter, the article received a 'yes' for that parameter; if it was not possible to find the information, the article received an 'unclear'; and if any type of bias was found the article received a 'no'. Articles that properly reported 5 to 6 items were classified as having a low risk of bias, those that reported 4 or 3 items were considered to have a medium risk of bias, and those that reported 1 to 2 items were classified as having a high risk of bias.

3. Results

Study Selection

After the database screening and removal of duplicates, 1254 studies were identified. After title screening, 281 studies remained, and this number was reduced to 22 (19 laboratorial and 3 clinical) after abstracts analysis. (Figure 2)

Figure 2 - Flowchart of study identification



Most included studies were judged as having a low risk of bias for clinical trials (Table 1) and for laboratory studies (Table 2). Most of parameters not properly reported received an unclear classification. Only one clinical study received a high risk of bias due to inconsistency with previously reported data.

Table 1 – Risk of bias for clinical trials included

CLINICAL TRIALS	SEQUENCE GENERATION	ALLOCATION	BLINDING OF PARTICIPANTS AND PERSONNEL	BLINDING OF OUTCOME ASSESSMENT	INCOMPLETE OUTCOME DATA	SELECTIVE REPORTING	OTHER BIAS
Mortazavi, 2012	?	●	●	●	●	●	●
Barros 2016	?	●	●	●	●	●	●
Souza, 2019	●	●	●	●	●	●	●

● Low risk of bias; ● Unclear risk of bias; ● High risk of bias.

Table 2 – Risk of bias for laboratory studies included

STUDIES	MATERIALS USED ACCORDING TO THE MANUFACTURER'S INSTRUCTIONS	SPECIMENS WITH SIZE STANDARDIZED	SAMPLE SIZE CALCULATION	SIMILAR PATTERN ON TESTING MACHINE	BLINDING OF THE OPERATOR OF THE TESTING MACHINE	CONTROL GROUP
Aggarwal, 2016	●	●	?	●	?	●
Ahn, 2015	●	●	?	●	?	●
Duan, 2011	?	●	?	●	?	●
Ekambaram, 2014	?	●	?	●	?	●
Faria-e-Silva, 2012	?	●	?	●	?	●
Huang, 2011	●	●	?	●	?	●
Kuhn, 2015	●	●	?	●	?	●
Fang Li, 2011	●	●	?	●	?	●
Li, 2016	●	●	?	●	?	●
Nagpal, 2015	●	●	?	●	?	●
Pei, 2012	●	●	?	●	?	●
Sadek, 2010	●	●	?	●	?	●
Sadek, 2010a	?	●	?	●	?	●
Sadek, 2010b	?	●	?	●	?	●
Sauro, 2011	?	●	?	●	?	●
Simões, 2014	●	●	?	●	?	●
Souza, 2018	●	●	?	●	?	●
Yang, 2016	?	●	?	●	?	●
Yesilyurt, 2015	●	●	?	●	?	●

● Low risk of bias; ● Unclear risk of bias; ● High risk of bias.

The forest plot for all laboratory studies and each variable is represented in Figure 3 to 10.

Figure 3 – Forest plot for all laboratory studies included

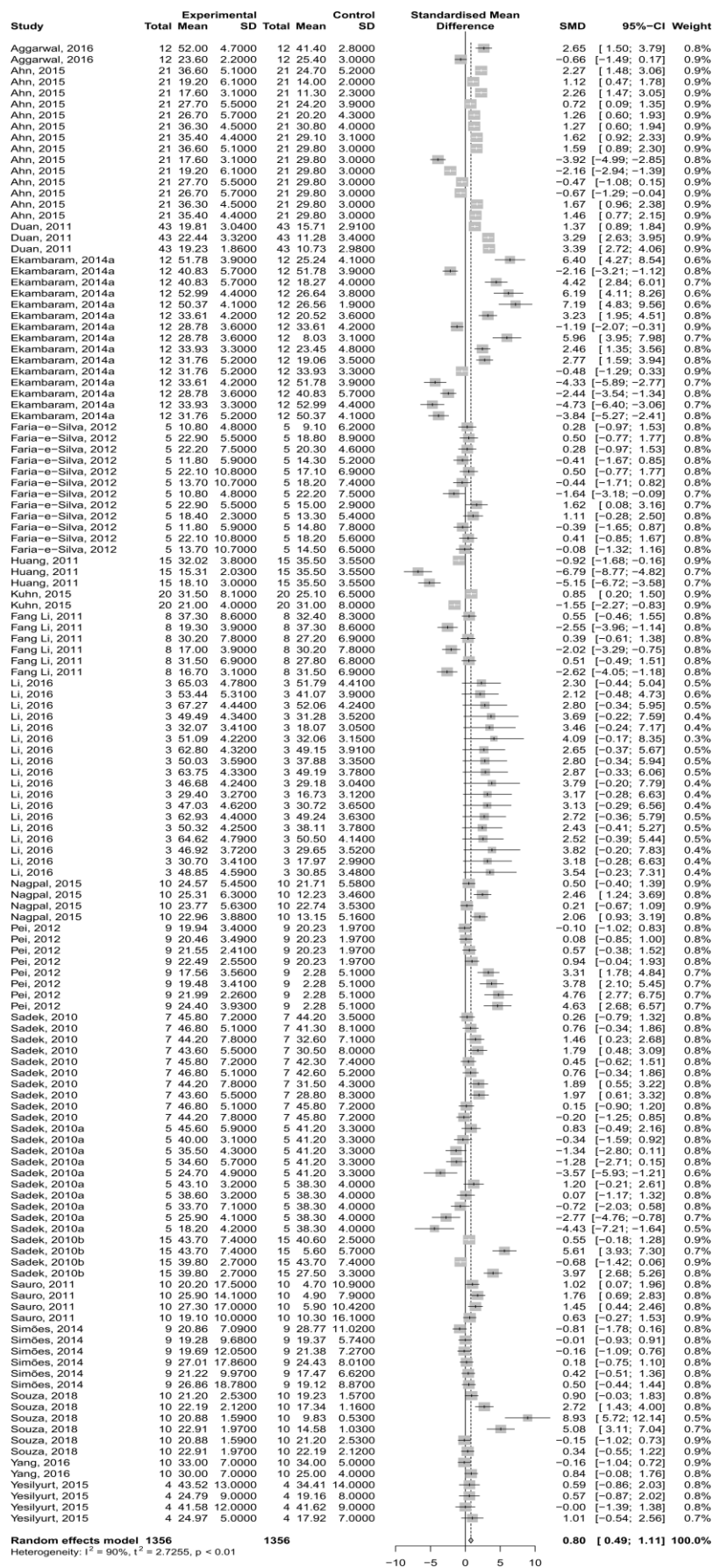


Figure 4 – Forest plot for the laboratory studies grouped by microtensile (MTBs) or shear bond strength (SBS) tests

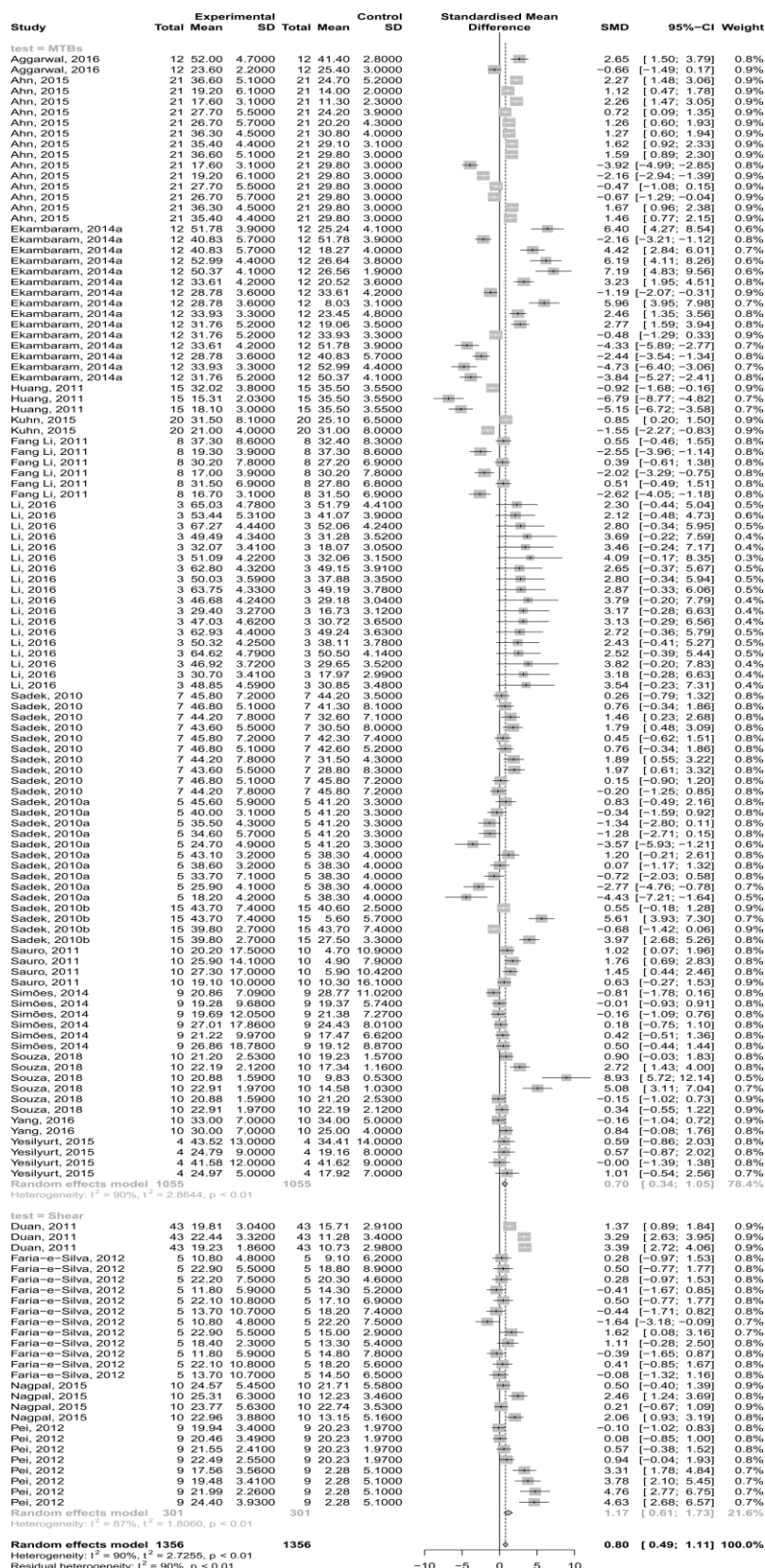


Figure 5 – Forest plot for the laboratory studies grouped by mode of application of EWBT

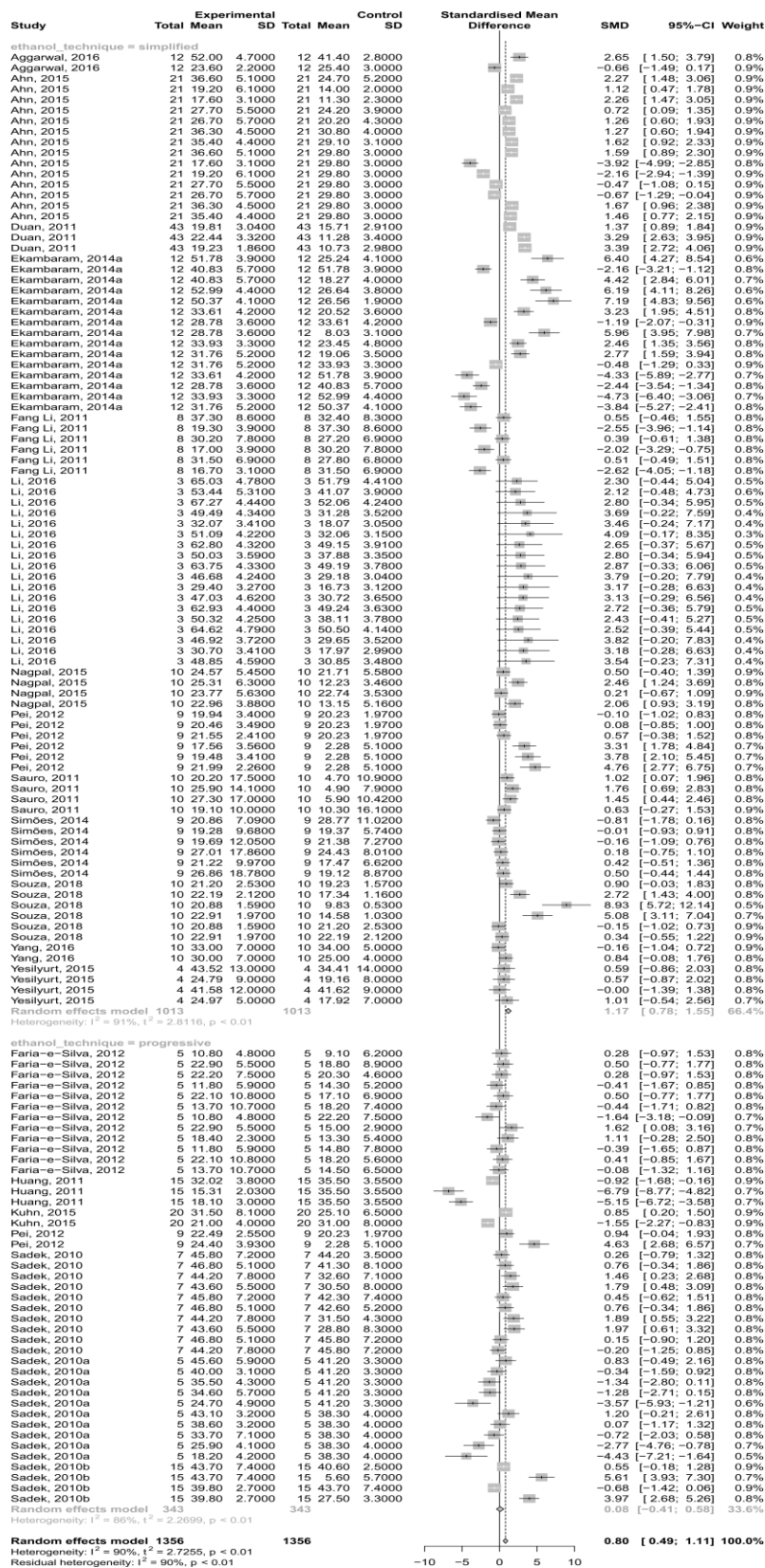


Figure 6 – Forest plot for the laboratory studies grouped by mode of application of adhesive system

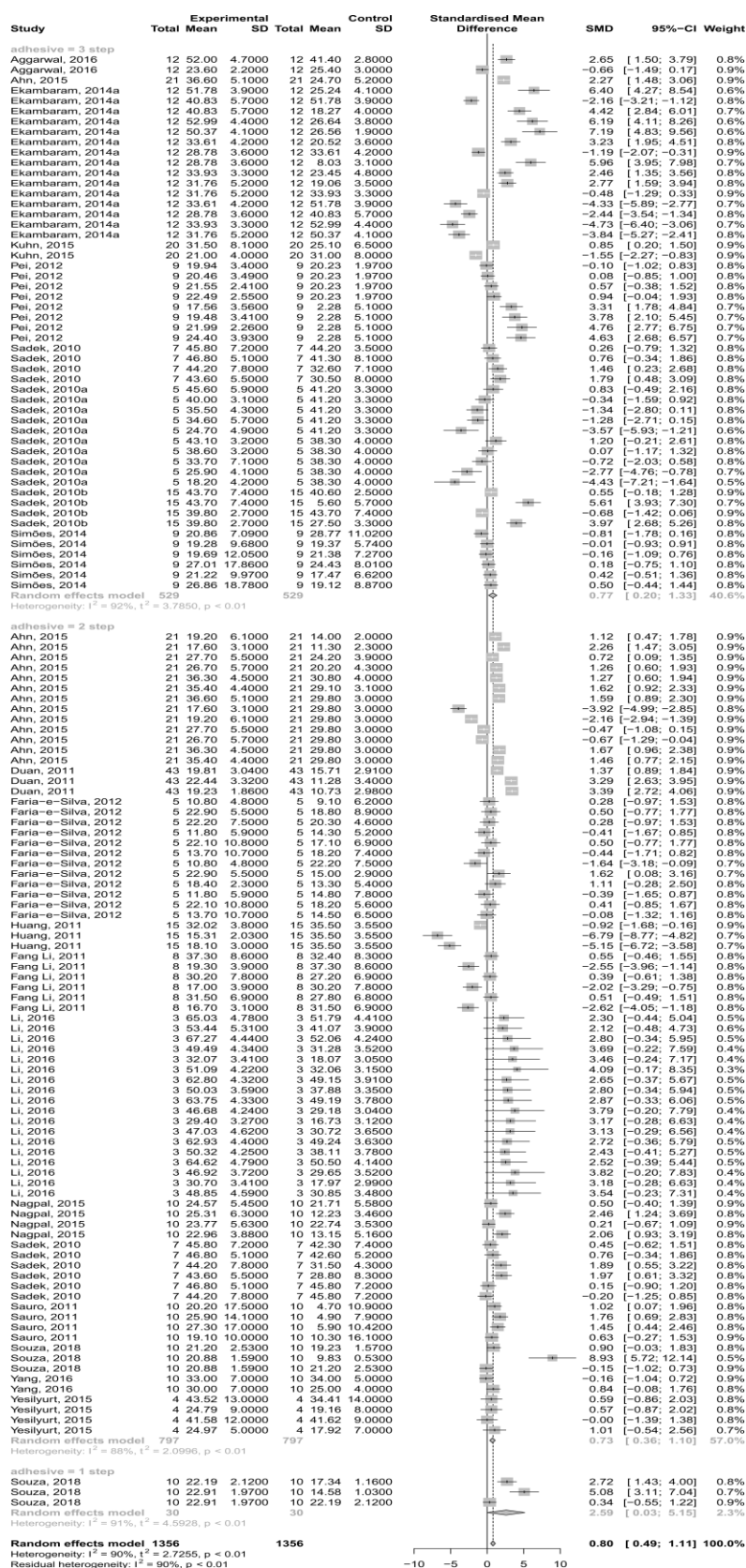


Figure 7 – Forest plot for the laboratory studies grouped by the presence or not of aging process on bond strength

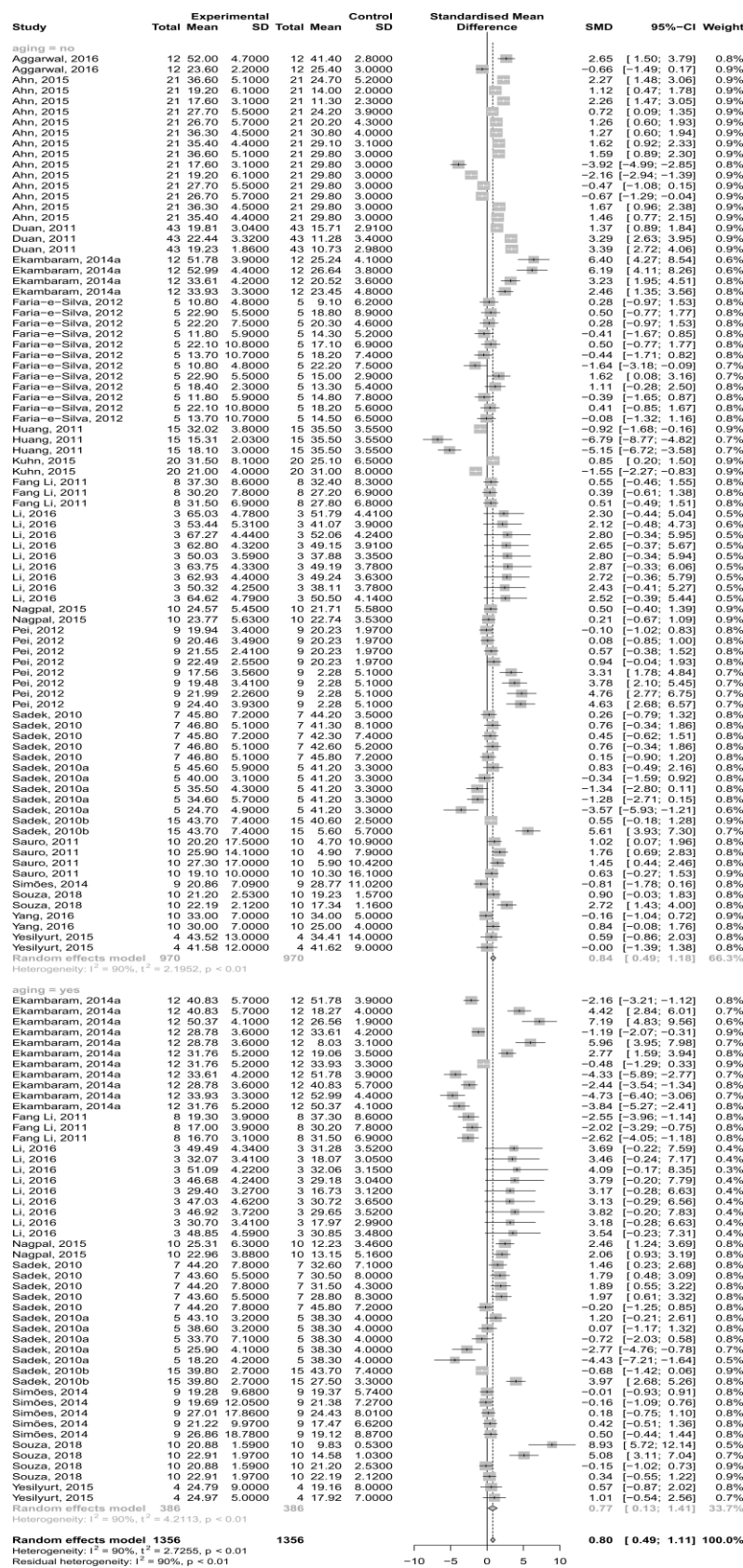


Figure 8 – Forest plot for the laboratory studies grouped by the use or not of CHX as pre-treatment associated to EWBT

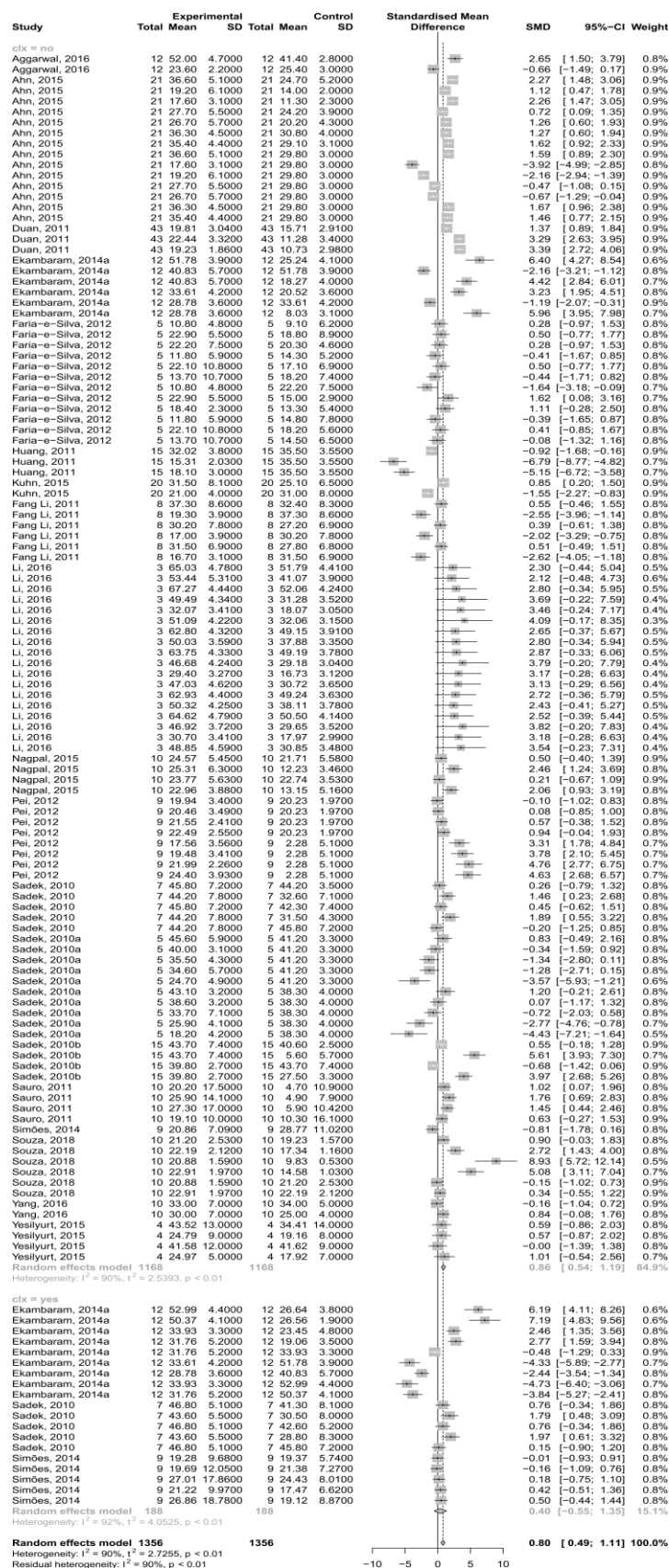


Figure 9 – Forest plot for the laboratory studies grouped by the use or not of hydrophobic primer after EWBT

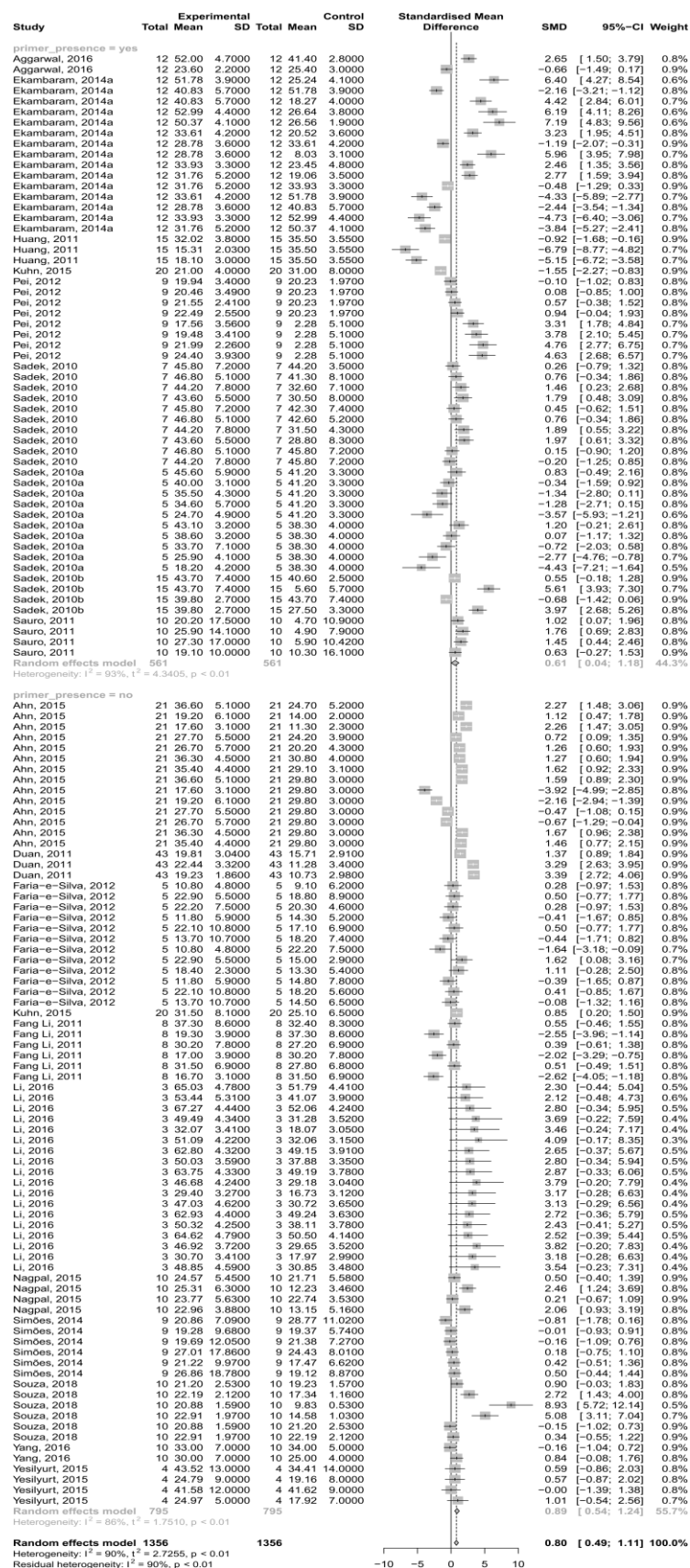
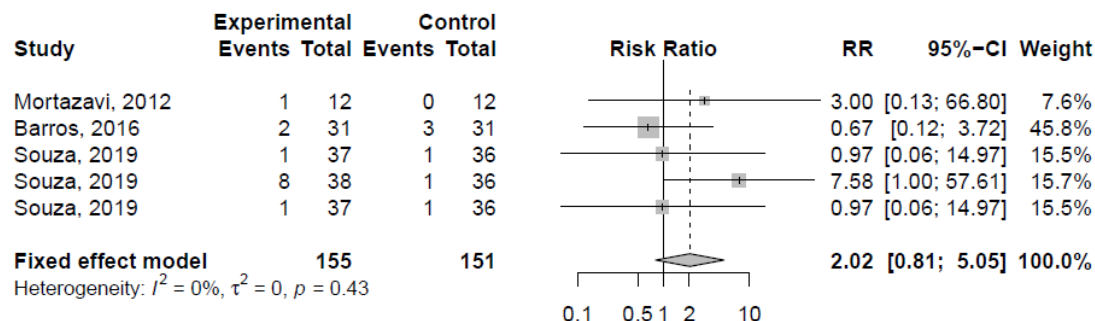


Figure 10 – Forest plot for the success (retention rates) of clinical trials



4. Discussion

EWBT was introduced in dentistry in an attempt to reduce the adhesive interface hydrolysis¹⁰. Although EWBT does not remove water completely from the dentin substrate¹⁰, some studies using this pretreatment reported a greater bond strength to dentin^{21, 22}, even after aging process¹², or while considering a better sealing capacity²³. Based on these laboratory results, EWBT may positively influence the interface adhesive to resist degradation. For studies with the EWBT that presented higher bond strength values, the authors relate the results with improved monomers impregnation, or with the possibility of carrying the monomers into dentin, besides the removal of water excess from the interface and the increase of interfibrillar spaces after this pretreatment^{10, 12, 24}. However, following the PICO question and taking into account all laboratory studies included, the overall results led to greater bond strength for control group (Figure 3). This effect was considered moderate with $SMD=0.80$. The

heterogeneity of all comparisons related to the laboratory studies was higher ($I^2=90\%$), and that fact might be related to the difference among the types of materials used, or tests performed, or testing parameters, or by the variability of the dentin substrate itself²⁵ (Figure 4 and 5). In addition, the risk of bias for the included laboratory studies was considered medium for 7 studies and low for 12 studies. None study reported two criteria evaluated, “sample size calculation” and “blinding of the operator of the testing machine”, based on this, unclear risk of bias was considered for these criteria, which may directly influence on the results of the study.

In relation to the mode of application of the adhesive systems, divergent results were observed. Some comparisons resulted in no difference between the mode of application of adhesives^{11, 26}, while for 2- or 3-step adhesives, the best results were obtained for control group^{19, 21}. The effect for adhesive mode of application was considered moderate for both protocols (SMD=0.73 and 0.77, respectively) (Figure 6). For self-etch approach, similar values were observed when associated or not with EWBT¹². This result may be related to the water remaining in the dentin, even after EWBT, once it is firmly bonded to collagen fibrils¹⁰. This may also facilitate bonding of the treated substrate with self-etch adhesives, in which there is a great amount of available hydrophilic monomers³. In addition, the adhesive system used by the authors of the reported study contained 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which is

stable to hydrolysis and the bond resulted from the acidic monomer and dentin may lead to durable retention of restoration in clinical conditions²⁷. Only one study that used EWBT associated to self-etch adhesive was included and based the results obtained, the authors suggest further studies evaluating this approach.

Other condition evaluated in this metanalysis was the presence of aging process. It was believed that after EWBT use, a hydrophobic hybrid layer is formed, which implies in lower hydrolysis of the adhesive interface over time^{11, 21}. In addition, an improved collagen fibrils protection associated with an increase of the interfibrillar spaces and better monomers impregnation are reported in the literature²⁴. However, this behavior was not observed in this metanalysis, since even after the aging process, the best results were observed for the control group, with moderate effect (SMD=0.77) (Figure 7). Based on this, others factors may be related to adhesive interface degradation overtime, such as MMPs or cysteine cathepsins with collagenolytic activity²⁸, mechanical fatigue²⁸ or the type of solvent content on adhesive²⁹.

In relation to the presence of CHX presence, laboratory studies have demonstrated higher bond strength values, greater structural integrity of the collagen network and decreased silver nitrate penetration^{30, 31}. Besides that, dentin demineralization produced by adhesive systems can activate different MMPs and some studies observed a capacity of CHX to inhibit the degradation

of collagen fibrils caused by MMPs³². The association between EWBT and CHX is promising since bond strength values were higher after this approach^{17, 33}. Although good results have been reported in laboratory studies, it is also reported that CHX may undergo leaching over time³⁴. The result of this metanalysis is indifferent when CHX is associated with EWBT and compared to EWBT alone. Both approaches resulted in adhesion results favoring the control groups (no EWBT) with moderate effect $SMD=0.40$ (Figure 8).

Moreover, although EWBT was proposed with hydrophobic adhesive alone, some laboratory studies employed a manipulated hydrophobic primer, which was used after the ethanol application^{11, 17, 19, 35}. Taking into account this condition, it was observed that in the absence of the hydrophobic primer the control group presented higher values, with high effect ($SMD=0.89$); however, in the primer presence this difference was lost (moderate effect, $SMD=0.61$) (Figure 9). It may be related to the dilution of the hydrophobic adhesive, which possibly improved its impregnation into the dentin. Regarding to EWBT mode of application, the superiority of control group was lost only for progressive technique. This may be related to the better water replacement into dentin substrate by ethanol and consequently a better impregnation of resin monomers³⁶.

For the clinical trials included in this metanalysis¹⁴⁻¹⁶, there was no difference between EWBT and water wet technique (Figure 10). This result may

be related to the short follow-up of the restorations, as the longest follow-up was 18 months¹⁶. The risk of bias for the clinical trials was considered low. One study was considered of high risk of bias due to the 'incomplete outcome data' criterium. The inconsistency was related to the fact that the results of tested groups presented 100% of success after 18 months, but in the 12 months follow up, it was around 90%³⁷. The authors considered the previously failed into the 18 months follow-up.

Based on the results obtained in this metanalysis, the authors understand that the difference found for the laboratory studies are because those are more controlled studies; with less bias the results might present a more homogeneous behavior. Therefore, when it comes to clinical trials, there is variability among the cavities/patients, as dentin type, occlusion, cavity shape and restorative protocol, and such variables may be related to the lack of differences observed.

Considering the limitations of this metanalysis and based on the PICO question, the authors suggest further clinical trials with longer follow-up should be performed to provide better evidence on the EWBT effectiveness.

5. Conclusion

Although most of laboratory studies EWBT presented lower results, when clinical performance was evaluated, no difference between EWBT and control group was observed. This result suggests that the EWTB should be further investigated to define a protocol and possible clinical indication.

6. References

1. Pashley DH, Tay FR, Breschi L, et al. State of the art etch-and-rinse adhesives. *Dent Mater* 2011;27(1):1-16.
2. Toledano M, Osorio R, Moreira MA, et al. Effect of the hydration status of the smear layer on the wettability and bond strength of a self-etching primer to dentin. *Am J Dent* 2004;17(5):310-4.
3. Van Meerbeek B, Yoshihara K, Yoshida Y, et al. State of the art of self-etch adhesives. *Dent Mater* 2011;27(1):17-28.
4. Perdigao J, Kose C, Mena-Serrano AP, et al. A new universal simplified adhesive: 18-month clinical evaluation. *Oper Dent* 2014;39(2):113-27.
5. Van Meerbeek B, De Munck J, Yoshida Y, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper Dent* 2003;28(3):215-35.
6. Perdigao J. Dentin bonding as a function of dentin structure. *Dent Clin North Am* 2002;46(2):277-301, vi.
7. Vallabhdas AK, Kumar CNV, Kabbinala P, et al. Evaluation of Hybrid Layer and Bonding Interface after Water Storage with and without the Usage of 2% Chlorhexidine: A Scanning Electron Microscope Study. *J Contemp Dent Pract* 2018;19(1):52-59.

8. Provenzi C, Collares FM, Cuppini M, et al. Effect of nanostructured zirconium dioxide incorporation in an experimental adhesive resin. *Clin Oral Investig* 2018;22(6):2209-18.
9. Hashimoto M, Hirose N, Kitagawa H, et al. Improving the durability of resin-dentin bonds with an antibacterial monomer MDPB. *Dent Mater J* 2018;37(4):620-27.
10. Pashley DH, Tay FR, Carvalho RM, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am J Dent* 2007;20(1):7-20.
11. Sadek FT, Braga RR, Muench A, et al. Ethanol wet-bonding challenges current anti-degradation strategy. *J Dent Res* 2010;89(12):1499-504.
12. Souza MY, R DIN, Bresciani E. Influence of ethanol-wet dentin, adhesive mode of application, and aging on bond strength of universal adhesive. *Braz Oral Res* 2018;32:e102.
13. Sauro S, Toledano M, Aguilera FS, et al. Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Part II: Effects of mechanical cycling load on microtensile bond strengths. *Dent Mater* 2011;27(6):563-72.

14. Mortazavi V, Samimi P, Rafizadeh M, et al. A randomized clinical trial evaluating the success rate of ethanol wet bonding technique and two adhesives. *Dent Res J (Isfahan)* 2012;9(5):588-94.
15. Souza MY, Jurema ALB, Caneppele TMF, et al. Six-month performance of restorations produced with the ethanol-wet-bonding technique: a randomized trial. *BOR* 2019.
16. Barros TAF DAJ, Braga EMF, Silva e Souza PdAR, Loretto SC, Souza Júnior MHSE Ethanol-Wet Bonding Technique: 18-month Clinical Evaluation. *Int J Odontostomatol* 2016;10((2)):267-76.
17. Ekambaram M, Yiu CK, Matinlinna JP, et al. Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine. *J Dent* 2014;42(6):709-19.
18. Li MZ, Wang JR, Liu H, et al. Effects of light curing modes and ethanol-wet bonding on dentin bonding properties. *J Zhejiang Univ Sci B* 2016;17(9):703-11.
19. Aggarwal V, Singla M, Sharma R, et al. Effects of simplified ethanol-wet bonding technique on immediate bond strength with normal versus caries-affected dentin. *J Conserv Dent* 2016;19(5):419-23.
20. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6(7):e1000097.

21. Ahn J, Jung KH, Son SA, et al. *Effect of additional etching and ethanol-wet bonding on the dentin bond strength of one-step self-etch adhesives. Restor Dent Endod* 2015;40(1):68-74.
22. Li F, Liu XY, Zhang L, et al. *Ethanol-wet bonding technique may enhance the bonding performance of contemporary etch-and-rinse dental adhesives. J Adhes Dent* 2012;14(2):113-20.
23. Sadek FT, Castellan CS, Braga RR, et al. *One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. Dent Mater* 2010;26(4):380-6.
24. Hosaka K, Nishitani Y, Tagami J, et al. *Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. J Dent Res* 2009;88(2):146-51.
25. Pongprueksa P, Senawongse P, Vongphan N. *Effect of dentinal tubule orientation on the modulus of elasticity of resin-infiltrated demineralized dentin. Dent Mater J* 2014;33(1):54-8.
26. Pei D, Huang X, Huang C, et al. *Ethanol-wet bonding may improve root dentine bonding performance of hydrophobic adhesive. J Dent* 2012;40(5):433-41.
27. Zhou J, Wurihan, Shibata Y, et al. *Quantitative/qualitative analysis of adhesive-dentin interface in the presence of 10-methacryloyloxydecyl dihydrogen phosphate. J Mech Behav Biomed Mater* 2019;92:71-78.

28. Perdigao J, Reis A, Loguercio AD. Dentin adhesion and MMPs: a comprehensive review. *J Esthet Restor Dent* 2013;25(4):219-41.
29. Ferreira JC, Pires PT, de Azevedo AF, et al. Morphology of the Dentin-resin Interface yielded by Two-step Etch-and-rinse Adhesives with Different Solvents. *J Contemp Dent Pract* 2017;18(10):947-58.
30. Ou Q, Hu Y, Yao S, et al. Effect of matrix metalloproteinase 8 inhibitor on resin-dentin bonds. *Dent Mater* 2018;34(5):756-63.
31. Simoes DM, Basting RT, Amaral FL, et al. Influence of chlorhexidine and/or ethanol treatment on bond strength of an etch-and-rinse adhesive to dentin: an in vitro and in situ study. *Oper Dent* 2014;39(1):64-71.
32. Carrilho MR, Carvalho RM, de Goes MF, et al. Chlorhexidine preserves dentin bond in vitro. *J Dent Res* 2007;86(1):90-4.
33. Gomes Franca FM, Vaneli RC, Conti Cde M, et al. Effect of Chlorhexidine and Ethanol Application on Long-term Push-out Bond Strength of Fiber Posts to Dentin. *J Contemp Dent Pract* 2015;16(7):547-53.
34. Barbour ME, Maddocks SE, Wood NJ, et al. Synthesis, characterization, and efficacy of antimicrobial chlorhexidine hexametaphosphate nanoparticles for applications in biomedical materials and consumer products. *Int J Nanomedicine* 2013;8:3507-19.
35. Kuhn E, Farhat P, Teitelbaum AP, et al. Ethanol-wet bonding technique: Clinical versus laboratory findings. *Dent Mater* 2015;31(9):1030-7.

36. Sadek FT, Pashley DH, Nishitani Y, et al. Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique. *J Biomed Mater Res A* 2008;84(1):19-29.
37. Araujo JF, Barros TA, Braga EM, et al. One-year evaluation of a simplified ethanol-wet bonding technique: a randomized clinical trial. *Braz Dent J* 2013;24(3):267-72.

3 CONSIDERAÇÕES GERAIS

A eficácia de uma adesão estável em longo prazo, entre substrato dentinário e sistemas adesivos, deve ser obtida a fim de garantir um resultado clínico significativo e duradouro. Desta forma, diversas pesquisas têm sido realizadas, visando melhorar a eficácia dos procedimentos adesivos. Somado a isso, novos materiais restauradores são formulados no intuito de melhorar a interação com o substrato dental. Além disso, pré-tratamentos dentinários também são motivos de estudo, sendo que o objetivo principal destes é a minimização da degradação da interface adesiva ao longo do tempo. Na literatura, há diversos pré-tratamentos descritos, como por exemplo, utilização de diferentes concentrações de clorexidina (Vallabhdas et al., 2018), inibidores de metaloproteinases da matriz (MMPs) (Hashimoto et al., 2018), formulação de adesivos experimentais (Provenzi et al., 2018) ou aplicação de etanol (Pashley et al., 2007). No entanto, devido a recente utilização deste último pré-tratamento, existe ainda discussão na literatura a respeito dos reais efeitos causados pela técnica da dentina úmida em etanol (EWBT) sobre o tecido dentinário, bem como da implicação clínica deste possível pré-tratamento.

EWBT foi proposta com o intuito de remover água do substrato dentinário, uma vez que a presença da água na interface ainda é desafiadora, pois para sistemas adesivos de condicionamento ácido total da cavidade, a presença desta se faz necessária para a formação de uma camada híbrida estável. Por outro lado, a água é a responsável pela hidrólise da interface ao longo do tempo (Ahn et al., 2015). Adicionalmente, a utilização da EWBT foi inicialmente proposta em associação com adesivos hidrófobos (Nishitani et al., 2006), acreditando na capacidade do etanol em remover a água que está ligada ao redor dos túbulos dentinários e com as fibrilas colágenas do substrato

dentinário. De acordo com estudos laboratoriais a associação da EWBT e adesivos hidrófobos apresentarem união satisfatória, esta ligação é favorecida devido ao uso de um *primer* hidrófobo, que é constituído de um *bond* diluído em 50% de peso de etanol (Sadek et al., 2010). Contudo este *primer* não é disponível comercialmente, o que dificulta a extrapolação do resultado desta associação para a prática clínica.

Entretanto, num estudo foi demonstrado que após a aplicação de etanol no substrato dentinário, este remove somente a água fracamente ligada, portanto pode-se afirmar que há presença de água remanescente após EWBT (Hosaka et al., 2009; Osorio et al., 2010; Pashley, et al., 2011). Baseado nisso, a associação de EWBT com adesivo hidrófilo torna-se uma alternativa. Considerando os resultados de um estudo que avaliou EWBT em associação com adesivo universal, independente do modo de aplicação, em que houve resultados de força de união satisfatórios, mesmo após envelhecimento termomecânico (Souza et al., 2018), sugere-se que EWBT pode interagir com a *smear layer*, modificando a matriz orgânica e auxiliar na penetração de monômeros adesivos, que consequentemente favorece uma proteção eficaz das fibrilas colágenas. Além disso, a ação de um adesivo universal no modo autocondicionante no substrato dentinário pode ser beneficiada, uma vez que o etanol serviria como um veículo, e também pode haver o favorecimento da ligação do 10-MDP aos íons cálcio, uma vez que os cristais de hidroxiapatita são mantidos no substrato (Souza et al., 2018).

Baseado nisso, características da interface adesiva após EWBT em associação com adesivo universal no modo autocondicionante foram avaliadas na tentativa de melhor explicar essa associação. Os maiores valores de nanodureza para o grupo que recebeu o pré-tratamento com etanol, aumenta a especulação de que há formação de uma camada híbrida estável, mesmo após envelhecimento, quando EWBT está associada com adesivos hidrófilos. Este

fato pode estar ligado ao aumento dos espaços interfibrilares, os quais permitem uma impregnação melhorada e em maior quantidade de monômeros resinosos, sendo que estes são responsáveis pela proteção das fibrilas colágenas à degradação, além de serem responsáveis em melhorar as propriedades mecânicas da interface adesiva (Hosaka et al., 2009; Reis et al., 2009).

Levando em consideração, os baixos scores de nanoinfiltração observados no estudo laboratorial, pode-se sugerir que a impregnação do sistema adesivo aconteceu de forma efetiva, uma vez que a qualidade da interface adesiva formada foi melhor para o grupo com pré-tratamento, fato que foi evidenciado pelos escores do grupo envelhecido. Com relação aos valores de nanodureza, não foram observadas alterações no grupo EWBT quando comparado os valores iniciais e após envelhecimento, além disso, a melhora da qualidade interface adesiva também pode estar ligado ao aumento do grau de conversão dos monômeros de resina, mesmo quando um adesivo hidrófilo é utilizado em associação com EWBT (Tjäderhane et al., 2013).

Embora bons resultados sejam relatados na literatura para os estudos laboratoriais, quando EWBT é aplicado clinicamente, em conjunto com adesivo hidrófobo e *primer* diluído, não houve diferença clínica entre os grupos após 12 meses ou 18 meses de avaliação (Mortazavi et al., 2012; Barros et al., 2016). Entretanto, a fim de se avaliar a eficácia de EWBT, a partir da utilização de uma técnica clinicamente aceitável, foi proposto um estudo clínico com o emprego da EWBT com adesivo hidrófobo comercialmente disponível, sendo que esta associação apresentou cerca de 20% de falha de retenção de restaurações em lesões cervicais não cariosas após 6 meses de avaliação, sendo estatisticamente diferente dos grupos com adesivos hidrófilos. Além disso, é possível observar que este comportamento permaneceu após 12 e 18 meses de avaliação. Estes resultados podem estar ligados à dificuldade de aplicação do *bond* (adesivo hidrófobo) pois este apresenta uma viscosidade maior do que os outros

materiais, portanto a infiltração deste adesivo pode ser dificultada (Souza et al., 2019).

Levando em consideração os estudos citados em que o *primer* hidrófobo foi aplicado previamente ao adesivo hidrófobo e que obtiveram bons resultados, podem indicar a necessidade da fabricação e disponibilização comercial de um *primer*, a fim de melhorar as expectativas clínicas do protocolo do pré-tratamento dentinário com etanol. Portanto, estudos clínicos com o objetivo de avaliar a eficácia de adesivos hidrófobos em associação com EWBT podem aumentar a evidência científica deste protocolo. Uma vez que a utilização deste *primer* é despercebida, porém de extrema importância quando se trata de EWBT, e a validação de um novo protocolo ou material se dá através de estudos clínicos com análises em longo prazo.

Por fim, um compilado de estudos clínicos bem delineados sobre determinado tema, pode originar uma revisão sistemática e metanálise. Sendo que, a partir da eliminação de vieses, é possível a realização de uma análise crítica, o que permite evidenciar um melhor protocolo a partir de uma combinação de resultados provenientes de diferentes estudos. Baseado nisso, uma revisão sistemática e metanálise sobre EWBT foi realizada a fim de se ter uma evidência científica deste pré-tratamento.

Com relação aos estudos laboratoriais incluídos pode-se observar que há na literatura cerca de 19 trabalhos que avaliaram a força de união à dentina após EWBT. Embora haja resultados divergentes, que podem estar diretamente ligados às diferenças de metodologia ou de associação de diferentes protocolos e/ou materiais, os estudos foram classificados como baixo risco de viés (Sauro et al., 2011; Simões et al., 2014; Yang et al., 2016). Além disso, após análise estatística, a evidência observada foi para o grupo controle sendo que, o efeito desta evidência foi considerado moderado (SMD = 0,80).

Diversas variáveis dentre os estudos incluídos foram verificadas e

analisadas, como por exemplo, a associação com clorexidina (Sadek et al., 2010; Ekambaram et al., 2014; Simões et al., 2014), utilização de *primer* hidrófobo (Sadek et al., 2010; Pei et al., 2012; Ekambaram et al., 2014; Aggarwal et al., 2016), envelhecimento artificial (Li et al., 2011; Ekambaram et al., 2016), diferentes modos de aplicação da EWBT (Duan et al., 2011; Faria-e-Silva et al., 2012; Ahn et al., 2015; Aggarwal et al., 2016) e utilização de diferentes protocolos adesivos (Souza et al., 2018). Dentre todas as variáveis analisadas, para a maioria, o efeito da evidência foi considerado moderado (SMD em torno de 0,73), sendo considerado baixo somente com relação ao modo de aplicação da EWBT (SMD = 0,08).

Para a metanálise dos estudos clínicos, somente 3 estudos foram encontrados e incluídos (Mortazavi et al., 2012; Barros et al., 2016; Souza et al., 2018), sendo que o risco de viés dos estudos também foi considerado baixo, embora tenha tido um estudo que foi considerado como alto risco para um dos critérios avaliados ('Dados incompletos de desfecho')(Barros et al., 2016). Por fim, com relação aos resultados, não houve diferença na utilização de EWBT.

REFERÊNCIAS*

Ahn J, Jung K-H, Son S, Hur B, Kwon Y-H, Park J-K. Effect of additional etching and ethanol-wet bonding on the dentin bond strength of one-step self-etch adhesives. *Restor Dent Endod*. 2015;40(1):68-74. doi: 10.5395/rde.2015.40.1.68.

Aggarwal V, Singla M, Sharma R, Miglani S, Bhasin, SS. Effects of simplified ethanol-wet bonding technique on immediate bond strength with normal versus caries-affected dentin. *J Conserv Dent*. 2016;19(5):419-23. doi: 10.4103/0972-0707.190009.

Araujo JF, Barros TA, Braga EM, Loretto SC, Silva e Souza Pde A, Silva e Souza MH. One-year evaluation of a simplified ethanol-wet bonding technique: a randomized clinical trial. *Braz Dent J*. 2013;24(3):267-72. doi: 10.1590/0103-6440201302128.

Barros TAF, Araujo JF, Braga EM, Silva e Souza Pde A, Silva e Souza MH. Ethanol-Wet Bonding Technique: 18-month Clinical Evaluation. *Int J Odontostomat*. 2016;10(2):267-76.

Borges AL, Borges AB, Xavier TA, Bottino MC, Platt JA. Impact of quantity of resin, C-factor, and geometry on resin composite polymerization shrinkage stress in Class V restorations. *Oper Dent*. 2014;39(2):144-51. doi: 10.2341/12-440-L.

Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, Dorigo EDS. Dental adhesion review: aging and stability of the bonded interface. *Dent Mater*. 2008;24(1):90-101. doi: 10.1016/j.dental.2007.02.009.

Cardoso MV, Coutinho E, Ermis RB, Poitevin A, Van Landuyt K, De Munck J, et al. Influence of dentin cavity surface finishing on micro-tensile bond strength of adhesives. *Dent Mater*. 2008;24(4):492-501. doi: 10.1016/j.dental.2007.04.011.

Duan SS, Ouyang XB, Pei DD, Huo YH, Pan QH, Huang C. Effects of ethanol-wet bonding technique on root dentine adhesion. *Chin J Dent Res*. 2011;14(2):105-11.

* Baseado em: International Committee of Medical Journal Editors Uniform Requirements for Manuscripts Submitted to Biomedical journals: Sample References [Internet]. Bethesda: US NLM; c2003 [atualizado 04 nov 2015; acesso em 25 jan 2016]. U.S. National Library of Medicine; [about 6 p.]. Disponível em: http://www.nlm.nih.gov/bsd/uniform_requirements.html

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 Jun;42(6):709-19. doi: 10.1016/j.jdent.2014.04.001.

Eliguzeloglu E, Eraslan O, Omurlu H, Eskitascioglu G, Belli S. The effect of cavity shape and hybrid layer on the stress distribution of cervical composite restorations. *Eur J Dent*. 2011;5(2):180-5.

Faria-e-Silva AL, Araújo JE, Rocha GP, de Oliveira Ada S, de Moraes RR. Solvent content and dentin bond strengths using water-wet, ethanol-wet and deproteinization bonding techniques. *Acta Odontol Scand*. 2013;71(3-4):710-5. doi: 10.3109/00016357.2012.715195.

Hashimoto M, Hirose N, Kitagawa H, Yamaguchi S, Imazato S. Improving the durability of resin-dentin bonds with an antibacterial monomer MDPB. *Dent Mater J*. 2018;37(4):620-7. Epub 2018 Jul 29. doi: 10.4012/dmj.2017-209.

Heymann HO, Sturdevant JR, Brunson WD, Wilder AD, Sluder TB, Bayne SC. Twelve-month clinical study of dentinal adhesives in class V cervical lesions. *J Am Dent Assoc*. 1988;116(2):179-83.

Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, et al. Durability of resin-dentin bonds to water vs ethanol-saturated dentin. *J Dent Res*. 2009 Feb;88(2):146–51. doi:10.1177/0022034508328910.

Kim J, Gu L, Breschi L, Tjäderhane L, Choi KK, Pashley DH, et al. Implication of ethanol wet-bonding in hybrid layer remineralization. *J Dent Res*. 2010 Jun;89(6): 575–80. doi:10.1177/0022034510363380.

Lenzi TL, Soares FZ, Raggio DP, Pereira GK, Rocha RO. Dry-bonding Etch-and-Rinse Strategy Improves Bond Longevity of a Universal Adhesive to Sound and Artificially-induced Caries-affected Primary Dentin. *J Adhes Dent*. 2016;18(6):475-82. doi: 10.3290/j.jad.a36670.

Levitch LC, Bader JD, Shugars DA, Heymann HO. Non-carious cervical lesions. *J Dent*. 1994;22(4):195-207.

Li F, Liu XY, Zhang L, Kang JJ, Chen JH. Ethanol-wet bonding technique may enhance the bonding performance of contemporary etch-and-rinse dental adhesives. *J Adhes Dent*. 2012 Apr;14(2):113-20. doi: 10.3290/j.jad.a21853.

Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *J Dent Res*. 2011;90(8):953-68. doi: 10.1177/0022034510391799.

Mortazavi V, Samimi P, Rafizadeh M, Kazemi S. A randomized clinical trial evaluating the success rate of ethanol wet bonding technique and two adhesives. *Dent Res J*. 2012 Sep;9(5):588-94. PMID: 23559924.

Nagpal R, Manuja N, Pandit I. Effect of ethanol wet bonding technique on the durability of resin-dentin bond with contemporary adhesive systems. *J Clin Pediatr Dent*. 2015;39(2):133-42.

Nishitani Y, Yoshiyama M, Donnelly A, Agee K, Sword J, Tay F, et al. Effects of resin hydrophilicity on dentin bond strength. *J Dent Res*. 2006;85(11):1016-21. doi: 10.1177/154405910608501108.

Osorio E, Toledano M, Aguilera FS, Tay FR, Osorio R. Ethanol wet-bonding technique sensitivity assessed by AFM. *J Dent Res*. 2010 Nov;89(11):1264-9. doi: 10.1177/0022034510376403.

Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am J Dent*. 2007;20(1):7-20. Epub 2007 Feb.

Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. *Dent Mater*. 2011;27(1):1-16. doi: 10.1016/j.dental.2010.10.016.

Pei D, Huang X, Huang C, Wang Y, Ouyang X, Zhang J. Ethanol-wet bonding may improve root dentine bonding performance of hydrophobic adhesive. *J Dent*. 2012;40(5):433-41. doi: 10.1016/j.jdent.2012.02.005.

Perdigão J, Loguercio A. Universal or multi-mode adhesives: why and how? *J Adhes Dent*. 2014a;16(2):193. doi: 10.3290/j.jad.a31871.

Perdigão J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, et al. A new universal simplified adhesive: 18-month clinical evaluation. *Oper Dent*. 2014b;39(2):113-27. doi: 10.2341/13-045-C.

Provenzi C, Collares FM, Cuppini M, Samuel SMW, Alves AK, Bergmann CP, et al. Effect of nanostructured zirconium dioxide incorporation in an

experimental adhesive resin. *Clin Oral Investig*. 2018;22(6):2209-18. Epub 2018 Jul. doi: 10.1007/s00784-017-2311-z.

Reis A, Klein-Junior CA, Accorinte Mde L, Grande RH, dos Santos CB, Loguercio AD. Effects of adhesive temperature on the early and 6-month dentin bonding. *J Dent*. 2009; 37:791-8. doi:10.1016/j.jdent.2009.06.007.

Sadek FT, Castellan CS, Braga RR, Mai S, Tjaderhane L, Pashley DH, et al. One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. *Dent Mater*. 2010;26(4):380-6. Epub 2010 Apr. doi: 10.1016/j.dental.2009.12.009.

Sauro S, Di Renzo S, Castagnola R, Grande NM, Plotino G, Foschi F, et al. Comparison between water and ethanol wet bonding of resin composite to root canal dentin. *Am J Dent*. 2011;24(1):25-30.

Scheffel DL, Sacono NT, Ribeiro AP, Soares DG, Basso FG, Pashley DH, et al. Immediate human pulp response to ethanol-wet bonding technique. *J Dent*. 2015 May;43(5):537–45. doi: 10.1016/j.jdent.2015.02.014.

Shetty MS SR, Mattigatti S, Managoli AN, Rairam GS, Patil MA. No carious cervical lesions: abfraction. *J Int Oral Health*. 2013;5(5):143-6.

Simões DM, Basting RT, Amaral FL, Turssi CP, França FM. Influence of chlorhexidine and/or ethanol treatment on bond strength of an etch-and-rinse adhesive to dentin: an in vitro and in situ study. *Oper Dent*. 2014;39(1):64-71. doi: 10.2341/12-486-L.

Souza MY, Di Nicoló R, Bresciani E. Influence of ethanol-wet dentin, adhesive mode of application, and aging on bond strength of universal adhesive. *Braz Oral Res*. 2018; 11;32:e102. doi: 10.1590/1807-3107bor-2018.vol32.0102.

Souza MY, Jurema ALB, Caneppele TMF, Bresciani E. Six-month performance of restorations produced with the ethanol-wet-bonding technique: a randomized trial. *Braz Oral Res*. 2019 [Epub ahead of print] doi: 10.1590/1807-3107bor-2019.vol33.0052.

Spencer P, Jonggu Park QY, Misra A, Bohaty BS, Singh V, Parthasarathy R, et al. Durable bonds at the adhesive/dentin interface: an impossible mission or simply a moving target? *Braz Dent Sci*. 2012;15(1):4-18.

Tassery H, Levallois B, Terrer E, Manton DJ, Otsuki M, Koubi S, et al. Use of

new minimum intervention dentistry technologies in caries management. *Aust Dent J*. 2013;58 Suppl 1:40-59. doi: 10.1111/adj.12049.

Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, et al. Bonding BisGMA to dentin—a proof of concept for hydrophobic dentin bonding. *J Dent Res*. 2007;86:1034-9. doi: 10.1177/154405910708601103.

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

Tjaderhane L. Dentin bonding: can we make it last? *Oper Dent*. 2015;40(1):4-18. doi: 10.2341/14-095-BL.

Tsujimoto A, Shimatani Y, Nojiri K, Barkmeier WW, Markham MD, Takamizawa T, et al. Influence of surface wetness on bonding effectiveness of universal adhesives in etch-and-rinse mode. *Eur J Oral Sci*. 2018;127(2):162-9. doi: 10.1111/eos.12596.

Vallabhdas AK, Kumar CNV, Kabbinala P, Nayak R, Rajakumari M, Shilpa T. Evaluation of Hybrid Layer and Bonding Interface after Water Storage with and without the Usage of 2% Chlorhexidine: A Scanning Electron Microscope Study. *J Contemp Dent Pract*. 2018;19(1):52-9. Epub 2018 Jan 1.

Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dent Mater*. 2011;27(1):17-28. doi: 10.1016/j.dental.2010.10.023.

Yang H, Guo J, Deng D, Chen Z, Huang C. Effect of adjunctive application of epigallocatechin-3-gallate and ethanol-wet bonding on adhesive-dentin bonds. *J Dent*. 2016;44:44-9. doi: 10.1016/j.jdent.2015.12.001.

APÊNDICE A – Ficha clínica de consulta inicial**FICHA CLÍNICA**

DATA DE ENTRADA: ____/____/____ MATRÍCULA: _____ # _____

Nome: _____

Data de nascimento: ____/____/____ Idade: _____ Gênero: _____

Tel: (____) _____ Celular: (____) _____

E-mail: _____

DADOS INICIAIS DA PESQUISA

DENTE	
SENSIBILIDADE PRÉVIA	não () com ar () tátil () alimentos ()
GENGIVA MARGINAL	
TIPO DE CAVIDADE	
DENTINA ESCLEROSADA	
OCLUSÃO	
TAMANHO DA CAVIDADE	Cérvico-oclusal: mm Profundidade: mm
MATERIAL RESTAURADOR	Controle - SEM ETANOL () Et + Hidrófobo () Et + Adesivo Universal () Et + Hidrófilo + Hidrófobo ()

Observações: _____

ANEXO A – Termo de Consentimento Livre e Esclarecido**TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

Caro(a) Senhor(a)

Eu, Eduardo Bresciani, Professor Adjunto do Departamento de Odontologia Restauradora da UNESP – Campus de São José dos Campos – Faculdade de Odontologia, situada à Av. Engº Francisco José Longo, 777 – São José dos Campos – SP, telefone para contato (012) 3947-9403, coordeno esta pesquisa cujo título é “Avaliação clínica da técnica da dentina úmida em etanol com adesivos hidrófilo, hidrófobo ou associação: estudo clínico randomizado controlado”. Esse trabalho é realizado pelo aluno de doutorado em Odontologia Restauradora, área de concentração Dentística, Mauricio Yugo de Souza, e tem o objetivo de avaliar o desempenho clínico de restaurações em cavidades do tipo Classe V (lesões localizadas nos dentes, próximo à gengiva, geralmente expondo parte da raiz).

Para o tratamento, quatro técnicas de adesão (colagem da restauração ao dente) serão testadas. Em três destas técnicas será utilizada a aplicação do etanol (álcool) como um passo intermediário. Cada dente a ser restaurado receberá, por meio de sorteio, um dos protocolos a serem testados.

Os benefícios desse tratamento são: possibilidade da diminuição de sensibilidade, o reestabelecimento da saúde e estrutura do dente, com devolução da estética perdida e possível aumento da sobrevida das restaurações.

Os riscos do tratamento são: trauma gengival e possível sensibilidade, condições que podem ocorrer em qualquer procedimento restaurador.

Informo que será garantida a liberdade da retirada do consentimento a qualquer momento e assim deixar de participar do estudo, sem penalização alguma. A manutenção do sigilo e da privacidade durante todas as fases da pesquisa também é garantida. Não haverá custo nem pagamento por sua colaboração.

Se houver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética (CEP) do Instituto de Ciência e Tecnologia de São José dos Campos – UNESP, situado à Av. Engº Francisco José Longo, 777 – CEP 12245-000 São José dos Campos – SP, telefone (012) 3947-9028, e comunique-se com a Coordenadora Profa. Dra. Denise Nicodemo.

Termo de Consentimento Livre e Esclarecido

Pelo presente instrumento que atende às exigências legais, o Sr. (a)

portador da cédula de identidade _____, após leitura minuciosa das informações constantes neste TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO, devidamente explicadas pelos profissionais em seus mínimos detalhes, ciente dos serviços e procedimentos aos quais será

submetido, não restando quaisquer dúvidas a respeito do lido e explicado, firma seu CONSENTIMENTO LIVRE E ESCLARECIDO concordando em participar da pesquisa proposta. Fica claro que o participante da pesquisa, pode a qualquer momento retirar seu CONSENTIMENTO LIVRE E ESCLARECIDO e deixar de participar desta pesquisa e ciente de que todas as informações prestadas tornar-se-ão confidenciais e guardadas por força de sigilo profissional (Art. 9º do Código de Ética Odontológica). Por fim, como pesquisador responsável pela pesquisa, comprometo-me a cumprir todas as exigências contidas no item IV.3 e IV.4 (se pertinente) da resolução do CNS/MS n. 466 de dezembro de 2012, publicada em 13 de junho de 2013.

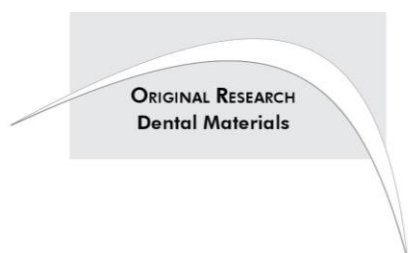
Por estarmos de acordo com o presente termo o firmamos em duas vias. A primeira via ficará de posse do participante da pesquisa e a segunda de posse do pesquisador.

São José dos Campos, ____/____/____.

Assinatura do Paciente

Assinatura do Pesquisador

ANEXO B – Comprovante de artigo aceito



Six-month performance of restorations produced with the ethanol-wet-bonding technique: a randomized trial

Maurício Yugo de SOUZA^(a)
 Ana Luiza Barbosa JUREMA^(a)
 Taciana Marco Ferraz CANEPPELE^(a)
 Eduardo BRESCIANI^(a)

^(a)Universidade Estadual Paulista – Unesp,
 Institute of Science and Technology,
 Department of Restorative Dentistry, São
 José dos Campos, SP, Brazil.

Abstract: This double-blind randomized controlled clinical trial evaluated the effectiveness of dentin pretreatment with 100% ethanol (EWBT – ethanol wet bonding technique) and different adhesive protocols in noncarious cervical lesions (NCCL) after 6 months. Patients presenting at least one NCCL were included. NCCLs (n=148) were randomly assigned to 4 groups: NE (Non-EWBT + three-step etch-and-rinse (Scotchbond Multi Purpose, 3M ESPE [MP])), E (EWBT + MP); EB (EWBT + [Bond – third step of MP]), and EU (EWBT + universal adhesive (Single Bond Universal, 3M ESPE). Conventional acid-etching (Condac 37%, FGM) and nanohybrid resin composite (Z350, 3M ESPE) were used. Trained and calibrated examiners (Kappa = 0.61) evaluated the restorations at baseline (7 days) and 6-month recall using the USPHS modified criteria. Data were subjected to Chi square ($\alpha = 0.05$). Differences in the success rate were found for the treatments ($p = 0.003$). E presented the lowest success rate compared with the other groups ($p < 0.02$). No significant differences were detected among NE, E, and EU ($p > 0.49$). The survival rates were 97.23%, 97.30%, 78.95%, and 100% for NE, E, EB, and EU, respectively. Regarding postoperative sensitivity, a significant reduction was found for groups E ($p = 0.027$) and EU ($p < 0.01$) after 6 months. After 6 months, EWBT associated to the hydrophobic adhesive system had the highest failure rate.

Keywords: Dentin; Ethanol; Adhesives; Tooth Abrasion.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
 Eduardo Bresciani
 E-mail: eduardo.bresciani@unesp.br

<https://doi.org/10.1590/1807-3107bor-2019.vol33.0052>

Submitted: January 16, 2019
 Accepted for publication: May 20, 2019
 Last revision: May 22, 2019

Introduction

Dental tissues undergo different responses to acid-etching procedures¹ and consequently to different adhesion patterns. For enamel, adhesion is facilitated because of the predominance of hydroxyapatite crystals and the consequent adequate formation of resin microtags.^{1,2} However, dentin consists of predominantly organic components, mainly collagen fibers, extension of odontoblasts, and dentinal fluid, which leads to a more variable and challenging bonding procedure.^{2,3,4}

A meta-analysis⁵ indicated that the best adhesion results to dentin were observed with the use of 2-step self-etch (moderate pH) and 3-step etch-and-rinse adhesives. The authors also concluded that additional studies are required to improve dental adhesion. Although new dental adhesives have been introduced, including adhesives named “universal”



ANEXO C - Comprovante de artigo enviado

Clinical Oral Investigations
HOME • LOGOUT • HELP • REGISTER • UPDATE MY INFORMATION • JOURNAL OVERVIEW
MAIN MENU • CONTACT US • SUBMIT A MANUSCRIPT • INSTRUCTIONS FOR AUTHORS • PRIVACY

Editorial Manager™
Role: Author Username: eduardob

Submissions Being Processed for Author Eduardo Bresciani, DDS, MS, PhD

Page: 1 of 1 (1 total submissions) Display 10 results per page.

Action ▲▼	Manuscript Number ▲▼	Title ▲▼	Initial Date Submitted ▲▼	Status Date ▲▼	Current Status ▲▼
View Submission View Reference Checking Results	CLOI-D-19-00848	18-month performance of ethanol-wet-bonding technique with different adhesive protocols: a randomized controlled clinical trial	12 Jun 2019	17 Jun 2019	With Editor

Page: 1 of 1 (1 total submissions) Display 10 results per page.

<< Author Main Menu

ANEXO D - Comprovante de artigo enviado

De: William Palin <W.M.Palin@bham.ac.uk>

Assunto: Re: IJAA/442W

Data: 26 de junho de 2019 13:33:46 BRT

Para: "eduardo.bresciani@unesp.br" <eduardo.bresciani@unesp.br>

Dear Dr Eduardo Bresciani

Ref: IJAA/442W

Thank you for submitting your work to the International Journal of Adhesion and Adhesives. Your paper has been sent for review and I will contact you again when I have heard the opinions of the reviewers.
In any correspondence please quote the reference shown above.

Sincerely,

William M. Palin
Subject Editor for Biomaterial Adhesion
International Journal of Adhesion and Adhesives
Professor of Biomedical Materials Science

ANEXO E - Certificado do Comitê de Ética em Pesquisa

UNESP - INSTITUTO DE
CIÊNCIA E TECNOLOGIA -
CAMPUS DE SÃO JOSÉ DOS



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO CLÍNICA DA TÉCNICA DA DENTINA ÚMIDA EM ETANOL COM ADESIVOS HIDRÓFILO, HIDRÓFOTO OU ASSOCIAÇÃO: ESTUDO CLÍNICO RANDOMIZADO CONTROLADO

Pesquisador: Mauricio Yugo de Souza

Área Temática:

Versão: 1

CAAE: 66097217.8.0000.0077

Instituição Proponente: Instituto de Ciência e Tecnologia de São José dos Campos - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.022.383

Apresentação do Projeto:

O presente trabalho irá avaliar a técnica da dentina úmida em etanol com adesivos hidrófilo, hidrófobo ou associação para o tratamento de lesões cervicais não cáries de Classe V (Lesão Cervical Não Cáries – LCNC) em caninos e pré-molares.

Objetivo da Pesquisa:

Avaliar por meio de um estudo clínico controlado randomizado duplo-cego, o desempenho da técnica úmida por etanol com diferentes protocolos adesivos.

Avaliação dos Riscos e Benefícios:

Riscos:

Possibilidade de sensibilidade pós-operatória e trauma na gengiva.

Benefícios:

Definição de um possível melhor protocolo de restauração adesiva em comparação com o tratamento controle (padrão)

Estética, saúde e reabilitação estrutural do dente em questão.

Comentários e Considerações sobre a Pesquisa:

A pesquisa tem como objetivo proporcionar uma melhora na técnica já existente de tratamento de

Endereço: Av. Engº Francisco José Longo 777

Bairro: Jardim São Dimas

CEP: 12.245-000

UF: SP

Município: SAO JOSE DOS CAMPOS

Telefone: (12)3947-9078

Fax: (12)3947-9010

E-mail: ceph@fosjc.unesp.br

**UNESP - INSTITUTO DE
CIÊNCIA E TECNOLOGIA -
CAMPUS DE SÃO JOSÉ DOS**



Continuação do Parecer: 2.022.383

lesões cervicais não cáries, aumentando as chances de sucesso e longevidade dessa restauração.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados todos os termos obrigatórios.

Recomendações:

Incluir no trabalho o cálculo amostral (power da amostra).

O pesquisador deverá se atentar ao seu cronograma para envio de relatório parcial e final, sob pena de não ter protocolos analisados pelo CEPH-ICT-CSJC-UNESP. No site do ICT-CSJC-UNESP - Comitê de Ética Envolvendo Seres Humanos, encontra-se formulários e instruções de envio dos relatórios

Conclusões ou Pendências e Lista de Inadequações:

Projeto de pesquisa bem fundamentado e com objetivos bem traçados.

Considerações Finais a critério do CEP:

O Colegiado acata o parecer do relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_874700.pdf	16/03/2017 14:52:38		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	16/03/2017 14:51:24	Mauricio Yugo de Souza	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.pdf	16/03/2017 14:46:38	Mauricio Yugo de Souza	Aceito
Folha de Rosto	folhaderosto.pdf	16/03/2017 14:45:15	Mauricio Yugo de Souza	Aceito

Situação do Parecer:

Aprovado

Necessita apreciação da CONEP:

Não

Endereço: Av. Engº Francisco José Longo 777
Bairro: Jardim São Dimas CEP: 12.245-000
UF: SP Município: SAO JOSE DOS CAMPOS
Telefone: (12)3947-9078 Fax: (12)3947-9010 E-mail: ceph@fosjc.unesp.br