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Carine Weber Pires

RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS AO ESMALTE ADJACENTE A CAVIDADES SUBMETIDAS À REMOÇÃO SELETIVA DE TECIDO CARIADO

Santa Maria, RS
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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Odontopediatria, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Ciências Odontológicas**.

Orientadora: Profa. Dra. Rachel de Oliveira Rocha

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DEDICATÓRIA

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aos meus pais, Dario e Bernadete, às minhas irmãs, Camile e Caroline,

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“Blackbird singing in the dead of night
Take these broken wings and learn to fly
All your life
You were only waiting for this moment to arise”

(John Lennon / Paul McCartney)

RESUMO

RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS AO ESMALTE ADJACENTE A CAVIDADES SUBMETIDAS À REMOÇÃO SELETIVA DE TECIDO CARIADO

AUTORA: Carine Weber Pires
ORIENTADORA: Rachel De Oliveira Rocha

A presente tese é composta por três artigos científicos, cujos temas principais são adesão e Odontologia minimanente invasiva. Artigo 1: A delimitação de área e o tipo de tubo pode impactar na resistência de união de adesivos aos substratos dentários? Esse trabalho avaliou a necessidade da delimitação da área adesiva e o tipo de tubo utilizado para a confecção dos espécimes de resina composta no ensaio de resistência de união ao microcisalhamento (µSBS). 120 incisivos bovinos foram preparados e randomizados em grupos de acordo com a) substrato: esmalte (n=60) e dentina (n=60); b) sistemas adesivos: Clearfil SE Bond –CSE, Single Bond Universal – SBU- nas estratégias com condicionamento ácido e autocondicionante; c) área adesiva: com delimitação e sem delimitação; d) tipo de tubo: amido e polietileno. Após a realização do teste de µSBS os dados obtidos foram submetidos a Análise de Variância de três vias e teste de Tukey (α=0,05). A delimitação da área não influencia os valores de resistência de união, independente do substrato. O tipo de tubo influencia os valores de resistência de união de modo material e substrato-dependente. Assim, como metodologia simplificada do µSBS, é possível o emprego do tubo de amido sem delimitação da área adesiva. Artigo 2: A resistência de união adesiva é semelhante aos dentes decíduos e permanentes? Uma revisão sistemática e metanálise. Esse trabalho elucidou a necessidade da inclusão de dentes decíduos e permanentes em estudos de resistência de união, diante das diferenças microestruturais entre ambos. Seu objetivo foi revisar sistematicamente a literatura de estudos laboratoriais para determinar se os valores de resistência de união obtidos em dentes decíduos e permanentes são semelhantes. A metanálise englobou 37 estudos, e os resultados mostraram que dentes permanentes apresentaram maiores valores de resistência de união do que dentes decíduos. Considerando individualmente cada substrato, a diferença foi significativa apenas para dentina. Logo, os valores de resistência de união obtidos em esmalte de dentes permanentes podem ser considerados na extrapolação para dentes decíduos. Artigo 3: União de três sistemas adesivos ao esmalte em torno de cavidades de cárie naturais após remoção seletiva de tecido cariado. Esse estudo avaliou a influência da condição do esmalte - hígido ou supostamente desmineralizado (adjacente a lesões de cárie cavitadas submetidas a remoção seletiva de tecido cariado) - na resistência de união de sistemas adesivos. 28 molares permanentes (n=7) com lesões de cárie em dentina foram preparados e dois sistemas adesivos – SBU (nas estratégias com condicionamento ácido e autocondicionante), Adper Single Bond Plus e CSE - foram aplicados no esmalte em torno da margem da cavidade e no esmalte hígido, distante da lesão de cárie. O ensaio de µSBS foi realizado e os dados foram submetidos a Análise de Variância de duas vias e teste de Tukey (α=0,05). Os valores de µSBS ao esmalte em torno das margens da cavidade foram menores do que os obtidos ao esmalte distante das margens, independente do sistema adesivo utilizado. Assim, o esmalte com suspeita de perda mineral pode prejudicar a adesão, sendo indicado removê-lo com instrumentos rotatórios em alta rotação antes de procedimentos adesivos.

Palavras-chave: Adesivos Dentinários. Cárie Dentária. Dente Decíduo. Esmalte Dentário.

ABSTRACT

BOND STRENGTH OF ADHESIVE SYSTEMS TO THE ENAMEL AROUND CAVITIES SUBMITTED TO SELECTIVE CARIES REMOVAL

AUTHOR: CARINE WEBER PIRES
ADVISER: RACHEL DE OLIVEIRA ROCHA

This work is composed by three studies, whose main themes are adhesion and minimally invasive Dentistry. Manuscript 1: Can area delimitation and tube type impact microshear bond strength of adhesives to dental substrates? This work evaluated the need of delimitation of the adhesive area and of tube type used in the creation of the composite resin specimens for microshear bond strength test (μSBS). 120 bovine incisors were prepared and randomized in groups according to a) substrate: enamel (n=60) and dentin (n=60); b) adhesive systems: Clearfil SE Bond-CSE, Single Bond Universal-SBU- etch-and-rinse and self-etching; c) adhesive area: with delimitation and without delimitation; d) tube type: starch and polyethylene. After the μSBS assay, data were submitted to three-way ANOVA and Tukey's test (α=0.05). The area delimitation did not influence the bond strength values, regardless of the substrate. The tube type influences the bond strength of material and substrate-dependent mode. Thus, as a simplified methodology of μSBS, it is possible to use starch tube without delimiting the adhesive area. Manuscript 2: Is adhesive bond strength similar to primary and permanent teeth? A systematic review and meta-analysis. This work elucidated the need for inclusion of primary and permanent teeth in studies of bond strength, due to the microstructural differences between both. The aim of this paper was to systematically review the literature for laboratory studies to determine whether the bond strength values obtained to primary teeth are similar to permanent ones. The meta-analysis included 37 studies and the results showed that permanent teeth presenting higher bond strength values than primary teeth. Considering each substrate individually, the difference was significant only for dentin. Therefore, the bond strength values obtained in permanent tooth enamel can be considered in extrapolation for primary teeth. Manuscript 3: Bonding of three adhesive systems to enamel surrounding real-life cavities after selective removal of carious tissue. This study evaluated the influence of enamel condition – sound or supposedly demineralized (adjacent to cavitated caries lesions submitted to selective removal of carious tissue) – in the bond strength of adhesive systems. 28 permanent molars (n=7) with dentin caries lesions were prepared and three adhesives – SBU (etch-and-rinse and self-etching strategies), Adper Single Bond Plus and CSE - were applied to the enamel around the margin of cavity and in the sound enamel, distant to caries lesion. The μSBS assay was performed and the data were submitted to two-way ANOVA and Tukey's test (α=0.05). The values of μSBS to the enamel surrounding cavities were smaller than to the enamel far from the margins, regardless of the adhesive system used. Thus, enamel with suspected of mineral loss may jeopardize the adhesion and it is indicated to remove this enamel with rotating instruments in high rotation before adhesive procedures.

Keywords: Dentin-Bonding Agents. Dental Caries. Tooth, Deciduous. Dental Enamel.

SUMÁRIO

1 INTRODUÇÃO

O esmalte é o tecido mineralizado mais duro do corpo humano. Sua composição é principalmente inorgânica (96%), e o restante do conteúdo consiste em água e componentes orgânicos (GWINNETT, 1992). A adesão a esse substrato foi avaliada primeiramente por Buonocore (1955), o qual demonstrou que utilizar ácido fosfórico na superfície do esmalte resultou na adesão da resina acrílica a esse substrato, fato que deu início à Odontologia adesiva. A partir disso, foi estabelecido que o contato do agente de condicionamento ácido com a superfície do esmalte resulta em uma camada morfologicamente porosa. Assim, a energia livre de superfície aumenta e a resina fluida de baixa viscosidade que contata a superfície é atraída para o interior dessas microporosidades por capilaridade. Nessas microporosidades são formadas projeções de resina (tags), que polimerizadas proporcionam união micromecânica com o tecido (GWINNETT; MATSUI, 1967; BUONOCORE; MATSUI; GWINNETT, 1968; VAN MEERBEEK et al., 2003).

A adesão aos substratos dentários pode ser estabelecida por sistemas adesivos que necessitam de condicionamento ácido prévio e por sistemas adesivos autocondicionantes, que não necessitam da etapa de condicionamento ácido já que possuem monômeros acídicos capazes de desmineralizar esmalte e dentina simultaneamente à infiltração do agente de união. Considerando a facilidade de uso e a menor sensibilidade técnica, os fabricantes têm diminuído o número de passos necessários na utilização desses adesivos. Assim como, clínicos estão cada vez mais utilizando adesivos simplificados (VAN MEERBEEK et al., 2003; CARDOSO et al., 2011).

Em busca de um procedimento clínico mais simplificado foram desenvolvidos sistemas adesivos mais versáteis, chamados universais ou multi-modo (HANABUSA et al., 2012; PERDIGÃO et al., 2012). Esses sistemas podem ser utilizados nas estratégias com condicionamento ácido total ou seletivo em esmalte ou autocondicionante. Recentemente, alguns estudos avaliaram o desempenho destes sistemas adesivos ao esmalte dentário e a maioria deles indica que os valores de resistência de união ao esmalte são superiores quando utilizados na estratégia de condicionamento ácido (DE GÓES et al., 2014; MCLEAN et al., 2015; ANTONIAZZI et al., 2016; CARDENAS et al., 2016; SUZUKI et al., 2016; VERMELHO et al., 2017).

Para avaliar resistência de união adesiva, o teste de resistência de união ao microcisalhamento tem sido amplamente utilizado como alternativa para substrato frágil, como o esmalte, ou materiais que podem ser afetados pela condição do teste ou pelo preparo da amostra (ARMSTRONG et al., 2010). Além disso, esse teste apresenta vantagens como protocolo relativamente simples, possibilidade em utilizar um menor número de dentes e espécimes com pequenas áreas adesivas, que são facilmente padronizadas pelo diâmetro do tubo (MCDONOUGH et al., 2002). Ainda, o teste de resistência de união ao microcisalhamento se mostra mais preciso do que o teste de resistência de união à microtração na distinção entre adesivos de padrão ouro em esmalte (EL ZOHAIRY et al., 2010).

Os sistemas adesivos devem ser eficazes quando utilizados tanto em esmalte como em dentina, apesar de serem substratos muito diferentes quanto à composição e à micromorfologia. Além disso, é de se esperar que os sistemas adesivos também apresentem desempenho similar nos substratos provenientes de dentes decíduos e permanentes, apesar das substanciais diferenças químicas e morfológicas entre eles (HIRAYAMA, 1990). Dentes decíduos são constituídos de esmalte com espessura mais delgada, menor conteúdo mineral, e maior densidade de prismas que o esmalte de dentes permanentes (ANGKER et al., 2004; OLIVEIRA et al., 2010). Essas diferenças ajudam a explicar a progressão mais rápida de lesões de cárie no esmalte decíduo em relação ao esmalte de dentes permanente (PITTS, 1983; ANDO et al., 2001; VANDERAS et al., 2003). Desta forma, parece ser importante que o desempenho de sistemas adesivos seja avaliado considerando o tipo de dente (decíduo e permanente), dado que existem estudos que demonstram valores significativamente maiores em dentes permanentes (HALLETT; GARCIA-GODOY; TROTTER, 1994; SHANTALA; MUNCHI, 1995; ARAS et al., 2013; LENZI et al., 2013), e outros que encontraram resistência de união semelhante (SHIMADA et al., 2002; ILIE et al., 2014) ou até mesmo performance superior dos sistemas adesivos em dentes decíduos (MALFERRARI; FINGER; GARCIA-GODOY, 1994).

Além disso e considerando que a união ao esmalte circundante está associada à longevidade da interface resina-dentina (TORII et al., 2002; TORKABADI et al., 2009; HEINTZE, 2013; TAKAMIZAWA et al., 2015) pode-se sugerir que a presença de desmineralização no esmalte pode comprometer a adesão a este substrato (TEDESCO et al., 2014). Neste contexto, ao realizar a técnica de remoção parcial de tecido cariado (RPTC), que na terminologia atual é denominada remoção seletiva de tecido cariado (RSTC) (INNES et al., 2016), a periferia da cavidade deve ter ausência de tecido cariado de modo a ser circundada por esmalte hígido, e na parede pulpar removemos seletivamente a dentina cariada. Considerando que esta técnica é realizada somente com instrumentos manuais ou rotatórios em baixa rotação (MALTZ et al., 2002; ORHAN; OZ; ORHAN, 2010; LULA et al., 2011; DALPIAN et al., 2014) e que o acesso à lesão é realizado com instrumentos rotatórios em alta rotação somente quando necessário (MALTZ et al., 2013), é de se esperar que o esmalte circundante possa apresentar algum grau de desmineralização. Poucos estudos contemplam o esmalte desmineralizado como substrato em avaliações de resistência de união (TEDESCO et al., 2014; ANTONIAZZI et al., 2016), justificando assim, a realização deste estudo.

Considerando os contextos expostos, no presente trabalho serão apresentados os artigos oriundos de três investigações científicas. O primeiro deles, intitulado "Can area delimitation and tube type impact microshear bond strength of adhesives to dental substrates?" investigou a influência da delimitação da área adesiva e do tipo de tubo utilizado na confecção do espécime de resina composta na resistência de união de sistemas adesivos por meio do ensaio de microcisalhamento, com o intuito de definir a metodologia empregada no estudo subsequente. O segundo artigo, intitulado "Is adhesive bond strength similar to primary and permanent teeth? A systematic review and meta-analysis", visou revisar sistematicamente a literatura de estudos laboratoriais para determinar se a resistência de união de sistemas adesivos a dentes decíduos é semelhante a dentes permanentes, a fim de elucidar a necessidade da inclusão tanto de dentes decíduos quanto permanentes em estudos que mensurem resistência de união. O terceiro estudo, intitulado "Bonding of three adhesive systems to enamel surrounding real-life cavities after selective removal of carious tissue", objetivou avaliar a resistência de união de três sistemas adesivos ao esmalte em torno de cavidades cariosas naturais após remoção seletiva de tecido cariado em dentes permanentes.

2 ARTIGO 1 - CAN AREA DELIMITATION AND TUBE TYPE IMPACT MICROSHEAR BOND STRENGTH OF ADHESIVES TO DENTAL SUBSTRATES?

Este artigo foi submetido ao periódico Journal of Applied Biomaterials & Functional Materials; ISSN: 2280-8000; Fator de impacto = 1.069; Qualis B2. O artigo está de acordo com as normas deste periódico, que estão descritas no ANEXO A.

Can area delimitation and tube type impact microshear bond strength of adhesives to dental substrates?

Parameters on microshear bond strength test

Carine W. Pires[1], Tathiane L. Lenzi[1], Fabio Z. M. Soares[2], Anelise F. Montagner[1], Rachel O. Rocha[3]

[1]Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
[2]Department of Restorative Dentistry, Federal University of Santa Maria, Brazil
[3]Department of Stomatology, Federal University of Santa Maria, Brazil

Corresponding author:

Rachel de Oliveira Rocha
Rua Floriano Peixoto, 1184 sala 211
Santa Maria, RS, Brazil
55 55 3220 9266
rachelrocha@smail.ufsm.br

ABSTRACT

Background

The need of delimiting the bonding area on microshear bond strength (µSBS) test remains an unclear issue. In addition, there are different types of tube to perform the resin composite cylinders on µSBS test. The aim of this study was to evaluate the influence of the adhesive area delimitation and type of tube used to build up specimens for µSBS testing of adhesive systems, to enamel and dentin.

Methods

120 bovine incisors were ground to achieve flat buccal enamel (n=60) and dentin (n=60) surfaces. Specimens of each substrate were randomly allocated into 6 groups (n=10) according to the adhesive system-a two-step self-etch (Clearfil SE Bond) and a universal adhesive (Scotchbond Universal Adhesive) applied in the self-etch and etch-and-rinse modes– and type of tube used to build up the specimens: starch and polyethylene. 4 resin composite cylinders (Filtek Z250) were made on each surface; in 2 of them the area delimitation was performed. The specimens were subjected to µSBS test after 24 h of water storage at 37°C. Data were submitted to three-way ANOVA and Tukey's *post hoc* tests(α=0.05).

Results

The delimitation of the adhesive area did not influence on the bond strength to dentin(p=0.349) nor to enamel(p=0.158). The type of tube differently affected the ranking of adhesives in both substrates.

Conclusions

The delimitation of the adhesive area does not influence on the µSBS to enamel and dentin, irrespective of the tube type used to build up the resin composite specimens. The tube type's impact on the bond strength depends on the dental substrate and adhesive system.

Keywords: dentin-bonding agents, adhesive systems, dentin, enamel, microshear bond strength.

Introduction

In vitro studies are important tools to predict the clinical performance of dental materials (1). Therefore, bond strength tests are useful and effective methods to evaluate the performance of adhesive systems (2). Microshear bond strength (µSBS) test has been widely used as an alternative for evaluating brittle substrates or materials that could be affected by test condition or specimen preparation (3). Moreover, µSBS test seems to be more accurate than microtensile bond strength test in distinguishing among gold standard adhesives on enamel (4). Further, this test presents advantages as ease of specimen preparation, simple test protocol, and small bonding areas, which are easily standardized by the diameter of the tube. Similarly, to microtensile test, µSBS test allows the evaluation of bond strength in different regions of the substrate, eliminating the interference of variability among teeth.

Despite of all the advantages, the need of delimiting the bonding area in µSBS test remains an unclear issue (5). When the adhesive is applied on the whole surface, it is difficult to ensure that testing area is restricted to the circumference of resin cylinder. Thus, irregular portions around the bonded area could be pulled out during the test (5). Previous studies suggested that the technique of adhesive area delimitation might lead to more reliable and reproducible results, since an overestimation of the actual bonding performance of adhesives was verified when no area delimitation technique was employed (5).

Additionally, other shortcoming inherent to µSBS test is the polyethylene tube attachment to the dental substrate and the difficulty in packing and adapting the composite into the tube (6,7). Since polyethylene tube is usually placed on substrate before adhesive polymerization (8), there may be an accumulation of adhesive around the polyethylene tube perimeter. Thus, the use of an adhesive tape to delimitate the adhesive area could avoid an accumulation of adhesive around and inside the tube (5).

Recently, the use of starch tube was proposed as an alternative method to build up microshear bond test specimens (9), aiming to decrease the aforementioned difficulties during preparation of specimens. In this sense, the loss of specimens is frequent due premature failures or exclusion because the presence of defects (air bubbles). Moreover, the sectioning of the tube with a knife blade for its removal is critical, due the possibility of stress induction on the specimen and even on the adhesive interface. It was demonstrated that tube type (starch or polyethylene) did not influence on the bond strength values to enamel (9).

Previous studies regarding the effect of the area delimitation (5) and of the tube type (9) investigated these variables on dentin or enamel, respectively. To the best of our knowledge, this is the first study that assessed if the adhesive area delimitation impacts on bond strength values when the starch tube is employed. Since differences in the composition and micromorphology between both substrates could impact on the findings, the aim of this *in vitro* study was to evaluate the influence of the adhesive area delimitation and type of tube to build up the composite resin specimens for microshear bond testing on bond strength of adhesive systems to enamel and dentin substrates. The research question was as follows: Can area delimitation and tube type impact microshear bond strength of adhesives to dental substrates?

Methods

Tooth Selection and Preparation

One hundred and twenty freshly extracted bovine incisors were used in this study after storage in chloramine at 4°C for a maximum of thirty days. The root portion was removed using a low-speed diamond disc in a cutting machine (Labcut 1010, Extec Co., Enfield, USA) and crowns were embedded in acrylic resin inside polyvinyl chloride rings (JET Clássico®, São Paulo, SP, Brazil). The buccal surfaces were grinded under water cooling using a 320-grit

SiC paper to obtain flat enamel (n=60) or dentin (n=60) surfaces and further polished using 600-grit SiC paper for 60 s to create a standardized smear layer.

Restorative procedures

The teeth of each dental substrate (enamel and dentin) were randomly assigned to three groups (n=20) according to the adhesive system: a two-step self-etch adhesive system (Clearfil SE Bond; Kuraray Noritake Dental, Tokyo, Japan) and a universal adhesive (Scotchbond Universal Adhesive; 3M ESPE, St Paul, MN, USA), applied in etch-and-rinse and self-etch strategies. Afterwards, the teeth were randomly assigned to two groups (n=10) according to the type of tube used to build up the specimens: starch tube (tube of pasta) (9) and polyethylene tube (Micro-bore TYGON S-54-HL Medical Tubing, Saint-Gobain Performance Plastics; Akron, OH, USA). Starch and polyethylene tubes have an inner diameter of 1.16 mm and 0.76 mm, respectively, and both are 1 mm height. Table 1 displays the main components and the application mode of the adhesive systems. Figure 1 illustrates a schematic diagram of the study.

Before the bonding procedures, an acid/solvent-resistant double-faced adhesive tape (Tectape, Delfitas; Manaus, AM, Brazil) that contained two aligned perforations prepared with an Ainsworth perforator (Wilcos; Petropolis, RJ, Brazil) was used. Two perforations were done with the first perforator's orifice, suitable for mandibular incisors, which was used to delimit the area for the polyethylene tube. Other two perforations were done using the third perforator's orifice, indicated for canines and premolars, were used for the starch tube. The tape was positioned on the distal half of the middle third of buccal surfaces, keeping the other half proximal uncovered (control group with no area delimitation) (5).

First, the adhesive procedure, including the acid etching procedure when necessary, was carried out on the distal half of the specimens, over the double-faced tape, so that the action of the adhesive was limited to the substrate exposed by the two perforations. After application of adhesive systems following the respective manufacturers' instructions, the upper protective plastic film was removed from the surface of the tape, and two polyethylene or starch tubes were positioned over the uncovered tape. Then, the adhesives were light-cured using a light emitting diode curing unit (Emitter B, Schuster, Santa Maria, RS, Brazil) with a spectrum range of 450-490 nm.. The device built-in radiometer was used to check the light output power. The tubes were carefully filled with resin composite (Filtek Z250, 3M ESPE, St Paul, MN, USA; shade A2) and light cured for 20 s. A second bonding procedure was performed on the other side of the sample, as previously described, but without adhesive delimitation approach. Care was taken during second adhesive procedure aiming to avoid contamination or additional light curing of the two first specimens. Thus, four cylindrical restorations specimens were built up on each buccal surface, two with area delimitation, and two without area delimitation. To avoid inter-operator variables, one trained operator carried out all bonding and restorative procedures at room temperature.

Microshear bond strength test

After storage in distilled water at 37°C for 24 h, the tubes were removed. The starch tubes were removed under a gentle water jet, since the tubes were almost totally dissolved after water storage. The polyethylene tubes were removed by cutting each tube into two hemi-cylinders using a knife blade.

The remaining double-faced adhesive tape was also removed. Specimens were examined under a stereomicroscope at 20× magnification (Discovery V20, Zeiss, Berlin, Germany), and those with interfacial gaps, bubble inclusion, or other defects were excluded and replaced.

To guarantee the blinding of the testing machine operator, the specimen was numbered according the sequence of the randomization. The specimens were individually attached to a universal testing machine (Emic, São José dos Pinhais, PR, Brazil). A thin steel wire (0.20 mm diameter) was looped flush between the load cell projection and resin composite cylinder, in contact with the lower half-circumference of the cylinder and touching the substrate surface. The cylinder was kept aligned with the center of the load cell, and the wire loop was parallel both to the load cell movement direction and to the bonding interface. This way, a shear load was applied at a crosshead speed of 1.0 mm/min, until failure occurred. The fracture load was recorded and the bond strength was expressed in MPa.

Failure mode

All debonded specimens were observed under stereomicroscope at 40× magnification to determine failure mode: adhesive/mixed (failure at the resin-enamel/resin–dentin interface or mixed with cohesive failure of the surrounding substrate) or cohesive (failure exclusively within the enamel/dentin or resin composite). The specimens that debonded during preparation (pre-testing failures) were also recorded.

Representative specimens (n = 5) for each experimental group were prepared for failure mode evaluation under scanning electron microscope (SEM). Specimens were dehydrated in ascending degrees of ethanol (50, 75 and 95% for 5 min each, and 100% for 3 h) and kept in vacuum for 24 h. Subsequently, they were gold sputtered and analyzed with SEM (Quanta 600 FEG, FEI Co., Hilsboro, OR, USA) operated in the secondary electron mode with 10 kV voltage.

Statistical analysis

The experimental unit in the study was the tooth. Thus, the means of µSBS values of the two specimens from the same tooth were averaged for statistical purposes.

Normal distribution of bond strength data and equality of variances were assumed after Kolmogorov-Smirnov and Cochran tests, respectively. The µSBS means were submitted to three-way ANOVA (tube type *vs*. adhesive system *vs*. area delimitation) and Tukey's *post hoc* test for each dental substrate separately. The Chi-square ($\chi 2$) test was used to compare failure mode (in percentages) among the experimental groups and pre-testing failures, considering the specimen as experimental unit for this analysis. The significance level was set at $p < 0.05$. All statistical analyses were performed using the Minitab software (Minitab Inc., State College, PA, USA).

Results

The microshear bond strength means (MPa) and standard deviations for dentin and enamel substrates are presented in Tables 2 and 3, respectively.

Considering the dentin substrate, only the main factor "adhesive system" ($p = 0.037$) and the cross-product interaction "adhesive system *vs*. tube type" ($p = 0.003$) were statistically significant. For enamel, the main factors "adhesive system" ($p = 0.000$), "tube type" ($p = 0.000$), as well as the cross-product interaction "adhesive system *vs*. tube type" ($p = 0.008$) were significant. The delimitation of the adhesive area did not influence on the bond strength to dentin ($p = 0.349$) nor to enamel ($p = 0.158$) [16.3 ± 5.0 MPa and 15.4 ± 5.3 MPa; 17.5 ± 5.5 MPa and 18.8 ± 6.2 MPa for without and with delimitation groups to dentin and enamel, respectively].

The µSBS values to dentin was not influenced by the type of tube considering each adhesive system separately. However, using the polyethylene tube, the universal adhesive used in etch-and-rinse mode presented significantly lower values than those obtained for other groups.

Conversely, for enamel, using the polyethylene tube no significant differences were found to μSBS values among the materials. Significant differences were found only with the starch tube. The universal adhesive applied as etch-and-rinse mode showed the highest μSBS values. Moreover, the μSBS values of the universal adhesive following self-etch approach to enamel were lower when using starch tube in comparison with polyethylene ones. This difference was not observed for the other materials (groups).

The distribution of the failure mode is summarized in Table 4. The χ^2 test indicated no statistically significant difference in the pre-testing failures and failure mode among the experimental groups. There was a predominance of adhesive/mixed failures in all groups.

Representative SEM images of specimens bonded to dentin and enamel substrates are presented in Figure 2. In general, the failures tended to occur within the limits of the composite cylinder, irrespective of tube type and area delimitation. In the groups without area delimitation, the presence of adhesive layer around the composite cylinder only occurred with polyethylene tube use. Mixed with cohesive failure of the neighbouring dentin substrate can be observed in groups with area delimitation. For enamel, mixed with cohesive failure in resin can be observed in all groups.

Discussion

Microshear test has been widely used in adhesion studies and is considered easy to execute (7). This test allows to obtain multiple specimens per tooth but without sectioning or trimming, which can induce early micro-cracking within the specimen as observed in microtensile test, being thus, useful to enamel, susceptible substrate to specimen preparation effects (3). However, there are methodological variations that make it difficult either to compare the results among the studies or to define a standard experimental design of easy, quick and reproducible execution (8). Since test methods could impact on the bond strength results (7), it has been required the assessing of test parameters on the µSBS experimental design. Therefore, this study assessed the impact of the adhesive area delimitation and the type of tube on the performance of different adhesive systems to enamel and dentin.

The adhesive area delimitation was not a significant factor for bond strength values in µSBS test to both enamel and dentin substrates. Even without the restriction of the adhesive area, only adhesive/mixed failures were observed. In a previous study on the influence of adhesive area delimitation in µSBS test to dentin substrate, overestimation of the µSBS values was found without area delimitation and cohesive failures were usual (5). According to the authors, the findings were probably due the extension of adhesive area beyond of the limits of the resin cylinder (5). However, it appears that even with the use of perforated tape for adhesive area delimitation, small areas of adhesive could still be seen around the cylinder (10). In this sense, area delimitation as an additional step on µSBS test may be questioned as it is time-and laboratorial-consuming and does not offer additional advantages to the microshear test method. Previous studies also did not include the adhesive area delimitation in microshear bond strength test both in enamel (11-13) and dentin (12,14,15).

Since the µSBS test was introduced, the polyethylene tube has been recommended to build up resin composite specimens (16), and several studies have used this methodology. Nonetheless, the use of polyethylene tube has been related to technical shortcomings, such as air bubble formation, peripheral marginal gaps and mostly to stress transferring to adhesive interface and pre-test failures during the tube removal, prior to mechanical test. Moreover, it is difficult to attach this tube to dental substrate and subsequently, to insert the resin composite into it (7).

Therefore, an easier handling starch tube was recently proposed, with no negative influence on the bond strength values to enamel (9). After 24 h of water storage, the starch tubes soften and loosen from resin composite specimens, without the use of scalpel blade and without any stress to the interface during its removal. In this context, the pre-testing failures when using starch tube might not be related to the procedure of tube removal itself, but to the poor adhesive performance, even in the immediate evaluation. In our study, no difference in the percentage of pre-testing failures was verified between tubes, probably because a single experienced operator prepared all specimens, regardless the type of tube.

Considering the µSBS to dentin, similar values were found when both polyethylene and starch tubes were compared, considering each material separately. However, considering the use of polyethylene tube, the µSBS values to dentin obtained for universal adhesive following etch-and-rinse mode were lower than those obtained for the same system in self-etch strategy and also for Clearfil SE Bond. In a recent systematic review (17) and in some other studies (18, 19) no significant differences were found between etch-and-rinse and self-etch strategies for Scotchbond Universal Adhesive applied in dentin using microtensile test. This way, the polyethylene tube could have been more inaccurate and more sensitive than starch tube to evaluate different bonding strategies in dentin.

For enamel, the use of the polyethylene tube was not able to inform significant differences between etch-and-rinse and self-etch strategies using Scotchbond Universal Adhesive, in contrast of the literature that etch-and-rinse adhesives perform better in enamel (17-19). In this sense, it has been indicated the enamel selective acid etching prior self-etch adhesive systems application to improve the bonding effectiveness (20-23), extending the recommendation for new multi-mode adhesives (17,24,25). Scotchbond Universal Adhesive and Clearfil SE Bond contain MPD (10-methacryloxydecyl dihydrogen phosphate) as acid monomer. Nevertheless, the universal adhesive presents a relatively low acidity (pH=2.7) and, consequently, reduced ability to demineralize the enamel in comparison with two-step self-etch system (pH=2.0). It might explain the higher bond strength values of Clearfil SE Bond to enamel in comparison with universal adhesive applied in the self-etch mode.

Since that in our study, the tube type influenced only on the results of the Scotchbond Universal Adhesive, but differently on each dental substrate, it can be stated that effect of tube type is material and substrate dependent. It is hypothesized that polyethylene tubing could modify the air-inhibition layer and the thickness of the adhesive layer, which may influence on the bond strength (7), while the starch tube may lead to less questionable outcomes, reducing misinterpretation. In addition, in a previous study (26), difficulties to build up composite resin specimens after the use of one-step adhesive system were reported. Therefore, is plausible associate our results to this hypothesis. Starch tube seems to be able to absorb adhesive system beyond the bonding area preventing the thickness of the adhesive layer by air-inhibition, considering that the adhesive is confined inside the tube.

Based on the results, the use of starch tube can be advantageous to build up resin composite specimens for microshear bond tests, since it is easy handling. Moreover, the starch tube seems be able to self-delimit the adhesive area, absorbing adhesive system and consequently, avoiding its presence beyond the inner diameter of the tube, as observed in SEM images even in specimens performed without adhesive area delimitation technique. Thus, it is preferable to use starch tube without adhesive area delimitation technique when performing microshear bond strength evaluations to dental substrates.

Conclusions

This study showed that the delimitation of the adhesive area does not influence on microshear bond strength to enamel and dentin, irrespective of the tube type used to build up the resin composite specimens. The tube type's impact on the bond strength depends on the dental substrate and adhesive system.

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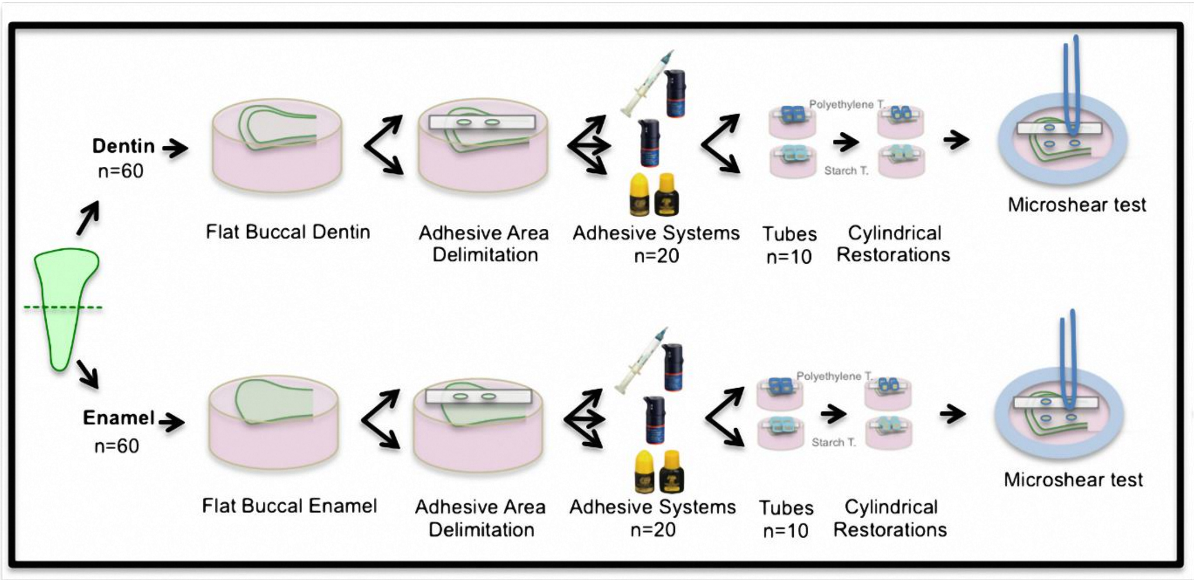

Figure 1 – Experimental design.

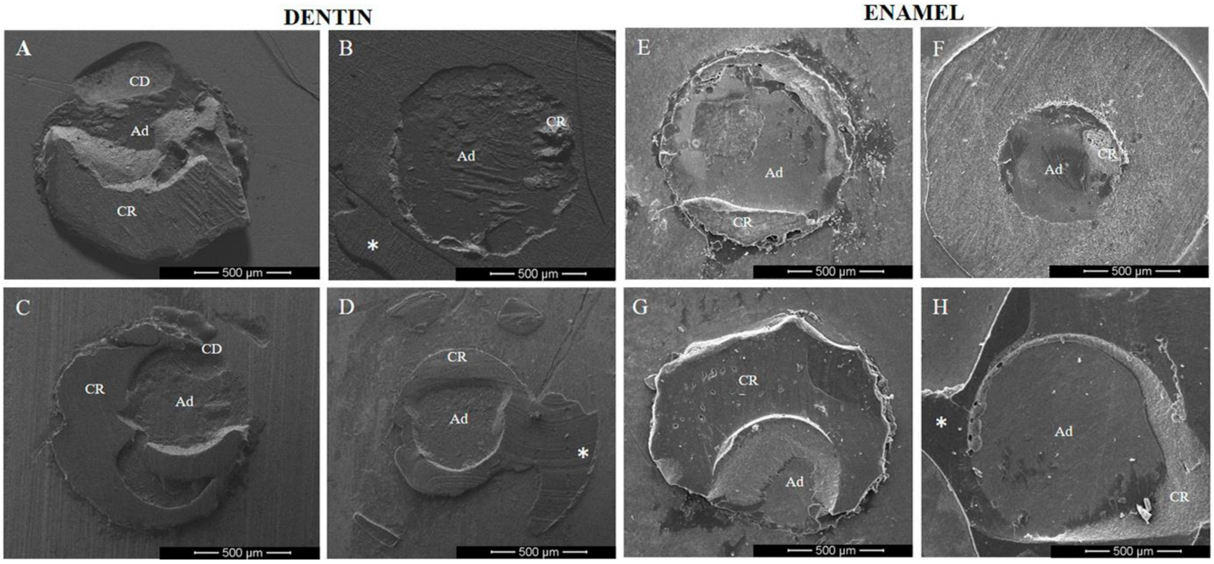

Figure 2 – Representative scanning electron microscope (SEM) images of specimens bonded to dentin with Scotchbond Universal Adhesive as etch-and-rinse mode with (A) and without (B) adhesive area delimitation when starch tube was used. SEM images of specimens bonded to dentin with Clearfil SE Bond with (C) and without (D) adhesive area delimitation when using tygon tube. Mixed with cohesive failure of the neighboring substrate can be observed in groups with area delimitation, although the adhesive layer was limited to resin circumference. SEM images of specimens bonded to enamel with Clearfil SE Bond with (E) and without (F) adhesive area delimitation when starch tube was used. SEM images of specimens bonded to enamel with Scotchbond Universal Adhesive as self-etch mode with (G) and withouth area delimitation when using polyethylene tube (H). Mixed with cohesive failure in resin can be observed in all groups. Note (*) the presence of adhesive around the polyethylene tube limits without area delimitation (D and H), while with the use of starch tube this was not observed (B and F). Ad: adhesive; CR: cohesive in resin; CD: cohesive in dentin.

Table 1. Composition and application mode of the adhesive systems tested.

Adhesive system	Main components	pH*	Self-etch strategy	Etch-and-rinse strategy
Scotchbond Universal Adhesive (3M ESPE, St. Paul, MN, USA)	Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	0.1 2.7	1. Keep dentin dry, do not overdry 2. Apply the adhesive for 20 s with vigorous agitation 6. Gentle air thin for 5 s 7. Light-cure for 10 s	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Air dry to remove excess of water 4. Keep dentin moist 5. Apply the adhesive as for the self-etch mode
Clearfil SE Bond (Kuraray Noritake Dental Inc., Tokyo, Japan)	*Primer:* MDP, HEMA, hydrophilic dimethacrylate, dl-campherquinone ,N,N-diethanol-*p*-toluidine, water *Bonding:* MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-campherquinone, N,N-diethanol-*p*-toluidine, silanated colloidal silica	2.0	1. Apply primer on dry dentin surface and left undisturbed for 20 s 2. Dry with air stream for 5 s to evaporate the volatile ingredients 3. Apply bond and gently air dry 4. Light-cure for 10 s	N.A

MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate; Bis-GMA: bisphenyl-glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate

*pH values were informed by manufacturers.

Table 2. The µSBS (MPa) means and standard deviations (±SD) to dentin substrate considering the cross-interaction product "adhesive system *vs*. tube type" (*).

Adhesive system	Starch Tube	Polyethylene Tube
Scotchbond Universal Adhesive Etch-and-rinse mode	16.3 ± 4.7^{A,a}	12.8 ± 3.5^{A,b}
Scotchbond Universal Adhesive Self-etch mode	13.9 ± 5.3^{A,a}	18.6 ± 3.4^{A,a}
Clearfil SE Bond	16.2 ± 5.2^{A,a}	17.4 ± 5.9^{A,a}

(*)Different superscript lowercase letters indicate statistically significant differences between rows ($p<0.05$). Equal superscript uppercase letters indicate no significant differences between the columns ($p>0.05$).

Table 3. The µSBS (MPa) means and standard deviations (±SD) to enamel substrate considering the cross-interaction product "adhesive system *vs*. tube type" (*)

Adhesive system	Starch Tube	Polyethylene Tube
Scotchbond Universal Adhesive Etch-and-rinse mode	22.1 ± 6.8^{A,a}	22.2 ± 3.6^{A,a}
Scotchbond Universal Adhesive Self-etch mode	11.4 ± 3.4^{B,c}	18.1 ± 3.9^{A,a}
Clearfil SE Bond	15.9 ± 5.0^{A,b}	19.3 ± 3.7^{A,a}

(*) Different superscript lowercase letters indicate statistically significant differences between rows. Different superscript uppercase letters indicate differences between the columns ($p < 0.05$).

Table 4. Distribution and percentage (%) of failure modes for the experimental groups (*)

Tube Area delimitation	Scotchbond Universal Adhesive Etch-and-rinse mode				Clearfil SE Bond				Scotchbond Universal Adhesive Self-etch mode				p value
	Starch		Polyethylene		Starch		Polyethylene		Starch		Polyethylene		
	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	
Dentin substrate													
Adhesive/mixed	20(100.0)	19 (95.0)	19(95.0)	18(90.0)	20(100.0)	20(100.0)	19(95.0)	16(80.0)	18(90.0)	19(95.0)	15(75.0)	15(75.0)	0.082
Pre-testing failures	0 (0.0)	1 (5.0)	1 (5.0)	2 (10.0)	0 (0.0)	0 (0.0)	1 (5.0)	4 (20.0)	2 (10.0)	1 (5.0)	5 (25.0)	5 (25.0)	
Enamel substrate													
Adhesive/mixed	19 (95.0)	20(100.0)	20(100.0)	17(85.0)	17 (85.0)	18 (90.0)	19(95.0)	18(90.0)	16(80.0)	17(85.0)	18(90.0)	15(75.0)	0.284
Pre-testing failures	1 (5.0)	0 (0.0)	0 (0.0)	3 (15.0)	3 (15.0)	2 (10.0)	1 (5.0)	2 (10.0)	4 (20.0)	3 (15.0)	2 (10.0)	5 (25.0)	

3 ARTIGO 2 - IS ADHESIVE BOND STRENGTH SIMILAR TO PRIMARY AND PERMANENT TEETH? A SYSTEMATIC REVIEW AND META-ANALYSIS.

Este artigo foi submetido ao periódico Journal of Adhesive Dentistry; ISSN: 1757-9988; Fator de impacto = 1.311; Qualis A2. O artigo está de acordo com as normas deste periódico, que estão descritas no ANEXO B.

Is adhesive bond strength similar to primary and permanent teeth? A systematic review and meta-analysis

Carine Weber Pires
Graduate
Post Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Execution and article writing.

Eloisa Barbieri Soldera
Undergraduate
School of Dentistry, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Execution of the methodology and proofread the manuscript.

Laura Izabel Lampert Bonzanini
Undergraduate
School of Dentistry, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Execution of the methodology and proofread the manuscript.

Tathiane Larissa Lenzi
Professor
Post Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Consulted on idea, hypothesis, proofread the manuscript.

Fabio Zovico Maxnuck Soares
Professor
Department of Restorative Dentistry, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Contribution substantially to discussion, proofread the manuscript.

Anelise Fernandes Montagner
Post Doctoral Student
Post Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Consulted on idea, hypothesis, proofread the manuscript.

Rachel de Oliveira Rocha
Associate Professor
Department of Stomatology, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Idea, contribution substantially to discussion, proofread the manuscript.

Corresponding author:
Rachel de Oliveira Rocha - rachelrocha@smail.ufsm.br
Rua Floriano Peixoto, 1184 sala 211, Santa Maria, RS, Brazil; 55 55 3220 9266

ABSTRACT

Purpose: This study aimed to systematically review the literature for laboratory studies to determine whether the bond strength of adhesive systems to primary teeth is similar to permanent ones.

Materials and Methods: This systematic review was conducted according to the PRISMA statement and registered in PROSPERO (CRD42014015160). A comprehensive literature search was conducted considering *in vitro* studies published up to June 2015 on PubMed/MEDLINE database, with no published year limit. Two reviewers independently selected trials, extracted data and assessed the risk of bias. From 422 elegible studies, 42 were full analyzed. Lastly, 37 were included in the systematic review and meta-analysis. A global comparison was performed with random-effects model at a significance level of $p<0.05$, and it was expressed by the difference of means between the groups. The mean bond strength values and respective standard deviations were tabulated and statistical analyses were conducted in RevMan 5.1 (The Cochrane Collaboration, Copenhagen, Denmark).

Results: There was significant difference between groups, with permanent teeth presenting higher bond strength than primary teeth ($p=0.0005$). When the enamel and dentin substrates were considered separately, the dentin presented the same trend ($p=0.002$), while for enamel there was no significant difference between the dentitions ($p=0.11$). The majority of the studies presented high bias risk.

Conclusion: The adhesives systems have higher bond strength values when applied in permanent teeth than in primary ones. This difference is valid when comparison is made in dentin (permanent vs. primary).

Keywords: permanent dentition, deciduous tooth, enamel, dentin, bond strength, systematic review.

INTRODUCTION

The adhesive systems have often been used in primary dentition based on acquired knowledge in permanent ones, since there are no protocols firmly established by manufactures for using it in primary teeth yet. Nevertheless, there are substantial chemical and morphological differences between primary and permanent teeth[25] that may impact on the adhesive performance. The dental substrates of primary teeth present lower thickness and minor mineral content in comparison with permanent ones.[1] Micro structurally, the aprismatic layer is more pronounced in primary enamel.[32] Additionally, tubule density is higher in primary dentin, and consequently, intertubular dentin area available for bonding is reduced.[33,65]

It has been expected that adhesion to primary enamel is not as reliable as to permanent teeth and that the mineral content of enamel plays an important role on mechanism of adhesion.[2] However, although studies have shown superior adhesive bond strength to permanent enamel,[34,50] some reports did not find differences on bond strength to sound enamel of primary and permanent teeth.[10,62,67]

Several studies also compared the bond strength to primary and permanent dentin using different bonding systems. The results varied among them, since some reports showed significantly lower bond strength values to primary dentin,[4,55,69] while others have found similar values[10,14,64] or even superior performance in primary dentin.[28] Thus, the comparison between the primary and permanent teeth is essential in investigations that evaluate the adhesive performance, because there is still uncertainty whether the adhesive systems have similar behavior in both dentitions with respect to immediate and longitudinal bond strength to enamel and dentin substrates.

Therefore, the aim of this study was to systematically review the literature for laboratory studies that compared the bond strength of adhesive systems on primary and permanent teeth investigating the follow research question: Is adhesive bond strength similar to primary and permanent teeth?

MATERIALS AND METHODS

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[42] and recorded in International Prospective Register of Systematic Review (PROSPERO- CRD42014015160).

Data Sources

A comprehensive literature search was undertaken through the PubMed/MEDLINE database to identify literature up to 30 June 2015, which compared the immediate or longitudinal bond strength of adhesive systems to primary and permanent dentitions. The search strategies were conducted by computer search of database, review of reference lists of all articles included and contact with authors and experts on this subject.

The subject search used a combination of controlled vocabulary and free text terms based on the following search strategy: ((((((((dentin bonding agents[MeSH Terms]) OR dentin bonding agents) OR dental bonding[MeSH Terms]) OR dental bonding) OR adhesive system*) OR bond*)) AND (((((((dentition, permanent[MeSH Terms]) OR dentition, permanent) OR permanent dentition) OR secondary dentition) OR adult dentition) OR permanent tooth) OR permanent teeth)) AND ((((((((((((tooth, deciduous[MeSH Terms]) OR tooth, deciduous) OR deciduous tooth) OR deciduous dentition*) OR primary dentition*) OR milk tooth) OR milk teeth) OR deciduous teeth) OR primary tooth) OR primary teeth) OR baby tooth) OR baby teeth).

The inclusion criteria were *in vitro* studies that evaluated bond strength to human dentin and/or enamel. Those that assessed bond strength of composite resins or another material (such as compomers, sealants) to coronary enamel and dentin, except brackets, were included. The comparison of primary and permanent teeth was also considered an inclusion criterion. Only studies in English were included.

The exclusion criteria were studies that did not present the bond strength outcome, and at least the immediate bond strength data and respective standard deviation and did not describe the use of any adhesive system. Papers that did not provide mean bond strength, in megapascals (MPa) and respective standard deviation, such data were excluded, even after e-mail request to authors (at least twice). When the same bond strength data were reported in different papers, only one investigation was considered. Moreover, when the methodology performed for primary and permanent teeth was different, the study was excluded.

Search Steps: Screening and Selection

Step 1: Titles and abstracts were reviewed independently by two authors (C.W.P and R.O.R) and selected for further review if they met the inclusion criteria. The inter-examiner agreement was calculated (Kappa = 0.88), indicating excellent agreement.

Step 2: Full-text articles of the studies selected in previous step were retrieved and reviewed independently by two authors (C.W.P and R.O.R). Those studies that did not show any exclusion criteria were maintained. The reference lists of selected articles to this step were evaluated, and the full texts of potentially interesting studies to the research question were evaluated.

In both steps, any disagreement was firstly solved by discussion between the reviewers (C.W.P and R.O.R). If discrepancies remained, a third author (F.Z.M.S.) was consulted.

Data Extraction

A protocol for data extraction was defined and evaluated by two authors (C.W.P. and R.O.R.) Data were independently extracted from full-text of included articles using a standardized form in a program (Office Excel 2013 Software, Microsoft Corporation, Redmond, WA, USA). From each study, publication details (title, authors and year), bonding procedures characteristics (category of adhesives, dental filling material), study methodology (sample size, type of test, storage time, type of tooth (e.g. molars, premolars, anterior teeth), substrate (enamel and/or dentin), type of surface, (cavity or flat) and condition of substrate (sound or carious), and outcome information (mean bond strength (MPa) and standard deviation) were extracted.

Assessment of Risk of Bias

Risk of bias was based on and adapted from a previous study.[57] The studies' quality was assessed according to the description of the following parameters: randomization of teeth, adhesive systems used according to the manufacturer's instructions, adhesive procedures performed by the same operator, description of sample size calculation, and blinding of the operator of the testing machine. If the authors reported the parameter, the paper had a "Yes" on that specific parameter; if it was not possible to find the information, the paper received a "No". Papers that reported 1 or 2 items were classified as having a high risk of bias, 3 or 4 as medium risk, and 5 as low risk. Disagreements between the reviewers regarding the classification of risk of bias were resolved by consensus.

Statistical Analysis

Most studies provided many bond strength values, since analyzed more than one adhesive system. Thus, it was necessary to combine the groups into a single data. The "formulae for combining groups" was used to combine two or more groups into a single group considering a single sample mean and standard deviation for each study.[24] Additionally, only the immediate bond strength values were regarded in the meta-analysis when the studies evaluated the sample after aging beyond the immediate evaluation.[35,47,50] The bond strength means and standard deviations from the included studies were pooled and analyzed in the meta-analysis that was carried out via random effects. Therefore, the means of the bond strength values of the studies were compared for obtaining the pooled effect estimates expressed as the mean difference among the groups. A p value ≤ 0.05 was considered statistically significant (Z test).

A global analysis was carried out from subgroup analyses. These analyses were based on comparison of bond strength values in dentin and enamel, independently. Permanent teeth were considered the control group and primary teeth were the experimental group. All analyses were performed using Review Manager Software 5.1 (The Cochrane Collaboration, Copenhagen, Denmark). Statistical heterogeneity of the treatment effect among studies was assessed using the Cochrane Q test and the inconsistency I^2 test. The values greater than 50% were considered as high heterogeneity.[24]

RESULTS

Study selection

The search strategy identified 422 potentially relevant records. After screening titles and abstracts, we retrieved 42 full-text papers for more detailed information and 7 of these papers were excluded. Other two studies were identified in reference lists of related reviews. Finally, 37 papers met the eligibility criteria and were included in the review. Flow diagram summarizes the process of studies selection and the reasons for exclusions (Figure 1). The characteristics of included studies are presented in Table 1.

Quality and risk of bias of the studies

Of the 37 studies included, the most studies presented high bias risk. Only one study showed medium risk. The results are showed in Table 2, according to the parameters considered in the analysis.

Meta-analysis

The meta-analysis is presented in Figure 2. For the dentin, 28 studies were included, while for enamel 12 studies were entered. Three studies that evaluated both substrates were regarded separately for each one.[10,27,30]

According to the global analysis, there was significant difference between groups (experimental vs. control), with permanent teeth presenting significantly higher bond strength values than primary teeth ($p = 0.0005$). When the enamel and dentin substrates were considered separately, the permanent dentin showed higher bond strength values than the primary dentin ($p = 0.002$), while for enamel there was no statistically significant difference between dentitions ($p = 0.11$). Substantial heterogeneity was observed to dentin ($I^2 = 95\%$), enamel ($I^2 = 98\%$) and global ($I^2 = 97\%$) analysis.

DISCUSSION

In this systematic review, besides to answer the question "Is adhesive bond strength similar to primary and permanent teeth?" it was analyzed singly the substrates, enamel and dentin. It is important to evaluate these substrates because there are differences in their composition and micromorphology that may influence on the performance adhesive. The enamel is the harder mineralized tissues of the body, presenting 96% of inorganic content, 4% of water and organic content.[20] The dentin is a more complex and moist substrate, with 70% of inorganic material, 12% of water and 18% of organic material.[41] Many laboratory studies compared the bond strength of both dentitions and shown different results. Most of them evaluated adhesion in dentin, probably because it is more critical due its significant amount of water, organic nature, tubular structure and presence of collagen network.[39,48,49] However, there was no consensus on this subject so far.

The meta-analysis demonstrated that bond strength of adhesive systems to primary and permanent teeth was not similar. Bond strength values were higher to permanent teeth. This finding may be justified by the differences previously described. Specific adhesive protocols must be determined to provide more effective treatments to both dentitions,[46] mainly for primary teeth. The researchers and the manufacturers must consider this, taking account that the same protocol for adhesive systems application to permanent teeth has also been suggested to primary teeth.

The present findings demonstrated that, there are significant differences between permanent and primary teeth for bond strength to dentin. This result was expected, since the differentiation between permanent and primary dentin may explained by some reasons. In comparison with permanent dentin, the tubule density is higher in primary dentin and intertubular dentin area is lower and it might jeopardize the bonding performance since the area available for bonding is reduced.[33] The lower bond strength to primary teeth compared to permanent ones could be influenced by the decreasing in concentration of calcium and phosphorous in peritubular and intertubular dentin of these teeth[9], as well as by the presence and higher prevalence of microcanals or giant dentin tubules which render this substrate wetter due the smaller area of solid dentin[65]. Moreover, the lower mineral content of primary teeth may be associated to higher reactivity to action of acidic conditioners, resulting in thicker hybrid layers[44,45] and to lower bond strength values, such as was found in the present review.[7,9,12,31,35,47,55,59,68,69,71,72]

However, for enamel, there was no difference between bond strength on permanent and primary teeth. Despite the primary teeth present lower mineral content than permanent teeth, it did not seem to significantly influence the mechanism of adhesion. The similar bond strength may be explained by the specimens' preparation performed to assess the bond strength to enamel. According to literature, the primary and permanent enamel are different. There is a thicker and uniform aprismatic layer in primary enamel than to permanent enamel.[16] This prismless layer has uniform crystallite structure that results in fewer tag formation, jeopardizing the flow and adhesion of the resin monomers into the tags.[63] In laboratory studies, as the included studies in this review, enamel surface is usually worn to obtain flat surface, which would be responsible for removing the aprismatic enamel layer and consequently, facilitate the exposure of enamel rods by the action of conditioning agent. Some authors recommended grinding this enamel surface before etching.[17,40] However, in some cases (aesthetic and functional repair of teeth, conservative restorations, sealant applications or bracket bonding) prismless enamel may be submitted to the adhesive procedure without previous preparation.[32] Prolonged etching time to improve adhesive performance to this intact enamel may not be necessary since studies found that preliminary acid etching (37% phosphoric acid) for 15 s can produce optimal bond strength in both dentitions[19] and satisfactory clinical results.[74] Moreover, clinically, cavity preparation often includes intact and ground enamel.[11] Considering that this review evaluated only *in vitro* studies, the similar performance of primary and permanent enamel might be valid just when enamel was ground.

The variability of selected studies must be taken into account. Different application techniques and different formulations among the adhesive systems might interfere in the performance of the materials.[54] In agreement to this affirmative, our review evaluated many studies that assessed several adhesives systems using different protocols during almost 30 years, although some of the materials evaluated are not in the market anymore. Moreover, other factors varied, as the type of bond strength test, type and age of teeth, the storage time and media. It may explain the high heterogeneity found in the analyses. Additionally, the majority of the studies showed high risk of bias, as well as in previous systematic review of *in vitro* studies.[57] Most of the authors did not describe important methodological details that were considered in the evaluation of risk of bias, such as, randomization, sample size calculation and blinding of operator of testing machine. It is important to emphasize that the further *in vitro* studies be carried out with this concern.

In descriptive analysis presented (Table 1), the shear bond strength was used in 70.27% of the studies. This is in agreement with previous study that shear testing was the method used in 46% of the studies on bond strength tests of resin composite to dentin.[5] Thereby, it has been a very popular test to screen new adhesive formulations on their bonding efficacy.[70] Microtensile test was probably least used by the difficulty of this technique in primary teeth, which are smaller and more sensitive to technique. Our review has a limitation by evaluating only laboratory studies, although this kind of study is very important to determine the behavior of adhesive systems and help in the choice of the best option before be used clinically.

Only one selected study for the meta-analysis evaluated the bond strength using carious teeth,[55] but they removed all traces of carious dentin using a low speed diamond. Therefore, their objective was to perform the evaluations in sound substrate. Tedesco et al.[67] assessed the effect of cariogenic challenge on bond strength of adhesive systems to sound and demineralized primary and permanent enamel. Even, there are other studies evaluating bond strength in sound and caries-affected dentin to primary and permanent teeth,[36,58] but they were not included in meta-analysis. Due to scarce number of studies evaluating carious substrate, we could not perform an analysis on it. Therefore, more studies are needed to evaluate the bond strength of adhesive systems on demineralized enamel or caries-affected dentin substrates, since the literature brings to date that the condition of the substrate appears to influence the bond strength.[36,58,67]

According to the scientific literature, after both etch-and-rinse and self-etch adhesive systems application, it may happen a deeper demineralization of the dentin and consequently an incomplete infiltration of resin monomers, creating a non-impregnated zone at the base of hybrid layer, that is more prone to degradation.[22,23] Moreover, in this review only the immediate bond strength values were regarded, since only two studies assessed degradation of resin-dentin bonds.[35,47] More studies comparing the degradation of resin-dentin bonds of adhesive systems to primary and permanent teeth are required.

A possible limitation of this study has to be stated, focusing only in PubMed/MEDLINE database and studies published in English language. Nevertheless, investigations have not shown that the occurrence of bias when articles were written in languages other than English in conventional medicine reviews, interfering only in alternative medicine studies.[43] Regarding databases choice, although EMBASE can result in a wider search, it also results in higher number of false positives (unnecessarily identified). Thus, PubMed/MEDLINE remains an optimal tool in biomedical electronic research and covers a wider journal range,[15] including papers available in other databases, as LILACS, SCIELO and Cochrane.

Based on the findings of our meta-analysis, the evidence about adhesive performance on permanent teeth cannot directly extrapolated to primary ones. Therefore, bond strength evaluations of new materials or adhesive protocols should be performed using primary teeth for providing more useful information for clinical evidence-based decision.

CONCLUSION

The primary and permanent teeth do not present similar dentin-resin bond strength, although the enamel-resin bond strength is similar between them.

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Clinical relevance: This meta-analysis emphasizes the necessity of performing the bond strength evaluations in primary teeth, considering that microstructural differences between permanent and primary substrates impact on adhesive performance.

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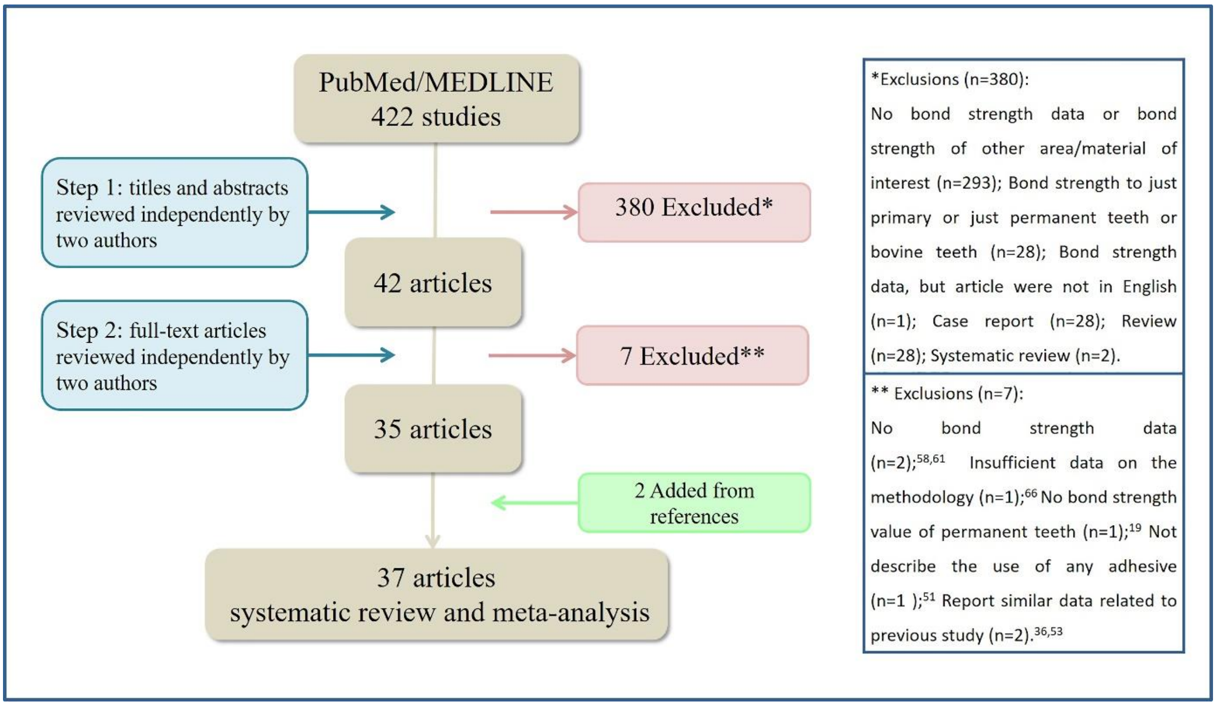

Fig 1 Flow-chart of study selection according to PRISMA statement.

Table 1. Detailed data from included studies in the Systematic Review

Study	Year	Country	Primary outcome	Substrate Condition	Surface type	Adhesive area	Storage time/media	Adhesives Systems	Restorative material
Fagan et al[14]	1986	United States	Dentin SBS	Sound	Flat	Not informed	Not informed	Bowen1s method - ferric oxalate, NTG-GMA and PMDM Scotchbond (3M ESPE, St.Paul,MN,USA)	Silux Enamel Bond Resin (3M ESPE, St.Paul,MN,USA)
Salama & Tao[55]	1991	United States	Dentin SBS	Carious	Flat	7.07mm²	24 h/isotonic saline - 37ºC	Gluma (Columbus Dental, St. Louis, MO, USA)	Lumifor (Columbus Dental, St. Louis, MO, USA)
Bordin-Aykroyd et al[4]	1992	England	Dentin SBS	Sound	Flat	7.07mm²	7 days/ distilled water - 37ºC	Scotchbond 2 (3M ESPE, Loughborough, UK) Gluma (Bayer Dental, Newbury, UK) Tenure (DenMat Corporation, Thomton Heath, UK)	Silux Plus (3M ESPE,St.Paul,MN,USA) Pekalux (Columbus Dental, St. Louis, MO, USA) Perfection (DenMat, Santa Maria, CA, USA)
Malferrari et al[38]	1994	Italy	Enamel SBS	Sound	Flat	7.07mm²	24h/ distilled water -23ºC	Gluma 2000 (Bayer AG, Germany)	Pekafill (Bayer AG, Germany)
Hallett et al[21]	1994	United States	Enamel SBS	Sound	Flat	Not informed	48h in distilled water/ thermocycled 1000x (5-55ºC)	Scotchbond Multi-purpose (3M ESPE ,St.Paul,MN, USA)	Filtek Z100 (3M ESPE, St.Paul,MN,USA)
Shanthala & Munshi[60]	1995	India	Enamel SBS	Sound	Flat	12.56mm²	28h/ distilled water at room temperature	Kulzer Enamel adhesive (Kulzer GmbH, Bereich Dental, Wehrheim, Germany)	Estilux Hibrid Vs (Heraeus-Kulzer, Dormagen, Germany)
Jumlongras & White[31]	1997	United States	Dentin SBS	Sound	Flat	7.07mm²	24h at room temperature in distilled water/ thermocycled for 450 cycles of water in (5-55ºC)	Optibond (Kerr Dental, Orange, CA, USA)	Herculite XR-V (Kerr Dental, Orange, CA, USA)
el Kalla & García-Godoy[12]	1998	United States	Dentin SBS	Sound	Flat	5mm²	72 h in distilled water room temperature/ thermocycled in distilled water at 4º and 60º for 1000 cycles/	Scotchbond Multi-purpose (3M ESPE, St.Paul,MN,USA) One Step (Bisco, Inc., Itasca, IL, USA) Prime & Bond 2.1 (Dentsply, Milford, DE, USA) EBS (ESPE, Dental Medizin, Seefeld, Germany)	Filtek Z100 (3M ESPE,St.Paul,MN,USA)
el Kalla & García-Godoy[13]	1998	United States	Dentin SBS	Sound	Flat	5mm²	72h stored distilled water at 37ºC/ thermocycled at 4ºC 60ºC for 1000 cycles	Prime & Bond (Dentsply, Milford, DE, USA) Hytac OSB (ESPE, Seefeld, Germany)	Dyract (Dentsply, Milford, DE, USA) Hytac (ESPE, Seefeld, Germany)
Çehreli & Usmen[8]	1999	Turkey	Enamel SBS	Sound	Flat	7.07mm²	48h at room temperature in distilled water/ thermocycled for 5000 cycles (5-55ºC)	Prime & Bond 2.0 (Dentsply, Milford, DE, USA) Syntac Single-Component (Ivoclar Vivadent, Amherst, NY)	Dyract (Dentsply, Milford, DE, USA) Compoglass (Ivoclar Vivadent, Amherst, NY)
Hosoya et al[27]	2000	Japan	Enamel and Dentin SBS	Sound	Flat	7.07mm²	24h stored wet in a box at room temperature	Imperva Fluorobond (Shofu Inc., Kyoto, Japan)	Lite-Fil IIA (Shofu Inc., Kyoto, Japan)
Hosoya et al[26]	2001	Japan	Dentin SBS	Sound	Flat	7.07mm²	24h stored wet in a box at room temperature	Imperva Fluorobond (Shofu Inc., Kyoto, Japan) Super-Bond D liner DUAL (Sun Medical Co., Moiyama, Japan)	Clearfill Photo Anterior (Kuraray, Osaka, Japan) Lite-Fil IIA (Shofu Inc., Kyoto, Japan)
Burrow et al[6]	2002	Australia	Dentin µTBS	Sound	Flat	1.13mm²	24 h at 37ºC in tap water	Prime&Bond NT (Dentsply, Milford, DE, USA) Single bond (3M ESPE,St.Paul,MN,USA)	Silux Plus (3M,St.Paul,MN,USA)
Shimada et al[62]	2002	Japan	Enamel µSBS	Sound	Flat	0,5mm²	24 h at 37ºC in water	Single Bond (3M ESPE,St.Paul,MN,USA) Clearfil SE Bond (Kuraray, Osaka, Japan)	Clearfil AP-X (Kuraray, Osaka, Japan)
Baghdadi[3]	2003	Syria	Dentin SBS	Sound	Flat	19.62mm²	24h/not informed storage media	Prime & Bond NT (Dentsply, Milford, DE, USA)	Dyract AP (Dentsply, Milford, DE, USA)
Senawongse et al[59]	2004	Thailand	Dentin µSBS	Sound	Flat	0.5mm²	24h in saline at 37ºC	Single Bond (3M ESPE, St.Paul, MN,USA) Clearfil SE Bond (Kuraray, Tokyo,Japan)	Clearfil AP-X (Kuraray, Tokyo,Japan)
Peutzfeldt & Nielsen[50]	2004	Denmark	Enamel SBS	Sound	Flat	3.46mm²	1 week/ 1 year/ in deionized water at 37ºC	Prompt L-Pop (3M ESPE, Seefeld, Germany)	Delton Light curing – CLEAR (Dentsply, York, Penn, USA)

Yoshimoto et al[73]	2004	Japan	Dentin SBS	Sound	Flat	7.07mm²	24h/water	Clearfil Photo bond (Kuraray, Tokyo,Japan)	Palfique Estelite (*Tokuyama* Dental Corporation, Tokyo, *Japan)*
Hosoya et al[29]	2005	Japan	Dentin SBS	Sound	Flat	7.07mm²	24h/ stored wet in a box at 37ºC	Clearfil SE Bond (Kuraray, Tokyo,Japan)	Clearfil AP-X (Kuraray, Tokyo,Japan)
Soares et al[64]	2005	Brazil	Dentin μTBS	Sound	Flat	0.4mm²	24h/ distilled water at 37◦C	One UP Bond F (Tokuyama Corp, Tokyo, Japan) Single Bond (3M ESPE, St.Paul,MN,USA) Clearfil SE Bond (Kuraray, Tokyo,Japan)	Filtek Z100 (3M ESPE, St.Paul,MN,USA)
Courson et al[9]	2005	Canada	Dentin SBS	Sound	Flat	3.14mm²	24h in water at 37ºC	Scotchbond Multipurpose Plus (3M ESPE, St.Paul,MN,USA) One Step (Bisco, Inc., Itasca, IL, USA) Scotchbond 1 (3M ESPE, St.Paul,MN,USA) Prime&Bond 2.1 (Dentsply, Milford, DE, USA) Optibond Solo Plus (Kerr Dental, Orange, CA, USA) Prime & Bond NT (Dentsply, Milford, DE, USA) Clearfil Linder Bond 2 (Kuraray, Tokyo,Japan) Clearfil SE Bond (Kuraray, Tokyo,Japan) Prompt L-Pop (3M ESPE,St.Paul,MN,USA)	Filtek Z100 (3M ESPE ,St.Paul,MN,USA)
Germán et al[18]	2005	Spain	Dentin SBS	Sound	Flat	6.69mm²	48 h in distilled water at 37ºC/ thermocycled 150 cycles (5-55ºC)	Adper Prompt-L-Pop (3M ESPE, St.Paul,MN,USA) Prime & Bond NT (Dentsply, Milford, DE, USA)	TPH Spectrum (Dentsply DeTrey, Konstanz, Germany) Compoglass (Ivoclar Vivadent, Amherst, NY)
Uekusa et al[69]	2006	Japan	Dentin μTBS	Sound	Flat	1mm²	24h/ distilled water	Clearfil tri-S Bond (Kuraray, Tokyo,Japan) One-Up Bond F Plus (Tokuyama Dental Corporation, Tokyo, Japan)	Clearfil AP-X (Kuraray, Tokyo,Japan) Palfique Estelite (*Tokuyama* Dental Corporation, Tokyo, *Japan)*
Mahdi et al[37]	2008	Iran	Dentin SBS	Sound	Flat	7.07mm²	stored at 37ºC in distilled water for 7-10 days/ thermo cycled 500x (5-55ºC)	Scotchbond Multipurpose Plus (3M ESPE, St.Paul,MN,USA)	Amalgam (Dr. Faghihi, Dental Co, Iran)
Da Costa et al[10]	2008	Brazil	Enamel and Dentin SBS	Sound	Flat	7.07mm²	stored in an oven (37ºC) at 100% humidity for 1h and immersed in distilled water (37ºC) for 23h	Single Bond (3M ESPE, St.Paul,MN,USA) AdheSE (Ivoclar Vivadent, Amherst, NY) Prompt-L-Pop (3M Espe,St.Paul,MN,USA)	Filtek Z250 (3M ESPE, St.Paul,MN,USA)
Can-Karabulut et al[7]	2009	Turkey	Dentin SBS	Sound	Flat	7.07mm²	stored at 37ºC for 24 h in distilled water/ thermocycled 500 cycles (5-55ºC)/ tested 24 h after thermocycled	Scotchbond Multi Purpose (3M ESPE, St.Paul,MN,USA) Gluma Confort Bond (Heraeus-Kulzer, Dormagen, Germany) Adper Prompt-L-Pop (3M ESPE, St.Paul, MN, USA)	Filtek Z100 (3M ESPE, St.Paul,MN,USA)
Yaseen & Subba Reddy[71]	2009	India	Dentin SBS	Sound	Flat	3.14mm²	24h at 37ºC in distilled water (Humidor)	Contax (DMG, Hamburg, Germany) Clearfil S3 bond (Kuraray, Tokyo,Japan)	Filtek Z350 (3M ESPE, St.Paul,MN,USA)
Ricci et al[52]	2010	Brazil	Dentin μTBS	Sound	Flat	0.81mm²	24h in distilled water	Adper Single Bond (3M ESPE, St.Paul,MN,USA) Prime&Bond NT (Dentsply, Milford, DE, USA) Excite DSC (Ivoclar Vivadent, Schaan Liechtenstein)	Filtek Z250 (3M ESPE, St.Paul,MN,USA)
Lenzi et al[35]	2012	Brazil	Dentin μTBS	Sound	Flat	0.8mm²	24h in distilled water at 37◦C/ aging(6months) in distilled water containing 0.4%	Adper Single Bond 2 (3M ESPE, St.Paul, MN, USA)	Filtek Z250 (3M ESPE, St.Paul, MN, USA)

							sodium azide at 37◦C		
Aras et al[2]	2013	Turkey	Enamel SBS	Sound	Flat	7.07mm²	24 h in deionised water at 37ºC/ thermo cycled 1000cycles (5-55ºC)	Gluma Confort Bond (Herause-Kulzer, Germany)	Charisma (Herause Kulzer, Germany)
Osorio et al[47]	2013	Spain	Dentin µTBS	Sound	Flat	1mm²	Immediate (24h) 3 months 6 months in distilled water containing 0.02% sodium azide (pH 7.0)	Adper Single Bond Plus (3M ESPE, St.Paul, MN, USA)	Tetric Evoceram (Ivoclar Vivadent, Schaan Liechtenstein)
Lenzi et al[34]	2013	Brazil	Enamel µSBS	Sound	Flat	0.45mm²	24h in distilled water at 37◦C	Adper Easy Bond (3M ESPE, Seefeld, Germany)	Filtek Z250 (3M ESPE,St.Paul,MN,USA)
Tedesco et al[67]	2014	Brazil	Enamel µSBS	Sound and Demineralized	Flat	0.45mm²	24 h in distilled water	Adper Single Bond (3M ESPE, St.Paul,MN,USA) Clearfil SE Bond (Kuraray, Tokyo,Japan)	Filtek Z250 (3M ESPE, St.Paul,MN,USA)
Ilie et al[30]	2014	Germany	Enamel and Dentin SBS	Sound	Flat	7.07mm²	24h in distilled water at 37 ºC/ thermal ageing - 5000 cycles (5-55ºC)	Adhese One F(Ivoclar Vivadent, Amherst, NY) Xeno V (Dentsply DeTrey, Konstanz, Germany)	Surefil SDR Flow (Dentsply DeTrey, Konstanz, Germany) Tetric Evo Ceram (Ivoclar Vivadent, Amherst, NY) Tetric Evo Ceram Bulk Fill (Ivoclar Vivadent, Amherst, NY)
Tuloglu et al[68]	2014	Turkey	Dentin SBS	Sound	Flat	3.14mm²	24h in water at 37ºC/ thermocycled 500 cycles (5-55ºC)	OptiBond All-In-One (Kerr Dental, Orange, CA, USA)	Vertise Flow (Kerr Dental, Orange, CA, USA) Filtek Ultimate (3M ESPE, St.Paul, MN, USA)
Yildiz et al[72]	2015	Turkey	Dentin µTBS	Sound	Flat	1mm²	24 h in water at 37ºC	Adper single bond 2 (3M ESPE, St.Paul, MN, USA)	Filtek Z250 (3M ESPE, St.Paul,MN,USA)
Santschi et al[56]	2015	Switzerland	Dentin SBS	Sound	Flat	1.8mm²	24 h at 37ºC/ 100% humidity	Xeno V+ (Dentsply DeTrey, Konstanz, Germany) Scotchbond Universal (3M ESPE; Neuss, Germany)	Filtek Z250 (3M ESPE, St.Paul,MN,USA)

Abbreviations: SBS: Shear Bond Strength; µTBS: Micro Tensile Bond Strength; µSBS: Micro Shear Bond Strength

Table 2. Studies' quality according to risk of bias

Study	Random	Manufacturer's instruction	Adhesive	Sample size	Blinding	Risk of bias
Fagan et al[14]	Yes	Yes	No	No	No	**High**
Salama & Tao[55]	No	No	No	No	No	**High**
Bordin-Aykroyd et al[4]	No	Yes	No	No	No	**High**
Malferrari et al[38]	Yes	No	No	No	No	**High**
Hallett et al[21]	Yes	Yes	No	No	No	**High**
Shanthala & Munshi[60]	No	Yes	No	No	No	**High**
Jumlongras & White[31]	Yes	Yes	No	No	No	**High**
el Kalla & García-Godoy[12]	Yes	Yes	No	No	No	**High**
el Kalla & García-Godoy[13]	Yes	Yes	No	No	No	**High**
Çehreli & Usmen[8]	No	Yes	No	No	No	**High**
Hosoya et al[27]	No	No	No	No	No	**High**
Hosoya et al[26]	No	No	No	No	No	**High**
Burrow et al[6]	No	Yes	No	No	No	**High**
Shimada et al[62]	Yes	Yes	No	No	No	**High**
Baghdadi[3]	Yes	Yes	No	No	No	**High**
Senawongse et al[59]	No	Yes	No	No	No	**High**
Peutzfeldt & Nielsen[50]	Yes	No	No	No	No	**High**
Yoshimoto et al[73]	No	No	No	No	No	**High**
Hosoya et al[29]	No	Yes	No	No	No	**High**
Soares et al[64]	Yes	Yes	No	No	No	**High**
Courson et al[9]	Yes	Yes	Yes	No	No	**Medium**
Germán et al[18]	No	Yes	No	No	No	**High**
Uekusa et al[69]	No	Yes	No	No	No	**High**
Mahdi et al[37]	No	No	No	No	No	**High**
Da Costa et al[10]	Yes	Yes	No	No	No	**High**
Can-Karabulut et al[7]	Yes	Yes	No	No	No	**High**
Yaseen & Subba Reddy[71]	No	Yes	No	No	No	**High**
Ricci et al[52]	Yes	Yes	No	No	No	**High**
Lenzi et al[35]	Yes	No	No	No	No	**High**
Aras et al[2]	Yes	No	No	No	No	**High**
Osorio et al[47]	No	Yes	No	No	No	**High**
Lenzi et al[34]	Yes	Yes	No	No	No	**High**
Tedesco et al[67]	Yes	Yes	No	No	No	**High**
Ilie et al[30]	No	Yes	No	No	No	**High**
Tuloglu et al[68]	Yes	Yes	No	No	No	**High**
Yildiz et al[72]	Yes	No	No	No	No	**High**
Santschi et al[56]	Yes	Yes	No	No	No	**High**

Random: randomization of teeth; Manufacturer's instruction: adhesive systems used according to the manufacturer's instructions; Adhesive: adhesive procedures performed by the same operator; Sample size: description of sample size calculation; Blinding: blinding of the operator of the testing machine.

Study or Subgroup	Primary Teeth Mean	SD	Total	Permanent Teeth Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Year
1.1.1 Dentin									
Fagan et al. 1986	4.81	2.94	17	4.7	2.88	20	2.7%	0.11 [-1.77, 1.99]	1986
Salama and Tao 1991	8.39	4.64	12	11.48	3.09	24	2.6%	-3.09 [-5.99, -0.19]	1991
Bordin-Aykroyd et al. 1992	6.2	4.8	12	8.8	4.9	12	2.4%	-2.60 [-6.48, 1.28]	1992
Jumlongras and White 1997	6.07	2.63	10	17.61	4.34	10	2.5%	-11.54 [-14.69, -8.39]	1997
el Kalla and García-Godoy 1998a	27.45	4.06	32	29.97	3.43	32	2.7%	-2.52 [-4.36, -0.68]	1998a
el Kalla and García-Godoy 1998b	27.7	4.11	16	26.1	3.26	16	2.6%	1.60 [-0.97, 4.17]	1998b
Hosoya et al. 2000	15.06	7.02	10	15.45	5.35	10	2.2%	-0.39 [-5.86, 5.08]	2000
Hosoya et al. 2001	14.65	8.73	16	15.85	6.99	20	2.2%	-1.20 [-6.46, 4.06]	2001
Burrow et al. 2002	18.15	3.87	12	21.6	4.49	6	2.4%	-3.45 [-7.66, 0.76]	2002
Bahgdadi 2003	15.21	5.25	10	13.32	6.6	10	2.2%	1.89 [-3.34, 7.12]	2003
Yoshimoto et al. 2004	9.28	3.03	5	6.08	1.76	5	2.6%	3.20 [0.13, 6.27]	2004
Senawongse et al. 2004	36.24	6.07	24	40.52	7.66	48	2.5%	-4.28 [-7.53, -1.03]	2004
Soares et al. 2005	61.53	5.41	18	59.87	6.09	18	2.5%	1.66 [-2.10, 5.42]	2005
Germán Cecilia et al. 2005	13.68	2.86	28	14.34	3.7	28	2.7%	-0.66 [-2.39, 1.07]	2005
Hosoya et al. 2005	27.8	8.7	8	21.3	3.6	10	2.0%	6.50 [0.07, 12.93]	2005
Courson et al. 2005	10.99	5.73	100	13.91	6.17	100	2.7%	-2.92 [-4.57, -1.27]	2005
Uekusa et al. 2006	42.65	9.98	30	52.15	8.7	30	2.3%	-9.50 [-14.24, -4.76]	2006
Mahdi et al. 2008	6.29	2.32	27	6.59	2.46	28	2.7%	-0.30 [-1.56, 0.96]	2008
Da Costa et al. 2008	21.45	6.39	45	23.4	7.26	45	2.6%	-1.95 [-4.78, 0.88]	2008
Yaseen and Subba Reddy 2009	16.1	7.15	32	21.5	6.78	32	2.5%	-5.40 [-8.81, -1.99]	2009
Can-Karabulut et al. 2009	14.36	0.8	21	19.57	2.16	21	2.7%	-5.21 [-6.20, -4.22]	2009
Ricci et al. 2010	41.87	11.3	12	43.63	10.6	12	1.7%	-1.76 [-10.53, 7.01]	2010
Lenzi et al. 2012	30.7	2.2	5	41.7	2.7	5	2.6%	-11.00 [-14.05, -7.95]	2012
Osorio et al. 2013	27.36	10.1	15	37.5	3.8	15	2.2%	-10.14 [-15.60, -4.68]	2013
Tologlu et al. 2014	12.15	2.14	20	30.65	2.87	20	2.7%	-18.50 [-20.07, -16.93]	2014
Ilie et al. 2014	16.91	8.71	120	16.36	6.93	120	2.7%	0.55 [-1.44, 2.54]	2014
Yildz et al. 2015	14.28	5.22	20	24.92	7.93	20	2.4%	-10.64 [-14.80, -6.48]	2015
Santschi et al. 2015	18.42	2.82	30	17.45	3.75	30	2.7%	0.97 [-0.71, 2.65]	2015
Subtotal (95% CI)			**707**			**747**	**69.3%**	**-3.30 [-5.44, -1.16]**	
Heterogeneity: Tau² = 29.92; Chi² = 586.49, df = 27 (P < 0.00001); I² = 95%									
Test for overall effect: Z = 3.03 (P = 0.002)									
1.1.2 Enamel									
Malferrari et al. 1994	19.55	4.37	40	16.32	4.55	40	2.7%	3.23 [1.27, 5.19]	1994
Hallett et al. 1994	11.8	7.35	15	17	5.06	15	2.3%	-5.20 [-9.72, -0.68]	1994
Shanthala and Munshi 1995	4.45	0.77	20	5.57	1.33	20	2.7%	-1.12 [-1.79, -0.45]	1995
Çehreli and Usmen 1999	7.12	0.78	20	17.75	0.98	20	2.8%	-10.63 [-11.18, -10.08]	1999
Hosoya et al. 2000	16.34	5.53	10	14.39	6.52	10	2.2%	1.95 [-3.35, 7.25]	2000
Shimada et al. 2002	39.85	6.23	20	42.8	7.74	20	2.4%	-2.95 [-7.30, 1.40]	2002
Peutzfeldt and Nielsen 2004	12.15	3.98	20	14.75	4.3	20	2.6%	-2.60 [-5.17, -0.03]	2004
Da Costa et al. 2008	19.75	4.96	45	19.77	4.85	45	2.7%	-0.02 [-2.05, 2.01]	2008
Lenzi et al. 2013	23.4	5.64	30	26.3	3.91	30	2.6%	-2.90 [-5.36, -0.44]	2013
Aras et al. 2013	14	2.89	10	25.46	3.71	20	2.6%	-11.46 [-13.88, -9.04]	2013
Ilie et al. 2014	11.56	6.14	120	12.84	5.57	120	2.7%	-1.28 [-2.76, 0.20]	2014
Tedesco et al. 2014	26.13	4.99	10	26.47	4.37	10	2.4%	-0.34 [-4.45, 3.77]	2014
Subtotal (95% CI)			**360**			**370**	**30.7%**	**-2.83 [-6.32, 0.65]**	
Heterogeneity: Tau² = 35.61; Chi² = 680.38, df = 11 (P < 0.00001); I² = 98%									
Test for overall effect: Z = 1.59 (P = 0.11)									
Total (95% CI)			**1067**			**1117**	**100.0%**	**-3.16 [-4.95, -1.37]**	
Heterogeneity: Tau² = 30.26; Chi² = 1315.53, df = 39 (P < 0.00001); I² = 97%									
Test for overall effect: Z = 3.46 (P = 0.0005)									
Test for subgroup differences: Chi² = 0.05, df = 1 (P = 0.82), I² = 0%									

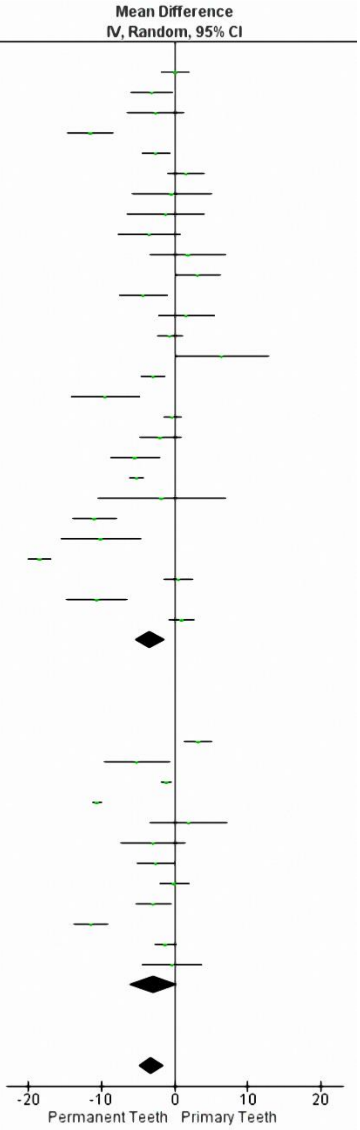

Fig 2 Results for the global analysis based on subgroup analysis in dentin and enamel using random-effects model. Statistically significant differences were found to dentin and to primary and permanent teeth in general ($p \leq 0.05$). While no statistically significant difference was found to enamel ($p \geq 0.05$).

4 ARTIGO 3 - BONDING OF THREE ADHESIVE SYSTEMS TO ENAMEL SURROUNDING REAL-LIFE CAVITIES AFTER SELECTIVE REMOVAL OF CARIOUS TISSUE

Este artigo foi submetido ao periódico Journal of Adhesive Dentistry; ISSN: 1757-9988; Fator de impacto = 1.311; Qualis A2. O artigo está de acordo com as normas deste periódico, que estão descritas no ANEXO B.

Bonding of three adhesive systems to enamel surrounding real-life cavities after selective removal of carious tissue

Carine Weber Pires
Graduate
Post Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Execution and article writing.

Tathiane Larissa Lenzi
Professor
Post Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Consulted on idea, proofread the manuscript.

Fabio Zovico Maxnuck Soares
Professor
Department of Restorative Dentistry, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Contribution substantially to discussion, proofread the manuscript.

Rachel de Oliveira Rocha
Associate Professor
Department of Stomatology, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 9715-270, Santa Maria, RS, Brazil
Contribution to the paper: Idea, contribution substantially to discussion, proofread the manuscript.

Corresponding author:
Rachel de Oliveira Rocha
Rua Floriano Peixoto, 1184 sala 211
Santa Maria, RS, Brazil
+55 55 3220 9266
rachelrocha@smail.ufsm.br

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ABSTRACT

Purpose: To evaluate the bond strength of three adhesive systems to enamel surrounding real-life carious cavities after selective carious tissue removal.

Materials and Methods: Twenty-eight permanent molars (n=7) with carious lesions in dentin were subjected to selective carious tissue removal and had their crowns sectioned longitudinally. Three adhesive systems (Scotchbond Universal Adhesive [SBU] used in either etch-and-rinse and self-etch strategies, Adper Single Bond Plus [ASB] and Clearfil SE Bond [CSE]) were applied on the enamel surrounding the cavity margins and on enamel distant of it (control substrate). Composite cylinders were built (0.72 mm^2) and after 24h, microshear bond strength (µSBS) test was performed. The µSBS values (MPa) were analyzed using two-way ANOVA and Tukey's *post hoc* tests ($\alpha = 0.05$).

Results: Bond strength values obtained in enamel surrounding carious cavities margins were significantly lower than that obtained in enamel distant of carious cavities margins ($p = 0.035$). The bonding strategy of SBU did not influenced the bond strength values that were higher than that obtained to ASB and similar to CSE when it was used in a self-etch mode.

Conclusion: The enamel surrounding carious cavities after selective carious tissue removal jeopardize the bonding of representative adhesive systems.

INTRODUCTION

Selective removal of carious tissue, formerly known as partial caries removal, is widely adopted as a minimally invasive approach in the restorative treatment of primary and permanent teeth to reduces the risk of pulp exposure,[9,17-19] treatment costs,[35] post-operative complications and the over extension of the cavities that weakens tooth structure increasing the risk of restoration failure.[44] Nevertheless, there is still little evidence to determine the risk of restorative failure after selective removal carious tissue.[12,29] A recent retrospective study did not find significant differences between selective and complete caries tissue removal in permanent teeth regarding restoration longevity.[4] In contrast, according to a clinical study in primary teeth, restorations placed after selective removal of carious tissue had a higher risk of restorative failure compared to that subjected to total caries removal.[8] However, although uncertainties remain concerning to left a thin layer of affected dentin under the restoration after selective excavation, this conduct did not affect the marginal integrity of placed restorations neither the secondary caries susceptibility of teeth in vitro.[30]

More attention has been given to the dentin-pulp complex when referring to the selective removal of carious tissue, including to arrest the caries process[16,17] and preservation of pulp vitality.[9,17-19] However, bonding to enamel should be a concern, since the stability of the resin-bonded dentin[41] and effectiveness of marginal sealing[11] depends on the bonding to surrounding enamel. Considering that in the selective removal approach the excavation of carious dentin is performed only with manual or rotary instruments at low speed[6,16] and the access to the lesion is performed with rotary instruments at high speed only when needed,[19] it is possible that the enamel surrounding the cavity margins present some degree of demineralization. Nevertheless, few studies have considered the demineralized enamel as a substrate in bond strength evaluations. Even so, bonding to demineralized enamel seems to be lower than to sound enamel,[2,40] probably due lower mineral content, higher porosity of the surface,[35] with widened intercrystalline spaces, and consequently a larger pore volume than sound enamel,[13,26] produce an unsatisfactory etching pattern and infiltration of resin monomers.[40]

Therefore, considering that enamel surrounding carious cavities after selective removal of carious tissue is probably demineralized, this study aimed to evaluate the bond strength of three adhesive systems to enamel surrounding real-life carious cavities after selective carious tissue removal, considering the null hypothesis that there is no difference in the bonding values regardless the enamel proximity of carious cavities.

MATERIALS AND METHODS

The influence of the enamel proximity to selectively excavated cavities on the bond strength values was assessed by microshear bond strength (μSBS) test. Enamel surrounding carious cavity was assumed as possibly demineralized and that enamel further lesion was considered as sound. The experimental design of the study is presented in Fig. 1. The research protocol received previous approval from the Local Ethics Committee.

Sample preparation

Twenty-eight extracted human permanent molars were selected from a pool of extracted teeth according the inclusion criteria: presence of carious cavities involving dentin on occlusal or occlusal-proximal surfaces; no restorations, cracks, enamel defects or signs of previous restorative intervention. Teeth were stored in 0.5% chloramine T solution at 4ºC for up thirty days before being used.

Teeth were randomly allocated into four groups (n=7) by a program to generate random number list (Random.org - Randomness and Integrity Services Ltd., Dublin, Ireland) according to the adhesive system: Scotchbond Universal Adhesive (SBU) (3M ESPE; St Paul, MN, USA) in both self-etch (SE) and etch-and-rinse (ER) application modes; Adper Single Bond 2 (ASB) (3M ESPE; St Paul, MN, USA) and Clearfil SE Bond (CSE) (Kuraray Noritake; Tokyo, Japan).

A single experienced operator performed the selective removal of the carious tissue by using low-speed metal burs and/or hand excavator, according to the size of the cavity. The decayed tissue of the sidewalls was completely removed,[19] while on the cavity floor carious tissue removal was continued until firm dentin remains over the pulp.[31]

The presence/absence of white spot lesion in the enamel was verified after the selective carious tissue removal. If the enamel surrounding carious cavities margins presented some visible white spot lesion the tooth was replaced by another. Afterwards, the crowns of all teeth were longitudinally sectioned at the center of the cavities using a low-speed water-cooled diamond saw in a cutting machine (Labcut 1010, Extec Co., Enfield, USA), for obtaining at least two sections (specimens) of each tooth. Each slice was glued with cyanoacrylate in PVC rings embedded with self-curing acrylic resin (JET Clássico®, São Paulo, SP, Brazil), constituting a specimen.

Bonding and restorative procedures

Specimens were manually ground with 600-grit SIC paper under running water for 60 s to create standardized flat enamel surfaces.[15]

The adhesive systems were applied to enamel according to the manufacturers' instructions (Table 1). Starch tubes (Isabela, Ind. E Com. Food, São Paulo, SP, Brazil) with 0.96 mm of internal diameter and 1.0 mm in height,[39] were positioned over the enamel surface prior the light curing the adhesives with a light emitting diode curing unit (Emitter B, Schuster, Santa Maria, RS, Brazil) with a light output of at least 1250 mW/cm^2. The device's own radiometer quantified the output power. The tubes were carefully filled with composite resin (Filtek Z250 XT, 3M ESPE, St Paul, MN, USA; shade A2) and light cured for 20 s. At least two cylinders of composite resin were built up in each specimen, one over the enamel surrounding carious cavity and one over the sound enamel. A single trained operator performed all adhesive and restorative procedures at room temperature.

Microshear Bond Strength Test

The specimens were stored in distilled water at 37°C for 24 h. After this period, the starch tubes were gently removed using air/water spray and a probe. Cylinders of composite resin were examined under a stereomicroscope (Stereo Discovery V20, Carl Zeiss do Brazil Ltda., Rio de Janeiro, RJ, Brazil) at 10× magnification, in order to verify the presence of bubbles, gaps in the interface or other defects. When it happened, all the specimens from the tooth were excluded and the tooth were replaced.

Each specimen was individually attached to a universal testing machine (Emic, São José dos Pinhais, PR, Brazil), equipped with a 100N load cell (10kgf), with 0.01N reading resolution and minimum sensitivity of 2N. A stainless steel wire (0.20 mm diameter) was looped around the composite resin cylinder, as closer as possible to the resin/enamel interface. A shear load was applied at a crosshead speed of 1.0 mm/min until failure. To avoid bias, a single and blinded operator carried out bond strength measurement procedures.

Failure mode

All debonded specimens were observed under stereomicroscope at 40× magnification, by a trained and blinded examiner, to determine failure mode: cohesive failure within enamel or resin and interfacial failure.[41] Only specimens with interfacial fractures were considered in the calculation of bond strength values.

Four representative specimens for each experimental group were prepared for failure mode evaluation under scanning electron microscope (SEM). Specimens were dehydrated in ascending degrees of ethanol (50, 75 and 90% for 5 min each, and 100% for 3 h)[22] and kept in vacuum for 24 h. Subsequently, they were gold sputter coated for SEM observation.

Statistical analysis

The tooth was considered the experimental unit. Thus, the µSBS values of all composite cylinders from the same tooth, according the enamel location, were averaged for statistical purposes. The sample size of 7 teeth per group was estimated previously considering an 80% power, a coefficient of variation of 20%, and assuming a two-sided 5% significance level for comparisons. Pretest failures (PTFs) that occurred during specimens' testing preparation were not included in the statistical analysis because were equally distributed in all groups.

The normal distribution of the data and equality of variances were confirmed with Kolmogorov-Smirnov and Cochran tests, respectively. The µSBS means were submitted to two-way ANOVA with pairwise comparisons (enamel location *vs.* adhesive system) and Tukey's *post hoc* tests at a significance level of 5%. All statistical analyses were performed using the Minitab software (Minitab Inc., State College, PA, USA).

RESULTS

Descriptive statistics, including means, standard deviations and the number of tested specimens per group are presented in Table 2. The cross-interaction product "enamel location *vs.* adhesive system" was not significant ($p = 0.62$). However, the main factors "enamel location" ($p = 0.03$) and "adhesive system" ($p < 0.00$) significantly influenced on the µSBS values. Bond strength of adhesive system to enamel surrounding carious cavities, assumed as demineralized enamel, was approximately 10% lower than that obtained to sound enamel. SBU presented similar values in ER (12.3±1.8) and SE (10.5±0.8) strategies, and higher values than those obtained with ASB (8.1±0.4). CSE (8.7±0.5) showed similar µSBS values to ASB and the SBU in the SE mode. PTFs occurred in all groups (Table 3).

All specimens showed interfacial failures, regardless of the enamel location (Fig. 2).

DISCUSSION

The current investigation has demonstrated that bond strength of adhesive systems to enamel assumed as demineralized was lower than those obtained to sound enamel. Thus, the proximity to the carious lesions can influence negatively the bond strength values to enamel. Moreover, since the use of artificial lesions decreased external validity,[30] the evaluation of the performance of adhesive systems to enamel surrounding real-life cavities, consequence of natural caries process, increase external validity of our findings, besides being original and clinically relevant.

The histological structure of demineralized enamel is different from sound enamel,[13,26,35] which should be related to reduced bond strength due unsatisfactory conditioning pattern and infiltration of resin monomers.[40] This fact may justify the lower bond strength of adhesive systems to enamel adjacent to dentin carious lesions. It is in line with previous studies that compared sound and demineralized enamel substrates, exhibiting significant differences in bond strength values.[2,40] Considering that the enamel condition may compromise the adhesion, may be suggested changes in the treatment of enamel surrounding cavities subjected to selective removal approach. Since the mineral loss suffered by enamel is not always clinically visible,[5] the enamel with suspected of mineral loss and unintentionally remained after selective removal of carious dentin may be removed prior the adhesive procedures with rotary instruments at high speed.

The enamel surrounding dentin carious lesion requires more attention in selective excavation technique. The presence of enamel margins is relevant, because dentinal margins showed more marginal imperfections, gaps and microleakage.[30] In addition, the morphology of the cavity must be considered in clinical setting, since closed occlusal lesions showing enamel cavitation presented higher dentin demineralization degree than open enamel lesions.[25] Therefore, closed cavitated lesions consist in protective areas favouring biofilm accumulation and progression of mineral loss. May be suggested that in closed cavitated lesions is more frequent the presence of enamel with some degree of mineral loss.

The enamel surface used in the present study was the inner, since the cut of slices was transversal. Moreover, the enamel surface was ground with 600-grit silicon carbide paper, which affected the nature of enamel smear layer, turning the substrate more receptive to bonding with self-etch systems.[21,27] Thereby, the performance of the Scotchbond Universal Adhesive in the both strategies was similar, in agreement with previous that evaluated universal adhesives.[14,28,42] Although, recent studies evaluating the performance of Scotchbond Universal Adhesive to enamel[2,3,10,20,37,38,43] stated that the enamel bond strength values are higher when used in etching strategy.

The results of the two-step self-etch adhesive system (Clearfil SE Bond) did not differ from bond strength values of the Scotchbond Universal Adhesive in the self-etch mode. Both adhesives contain MPD (10-methacryloxydecyl dihydrogen phosphate) as acidic monomer. This monomer contains phosphate groups able of producing ionic chemical bonds with calcium in hydroxyapatite, which may explain the similar performance. Previous studies also found similar bond strength to these adhesive systems.[2,10] Whereas, both two-step self-etch system (Clearfil SE Bond) and etch-and-rinse adhesive (Adper Single Bond 2) presented similar performance on permanent enamel, in accordance with previous studies.[21,39,40] They are considered gold standard adhesives and are frequently used in bond strength studies.[7,24,33,36]

In the evaluation of failure mode was found only interfacial failures for all experimental groups. Moreover, in general, the failures occured within the limits of the composite cylinder**.** It was expected, since in this study was used a microshear bond strength test to evaluate bond strength**.** This method is associated to few cohesive failures in tooth substrate or composite.[1,23] The frequency of pretest failure was very low and it was not prevalent in a specific group. The use of starch tubes to build up the specimens may be the reason to low occurrence of premature failure. The procedure of starch tube removal is very easy, not requires a blade or other cutting instrument, not causing pressure or stresses in the interface of resin composite cylinder, which is a great advantage.[39]

This study has limitations, such as the lack of an initial quantitative assessment of enamel surrounding cavities, and the use of only visual inspection to assess the enamel condition. Moreover, the enamel surrounding carious cavities was assumed as demineralized, because there was the suspicion that it presented mineral loss before the carious process in which it was involved.

Within the limitations of an *in vitro* study, the results obtained suggested more attention and changes in the treatment of this enamel surrounding carious cavities, since the permanence of undetected demineralized enamel might be the cause of failures in adhesive restorations performed after selective caries removal. Further studies evaluating the adjacent enamel to cavities submitted to selective excavation approach are required.

CONCLUSION

In conclusion, the bond strength of adhesive systems to enamel assumed as demineralized is lower than the sound enamel. The proximity to cavities undergoing to selective caries removal influences the bond strength values to enamel, regardless of the adhesive system.

Acknowledgments

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Clinical relevance: Lower bond strength values were found to enamel surrounding carious lesions, which suggest that enamel with suspected of mineral loss may be removed in the selective removal of carious tissue approach, prior to the restorative procedures.

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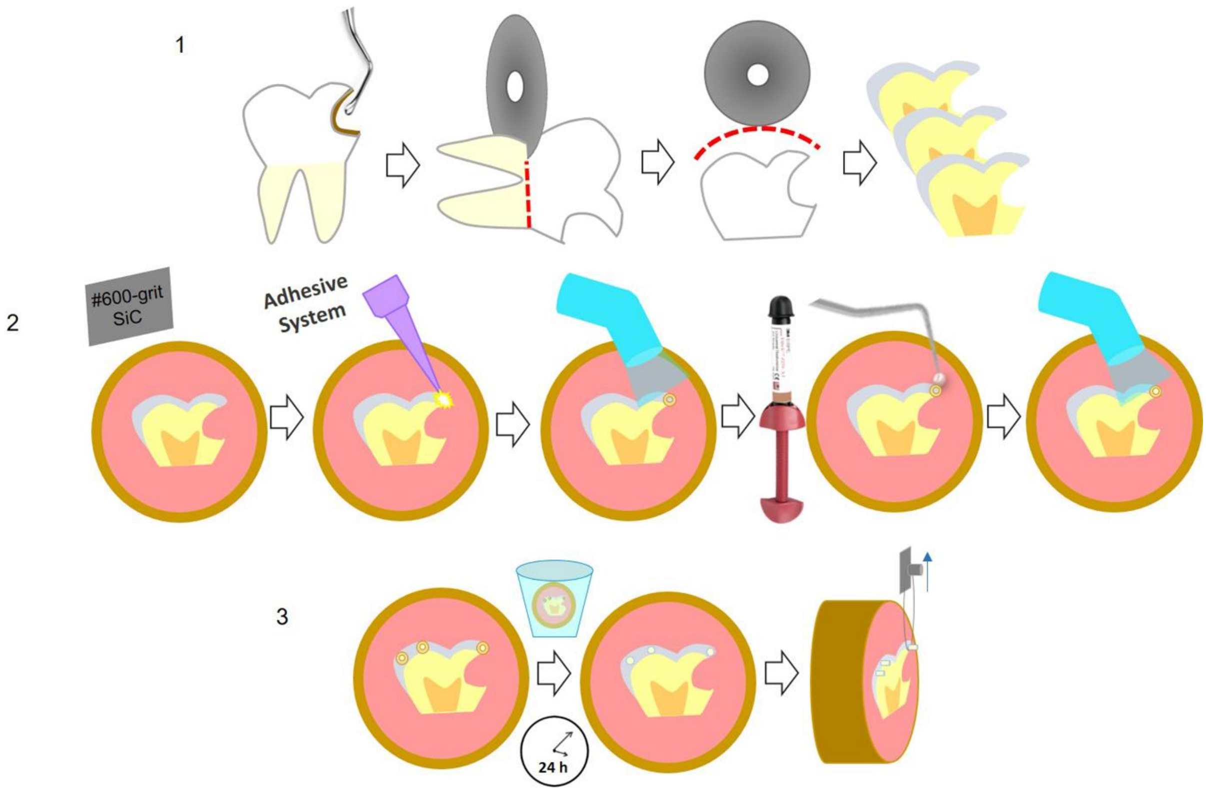

Fig. 1. Experimental design of the study: 1 - Sample preparation. 2 - Restorative procedures. 3 - Microshear bond strength test.

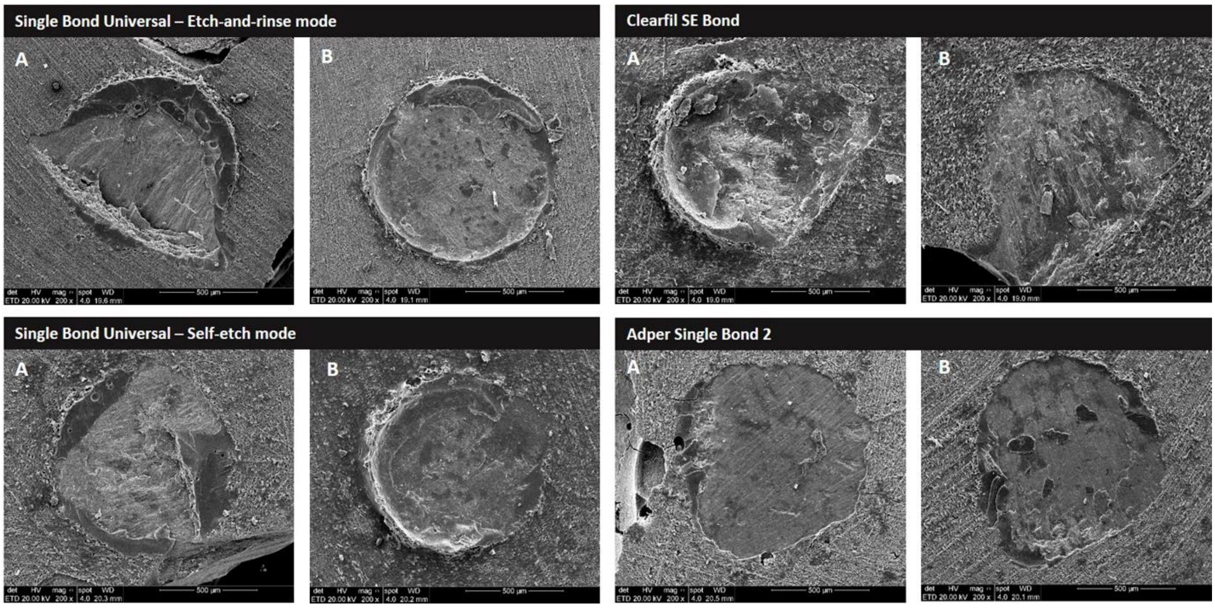

Fig. 2. Representative SEM images of specimens with interfacial failures. All the adhesive systems evaluated showed interfacial failures when bonded to adjacent enamel to carious lesion (A) and sound enamel (B).

Table 1
Materials used in the study

Material	Manufacturer	Lot. No.	Classification	Composition	Application mode
Scotchbond Universal Adhesive	3M/ESPE (St. Paul, MN, USA)	1508500365	Self-etch	MDP phosphate monomer, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive to the enamel with a microbrush and rub it in for 20 s 2. Gentle air thin for 5 s 3. Light-cure for 10 s
			Etch-and-rinse	37% phosphoric acid (DFL, Rio de Janeiro, RJ, Brazil) MDP phosphate monomer, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	1. Apply etchant for 15 s 2. Rinse for 15 s 3. Air dry to remove excess of water 4. Apply the adhesive as for the self-etch mode
Adper Single Bond 2	3M/ESPE (St. Paul, MN, USA)	1501300547	Two-step etch-and-rinse adhesive	37% phosphoric acid (DFL, Rio de Janeiro, RJ, Brazil) BIS-GMA, HEMA, dimethacrylate, amines, methacrylic copolymer of polyacrylic and polyitaconic acids, ethanol, water, photoinitiator	1. Apply etchant for 15 s 2. Rinse for 15 s 3. Air dry to remove excess of water 4. Apply 2 consecutive coats of adhesive for 15 s with gently agitation 5. Gentle air for 5 s to evaporate the solvent 6. Light-cure for 10 s
Clearfil SE Bond	Kuraray Noritake, Tokyo, Japan	051550	Two-step self-etching adhesive	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, di-canphoroquinone, aromatic tert-amine, water Bond: 10-MDP, bis-GMA, HEMA, hydrophiiic dimethacrylate, photoinitiator, aromatic tert-amine, sinaleted colloidal silica	1. Apply primer to enamel and leave in place for 20s. 2. Blow dry for 20s at a distance of 20 cm. 3. Apply bond for 20s 4. Gently air dry for 5s 5. Light-cure for 10s

According to manufacturers' information. Abbreviations: bis-GMA: bisphenol A glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate.

Table 2
The µSBS (MPa) means and standard deviations [number of tested cylinders/pretest failures] for all experimental groups.

Adhesive system (etching mode)	Enamel location	Mean (SD)	Composite Resin cylinder
Scotchbond Universal Adhesive (ER)	Surrounding carious cavities	11(1.6)	21/2
	Sound enamel	14(2.4)	23/3
Scotchbond Universal Adhesive (SE)	Surrounding carious cavities	9.9(1.6)	19/2
	Sound enamel	11(2.4)	25/2
Adper Single Bond 2	Surrounding carious cavities	7.8(2.1)	19/1
	Sound enamel	8.4(1.3)	21/2
Clearfil SE Bond	Surrounding carious cavities	8.3(2.6)	17/3
	Sound enamel	9.0(3.3)	22/1

Table 3

The µSBS (MPa) means and standard deviations [number of tested cylinders/premature failures] considering the factors "adhesive system" and "enamel location".

Adhesive System (etching mode)	MPa
Scotchbond Universal Adhesive (ER)	12.3 ± 1.8[a] [44/5]
Scotchbond Universal Adhesive (SE)	10.5 ± 0.8[ab] [44/4]
Adper Single Bond 2	8.1 ± 0.4[c] [40/3]
Clearfil SE Bond	8.7 ± 0.5[bc] [39/4]
Enamel location	**MPa**
Surrounding to carious cavities	9.3 ± 1.5[a] [76/8]
Sound enamel	10.5 ± 2.3[b] [91/8]

(*) Different superscript lowercase letters indicate statistically significant differences between rows ($p<0.05$).

5 DISCUSSÃO

Materiais e sistemas adesivos permitem a preservação da estrutura dentária devido suas propriedades adesivas, e, desse modo, têm sido amplamente utilizados por condizerem com o conceito de Odontologia Minimamente Invasiva (DHAR et al., 2015). Assim, pode-se dizer que as abordagens de mínima intervenção e adesiva andam lado a lado (FRENCKEN et al., 2012). Nesse contexto, os três artigos realizados interligam-se com o propósito principal de avaliar a performance *in vitro* de sistemas adesivos a um dos substratos envolvidos na técnica de RSTC, o esmalte adjacente à cavidade cariosa.

O artigo ***"Can area delimitation and tube type impact microshear bond strength of adhesives to dental substrates?"*** investigou parâmetros metodológicos do teste de resistência de união ao microcisalhamento, o qual é indicado para avaliação de substratos friáveis, como o esmalte, ou de materiais que podem ser afetados pela condição do teste ou pelo preparo da amostra (ARMSTRONG et al., 2010). Os resultados apresentados no artigo simplificam ainda mais o protocolo deste teste, pois sugere que a etapa de delimitação da área adesiva é dispensável, uma vez que não influenciou na resistência de união ao esmalte e à dentina, contrastando com estudo prévio (SHIMAOKA et al., 2011). Além disso, os achados referentes ao tipo de tubo utilizado para construir os espécimes em resina composta mostraram que este fator influenciou nos resultados de apenas um dos sistemas adesivos avaliados, o Single Bond Universal, e de forma diferente em cada substrato, sendo material e substrato dependente. Portanto, diante das deficiências técnicas relacionadas ao tubo de polietileno, como formação de bolhas de ar, transferência de estresse para a interface adesiva e falhas pré-teste durante a remoção do tubo (FOONG et al., 2006; TEDESCO et al., 2013), o uso do tubo de amido mostra-se vantajoso. Neste tubo é mais fácil inserir resina composta e ele pode ser removido sob um jato de água suave sem gerar estresse na interface adesiva (TEDESCO et al., 2013). Ainda, parece ser capaz de autolimitar a área adesiva, absorvendo o sistema adesivo e consequentemente, evitando sua presença além do diâmetro interno do tubo.

Os resultados apresentados pelo artigo ***"Is adhesive bond strength similar to primary and permanent teeth? A systematic review and meta-analysis"*** mostraram que, de modo geral, a resistência de união adesiva a dentes permanentes é superior aos dentes decíduos. Considerando separadamente os substratos, os sistemas adesivos apresentam maior resistência de união à dentina permanente quando comparada à dentina decídua. Porém, não houve diferença entre o esmalte decíduo e permanente, embasando a inclusão de somente dentes decíduos ou somente dentes permanentes em estudos que mensurem resistência de união a esse substrato. A relevância desses achados reside na necessidade de pesquisadores e fabricantes analisarem protocolos de aplicação de sistemas adesivos para dentes decíduos e dentes permanentes, separadamente, a fim de fornecer evidência para tomada de decisão clínica.

Faz-se importante enfatizar que a semelhança relatada entre o esmalte decíduo e permanente é válida apenas quando o esmalte for abrasionado. Além disso, apenas substratos hígidos foram considerados na metanálise, devido escassez de estudos selecionados que avaliaram também esmalte desmineralizado (TEDESCO et al., 2014) ou dentina desmineralizada (LENZI et al., 2012; SCHEFFEL et al., 2013). Em vista disso, a literatura carece de mais estudos que avaliem a performance de sistemas adesivos a substratos desmineralizados, já que essa condição parece influenciar a resistência de união (LENZI et al., 2012; SCHEFFEL et al., 2013; TEDESCO et al., 2014; NICOLOSO et al., 2016).

Também é importante considerar que a maioria dos estudos incluídos na revisão sistemática avaliou adesão à dentina, provavelmente por ser mais crítica a união a esse substrato devido sua natureza orgânica, quantidade significativa de água, estrutura tubular e presença de rede de colágeno (MARSHALL et al., 1997; PASHLEY, CARVALHO, 1997; PERDIGÃO, 2010). Do mesmo modo, tem-se direcionado mais atenção à dentina no que se refere à RSTC (LULA et al., 2009; 2011; MALTZ et al., 2012; 2013; FRANZON et al., 2014). Entretanto, a adesão ao esmalte também deve ser uma preocupação, pois a estabilidade da união dentina-resina (TORKABADI et al., 2009) e a eficácia do selamento marginal (HEINTZE, 2013) dependem da adesão ao esmalte da parede circundante à cavidade. Ademais, acredita-se que este esmalte desempenha um papel protetor contra a degradação da interface resina-dentina (DE MUNCK et al., 2003).

Considerando-se o exposto, fica clara a necessidade de se avaliar a condição do esmalte em que se estabelece a união. Neste contexto, em grande parte das situações clínicas nas quais realiza-se a abordagem operatória de lesões de cárie baseada na filosofia de mínima intervenção, o esmalte marginal pode estar desmineralizado, ser deixado de forma não intencional, e interferir na união dos materiais adesivos à cavidade. Em vista disso, o terceiro artigo, intitulado ***“Bonding of three adhesive systems to enamel surrounding real-life cavities after selective removal of carious tissue***”, objetivou avaliar a resistência de união de três sistemas adesivos ao esmalte circundante a cavidades cariosas naturais após RSTC em dentes permanentes. O uso de apenas dentes permanentes foi justificado pela revisão sistemática que compõe a presente tese e pela maior espessura do esmalte nesses dentes, permitindo a realização do teste de resistência de união ao microcisalhamento neste substrato. Ainda, a proposta de um estudo que avalie a performance de sistemas adesivos em esmalte adjacente a cavidades cariosas naturais é original e de relevância clínica.

Os resultados apresentados neste estudo demonstraram que a resistência de união de sistemas adesivos ao esmalte adjacente à cavidade de cárie, assumido como esmalte desmineralizado, foi menor do que ao esmalte hígido. Esses achados são corroborados por estudos que verificaram que o esmalte desmineralizado prejudica a resistência de união (TEDESCO et al., 2014; ANTONIAZZI et al., 2016). Acredita-se que isso se deve ao menor conteúdo mineral desse esmalte, maior porosidade da superfície (SILVERSTONE, 1973), com amplos espaços intercristalinos e, consequentemente, um maior volume de poros do que no esmalte hígido (PALAMARA et al., 1986; KIDD, FEJERSKOV, 2004), produzindo padrão de condicionamento e infiltração de monômeros resinosos insatisfatórios (TEDESCO et al., 2014).

Comparada à remoção completa de tecido cariado, a técnica de RSTC reduz os custos, a dor do paciente, o risco de danos à polpa dentária e mantém os dentes vitais por mais tempo (SCHWENDICKE et al., 2013; SCHWENDICKE, PARIS, STOLPE, 2014). Além do mais, é uma técnica menos demorada e considerada *user-friendly*, adjunta ao uso de sistemas adesivos universais. Diante das vantagens desta técnica, são necessários estudos clínicos que avaliem, como desfecho primário, falha restauradora após RSTC. Até o presente momento, as evidências que determinaram a longevidade de restaurações adesivas em dentes decíduos (FRANZON et al., 2015) ou permanentes (CASAGRANDE et al., 2017) tratados com RSTC são limitadas (RICKETTS et al., 2013; HOEFLER et al., 2016).

Portanto, na presente tese, são sugeridas reflexões e mudanças na abordagem operatória do esmalte circundante a cavidades submetidas à técnica de RSTC. Assim, uma vez que a perda mineral nem sempre é visível clinicamente, o esmalte com suspeita de perda mineral pode ser removido com instrumentos rotatórios em alta rotação antes dos procedimentos adesivos. Também, sugere-se que a presença de esmalte desmineralizado circundante a cavidades cariosas pode prejudicar a adesão e causar falhas em restaurações realizadas após RSTC.

Apesar de ser um estudo *in vitro*, consiste em uma importante ferramenta para predizer a performance clínica dos materiais (VAN MEERBEEK et al., 2010) e auxiliar na escolha da melhor opção a ser usada clinicamente. Assim sendo, estudos clínicos que avaliam o esmalte adjacente às cavidades submetidas à abordagem de remoção seletiva são necessários.

6 CONCLUSÃO

Com base nas investigações científicas apresentadas nessa tese, conclui-se que:

- A delimitação da área adesiva não influencia na resistência de união ao microcisalhamento ao esmalte e à dentina, independentemente do tipo de tubo (amido ou polietileno) usado na confecção dos espécimes em resina composta. A influência do tipo de tubo na resistência de união depende do sistema adesivo e do substrato. Assim, o tubo de amido mostra-se vantajoso, uma vez que facilita a inserção da resina composta, pode ser removido sob um jato de água suave sem gerar estresse na interface adesiva, e parece ser capaz de autolimitar a área adesiva, absorvendo o sistema adesivo e consequentemente, evitando sua presença além do diâmetro interno do tubo. Para tanto, visando a simplificação do teste de resistência de união ao microcisalhamento, recomenda-se utilizar o tubo de amido sem delimitar a área adesiva.
- A resistência de união de sistemas adesivos é maior em dentes permanentes que em dentes decíduos. Considerando separadamente os substratos, os sistemas adesivos apresentam maior resistência de união à dentina de dentes permanentes quando comparada à dentina de dentes decíduos. Essa diferença não é significativa em esmalte, dado que os valores de resistência de união foram similares ao esmalte de dentes decíduos e permanentes. Desse modo, a performance adesiva em dentes permanentes não pode ser diretamente extrapolada para dentes decíduos, sendo necessários protocolos adesivos específicos para cada tipo de dente, considerando suas diferenças microestruturais. Pesquisas sobre o desempenho de sistemas adesivos devem ser realizadas em dentes decíduos, em especial no substrato dentinário, a fim de fornecer informações úteis para a prática clínica baseada em evidências.
- A resistência de união ao esmalte circundante a lesões de cárie cavitadas submetidas à remoção seletiva de tecido cariado, assumido como desmineralizado, é menor do que ao esmalte hígido, independente do sistema adesivo utilizado. Assim, o esmalte com suspeita de perda mineral pode ser removido antes dos procedimentos adesivos a fim de evitar prejuízos a adesão e possivelmente contribuir para falhas restauradoras.

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ANEXO A – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO JOURNAL OF APPLIED BIOMATERIALS & FUNCTIONAL MATERIALS

SUBMISSION GUIDELINES

Manuscripts must be submitted online at www.editorialmanager.com/jabfm.

First-time users: Click the "Register" button from the menu and enter the requested information. You will receive an email with your username and password. Keep a copy of this information for future reference. If you wish to change any of the data in your profile, log in with your current username and password and update your profile. You only need to register once and should always use the same email address. If you think you have registered before please contact the Editorial Office before you register again.

We recommend that each time authors log in to the system, they check the email address registered for correspondence.

Submit your manuscript according to the Instructions to Authors. Refer to the Author Tutorial, which offers detailed instructions on initial and revised submission.

Once you have initiated a submission, you can leave it and continue later, as the system will save your information. The corresponding author will be able to track the progress of the submission through the system. Should you have any difficulties, please contact the Editorial Office.

You must submit separate files for the following:

- Manuscript file
- Figures
- Supplementary materials, if included in your manuscript
- Copyright Transfer and Author Responsibility Form

Manuscript preparation

Submissions need to be of sufficient editing quality in order that they are easily interpreted by the readership of the Journal. If submitted work does not meet this standard, it will be returned to the authors. The Journal follows the AMA Manual of Style for manuscripts submitted to biomedical journals.

Set your document as A4 (International Standard: ISO 216) paper, use double line spacing, Arial font size 12, number all pages, do not justify the right margin, and do not use line numbers. Save your manuscript as a Word document (.doc, .docx, or previous).

Structure your manuscript file as follows: Title page, Abstract and key words, Text, Acknowledgments, References, Tables, Figure legends.

Title page

The first page (title page) of your manuscript file must include the following information:

- Full title (max 135 characters including letters and spaces), which must be concise and informative.
- Short title (max 75 characters, including letters and spaces).
- All authors listed as first name, initials, and last name (i.e., Paul M. Smith) with highest academic or medical degree first.
- Institutional affiliation for each author, using superscripts and not symbols (e.g., Paul M. Smith[1]).
- Corresponding author's information (full mailing address, phone and fax numbers, email address); this is usually the submitting author.
- Online-only supplementary material, with a short description.
- If you are submitting a manuscript that has been rejected previously, please inform the Journal of the previous review comments, and subsequent revision of the manuscript.
- Manuscript word count (excluding figures and tables).

Abstract and key words

The abstract must not exceed 250 words and must be structured and divided in the sections indicated in each article type.

Below the abstract, identify 3 to 6 key words in alphabetical order under which you believe the article should be indexed. Use terms from the Medical Subject Headings list from lndex Medicus whenever possible. A library of terms is available at http://www.nlm.nih.gov/mesh/meshhome.html.

Manuscript text

Divide the text into the sections described above depending on content type. Use commas (,) to separate thousands and full stop (.) for decimals (e.g. 12,354.55). Include tables in the manuscript file, after the references. Number all figures (graphs, charts, photographs, and illustrations) in the order of their citation in the text. Figures must be submitted as separate files and not embedded in the Word document.

Units of measure

Laboratory values are expressed using conventional units of measure, with relevant Système International (SI) conversion factors expressed secondarily (in parentheses) only at first mention. Articles that contain numerous conversion factors may list them together in a paragraph at the end of the Methods section. In tables and figures, a conversion factor to SI units should be provided in a footnote or legend. The metric system is preferred for the expression of length, area, mass, and volume. For more details, see the Units of Measure conversion table on the website of the AMA Manual of Style.

Names of drugs, devices, and other products

Use non-proprietary names of drugs, devices, and other products, unless the specific trade name of a drug is essential to the discussion. In such cases, use the trade name once and the generic or descriptive name thereafter. Do not include trademark symbols.

Abbreviations

Use only standard abbreviations: the full term for which an abbreviation stands for should precede its first use in the text. Do not use abbreviations in the title. All abbreviations must be spelled out when they are used for the first time in the abstract and again when they are used for the first time in the text. Abbreviations should appear first in parentheses immediately after the term or phrase to which they refer. Every abbreviation used in any table or figure should be defined in each corresponding legend. Please refer to the AMA Manual of Style for a listing of acceptable abbreviations and acronyms.

Acknowledgments

List in this section:

- Any substantial contribution when provided by a person different from the author and list all other persons who do not fulfil authorship criteria.
- The assistance of medical writing experts.
- All participating group authors who do not meet the full authorship criteria.
- All sources of funding for the manuscript and the financial disclosures for all authors.

Written permission must be obtained to include the names of all individuals included in the Acknowledgments section.

If the manuscript has been presented at a meeting, please indicate in this section its name, location, and date.

References

Authors are responsible for the accuracy and completeness of their references and for correct text citation. Personal communications, unpublished data, abstracts, and oral or poster presentations should be limited and incorporated in parentheses within the text without a reference number. A signed permission should be included from each individual identified in a personal communication or as a source for unpublished data, as well as the date of communication.

References should follow the text and begin on a separate page.

- References must be double line spaced and numbered consecutively in order of appearance within the text, using the automated numbering tool of Word.
- Identify references in text, tables, and legends in Arabic numerals in parentheses, i.e. (7).
- List all authors when six or fewer; when seven or more, list only the first three and add et al.
- References used within tables or figure legends should be included in the reference list and numbered in consecutive order according to the table/figure citation in the text.
- Journals' names should be abbreviated according to Index Medicus/Medline. If there is any doubt about abbreviation of a journal name, it should be spelled out completely.
- Any references to studies (including books or articles) that have been accepted for publication, but not yet published, should indicate where they will be published and have the term "in press" in the reference in place of volume and page numbers. These must be updated prior to publication, if possible.

- Do not add a discussion or comment to a reference.
- Suffixes such as Jr, Sr, and III follow author's initials.

Tables

Submit tables in your manuscript file after references. Do not submit them as separate files. As a general rule, tables should not unnecessarily offer duplicate information given within the text. Starting on a new page, type each table on a separate sheet, using double line spacing. Tables should be created in a Word document using the table tool. Do not format tables as columns or tabs and do not submit tables as figures. Tables should be numbered consecutively in Roman numerals by order of citation in the text. Each table must include title, appropriate column headings, and explanatory legends, including definitions of any abbreviations used. References used within tables should be included in the reference list and numbered in consecutive order according to the table citation in the text. Identify statistical measures of variations such as SD and SEM. Follow the guidelines for creating tables.

Figure legends and legends for supplementary material

At the end of the manuscript, include a short title and a legend for each figure. When symbols, arrows, numbers, or letters are used to identify parts of the figures, identify and explain each one clearly in the legend. For photomicrographs, include the type of specimen, original magnification or a scale bar, and stain in the legend. For gross pathology specimens, label any rulers with unit of measure. Digitally enhanced images (CT/MRI, blots, photographs, photomicrographs, ultrasound images, x-ray films, etc.) must be clearly identified in the figure legends as digitally processed images. References used within figure legends should be included in the reference list and numbered in consecutive order according to the figure citation in the text.

Any figure that has been published elsewhere should have an acknowledgment to the original source; a copy of the permission to publish the figure, signed by the copyright holder, must accompany the submission.

Figures and illustrations

Number all figures (graphs, charts, photographs, and illustrations) in the order of their citation in the text. Include a title for each figure (a brief phrase, preferably no longer than 10-15 words). Do not embed figures in the Word document. Figures must be submitted as individual .jpg or .tif files and have a high enough resolution for publishing. Do not submit figures as Word, Powerpoint or PDF files.

Clinical photographs that identify an individual must be accompanied by a signed statement by the patient or legal guardian granting permission for publication of the pictures for educational purposes or must be masked to prevent identification of the patient.

Please refer to the Artwork Guidelines for more details.

Color figures

Authors may use color figures. If a manuscript has been submitted, reviewed, and accepted with color figures, then it MUST be published with color figures.

Image integrity

Preparation of scientific images (clinical images, radiographic images, micrographs, gels, etc.) for publication must preserve the integrity of the image data. Digital adjustments of brightness, contrast, or color applied uniformly to an entire image are permissible as long as these adjustments do not selectively highlight, misrepresent, obscure, or eliminate specific elements in the original figure, including the background.

Supplementary material

Authors may submit supplementary material to accompany their article for online-only publication. This material should be important to the understanding and interpretation of the report and should not repeat material within the print article. The amount of supplementary material should be limited and justified. Supplementary material should be original and not previously published and will undergo editorial and peer review with the main manuscript. Supplementary material must be listed on the title page of your submission and should be cited in the manuscript text in parentheses, in a similar way as when citing a figure or a table. Provide a legend for each supplementary material submitted.

If the manuscript is accepted for publication and if the supplementary material is deemed appropriate for publication by the editors, it will be posted online. This material will not be edited or formatted; thus, the authors are responsible for the accuracy and presentation of all such material.

Appropriate content

Tables
Expanded datasets and spread sheets, should be supplied in their original format or as PDF. Type text using Arial font size 10, and single line spaced. The table title should be set in Arial font size 12, and bold. Headings within tables should be set in Arial font size 10, and bold. Table footnotes should be set in Arial font size 8, and single line spaced. See also instructions for Tables. If a table runs on to subsequent pages, repeat the column headings at the top of each page. Wide tables may be presented using a landscape orientation.
Figures
Additional digitized figures and illustrations, should be supplied according to the technical specifications for figures.

REVIEW AND PUBLICATION PROCESS

Manuscripts submitted online at http://www.editorialmanager.com/jabfm are assigned a unique number upon submission and all correspondence and enquiries regarding the manuscript must include this ID number. Manuscripts that do not adhere to the guidelines for submission will be returned to the corresponding author for technical revision before undergoing the peer review process. Manuscripts with insufficient priority for publication will be rejected promptly.
All correspondence related to the submission, including editorial decisions, will be sent by email to the corresponding author. In case of revision, detailed revision instructions will be sent and a point-by-point response will be required. The Journal does not reveal the identity of its reviewers.
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Editing and page proofs
Accepted manuscripts will be edited by a professional style editor. The corresponding author will receive proofs, which will include all changes made by the style editor, and must be reviewed within 48 hours. It is the corresponding author's responsibility to ensure that there are no errors in the proofs.
Authorship changes including adding or removing authors cannot be submitted at proof stage. The publisher is not authorized to make such changes and it will delay the publication of your manuscript (see Authorship criteria).
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Offprints
Corresponding authors will receive an email including links to an offprint order form with the page proofs which can be shared with co-authors. Offprints are normally shipped 2 to 4 weeks after publication of the issue in which the article appears.
Errata, corrections, and retractions
In case an article has been published with major errors, it is often necessary to publish an erratum in the Journal. Requests to publish corrections should be sent to the Editorial Office.

ANEXO B – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO JOURNAL OF ADHESIVE DENTISTRY

GUIDELINES FOR AUTHORS

The Journal of Adhesive Dentistry is a bi-monthly journal that publishes scientifically sound articles of interest to practitioners and researchers in the field of adhesion to hard and soft dental tissues. The Journal publishes several types of peer-reviewed original articles:

SUBMISSION INSTRUCTIONS

Submission of manuscripts in order of preference:

1. Submission via online submission service (www.manuscriptmanager.com/jadd). Manuscript texts should be uploaded as PC-word files with tables and figures preferably embedded within the PC-word document. A broad range of file formats are acceptable. No paper version required but high resolution photographs or illustrations should be sent to the editorial office (see below). Online submissions are automatically uploaded into the editorial office's reviewer assignment schedule and are therefore processed immediately upon upload.

2. Submission via e-mail as a PC-word document (wintonowycz@quintessenz.de). Illustrations can be attached in any format that can be opened using Adobe Photoshop, (TIF, GIF, JPG, PSD, EPS etc.) or as Microsoft PowerPoint Documents (ppt). No paper version required but high resolution photographs or illustrations should be sent to the editorial office.

3. One paper copy of the manuscript plus a floppy diskette or CD-ROM (mandatory) containing a PC-word file of the manuscript text, tables and legends.

Figures should be included on the disk if possible in any format that can to be opened using Adobe Photoshop, (TIf, GIf, JPG, PSD, EPS etc.) or as a Microsoft PowerPoint Document (ppt)

Illustrations that cannot be sent electronically will be scanned at the editorial office so that they can be sent to reviewers via e-mail along with the manuscript to expedite the evaluation process. Resubmitted manuscripts should also be submitted in the above manner. Please note that supplying electronic versions of your tables and illustrations upon resubmission will assure a faster publication time if the manuscript is accepted.

Review/editing of manuscripts. Manuscripts will be reviewed by the editor-in-chief and at least two reviewers with expertise within the scope of the article. The publisher reserves the right to edit accepted manuscripts to fit the space available and to ensure conciseness, clarity, and stylistic consistency, subject to the author's final approval.

Adherence to guidelines. Manuscripts that are not pre pared in accordance with these guidelines will be returned to the author before review.

MANUSCRIPT PREPARATION

• The Journal will follow as much as possible the recommendations of the International Committee of Medical Journal Editors (Vancouver Group) in regard to
preparation of manuscripts and authorship (Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126: 36-47).

• **Title page.** The first page should include the title of the article (descriptive but as concise as possible) and the name, degrees, job title, professional affiliation, contribution to the paper (e.g., idea, hypothesis, experimental design, performed the experiments in partial fulfillment of requirements for a degree, wrote the manuscript, proofread the manuscript, performed a certain test, consulted on and performed statistical evaluation, contributed substantially to discussion, etc.) and full address of all authors. Phone, fax, and e-mail address must also be provided for the corresponding author, who will be assumed to be the first listed author unless otherwise noted. If the paper was presented before an organized group, the name of the organization, location, and date should be included.

• **3-8 keywords.**

• **Structured abstract.** Include a maximum 250-word structured abstract (with headings *Purpose, Materials and Methods, Results, Conclusion*).

• **Introduction.** Summarize the rationale and purpose of the study, giving only pertinent references. Clearly state the working hypothesis.

• **Materials and Methods.** Present materials and methods in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made. Indicate the statistical methods used, if applicable.

• **Results.** Present results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables or illustrations; emphasize only important observations.

• **Discussion.** Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results section. Relate observations to other relevant studies and point out the implications of the findings and their limitations.

• **Acknowledgments.** Acknowledge persons who have made substantive contributions to the study. Specify grant or other financial support, citing the name of the supporting organization and grant number.

• **Abbreviations.** The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.
• **Trade names.** Generic terms are to be used when ever possible, but trade names and manufacturer should be included parenthetically at first mention.
• **Clinical Relevance.** Please include a very brief (2 sentences or 3 lines) clinical relevance statement.

REFERENCES

• **All references must be cited** in the text, according to the alphabetical and numerical reference list.
• **The reference list** should appear at the end of the article, in alphabetical and numerical sequence.
• **Do not include unpublished data** or personal communications in the reference list. Cite such references parenthetically in the text and include a date.
• **Avoid using abstracts** as references.
• **Provide complete information** for each reference, including names of all authors. If the reference is part of a book, also include title of the chapter and names of the book‘s editor(s).

ILLUSTRATIONS

• All illustrations must be numbered and cited in the text in order of appearance.
• Submitted figures should meet the following minimum requirements:
– High-resolution images should have a width of 83 mm and 300 dpi (for column size).
– Graphics (bar diagrams, schematic representations, drawings) wherever possible should be produced in Adobe Illustrator and saved as AI or EPS files.
– All figures and graphics should be separate files – not embedded in Word or Power Point documents.

Upon article acceptance, high-resolution digital image files must be sent via one of the following ways:

1. As an e-mail attachment, if the files are not excessively large (not more than 10 MB), to our production department: Steinbrueck@quintessenz.de
2. Online File Exchange Tool: Please send your figures with our Online File Exchange Tool. This web tool allows you to upload large files (< 350.0 MB) to our server. Please archive your figures with a maximum size of 350 MB first. Then upload these archives with the following link: http://files.qvnet.de/JAD/, password: IAAD. Please name the archive with your name and article number so we can identify the figures.

Line drawings–Figures, charts, and graphs should be professionally drawn and lettered large enough to be read after reduction. Good-quality computer-generated laser prints are acceptable (no photocopies); also provide electronic files (eps, ai) if possible. Lines within graphs should be of a single weight unless special emphasis is needed.

Legends–Figure legends should be grouped on a separate sheet and typed double-spaced.

TABLES

• Each table should be logically organized, on a separate sheet, and numbered consecutively.
• The title and footnotes should be typed on the same sheet as the table.

MANDATORY SUBMISSION FORM

The Mandatory Submission Form, signed by all authors, must accompany all submitted manuscripts before they can be reviewed for publication. Electronic submission: scan the signed form and submit as JPG or TIF file.

PERMISSIONS & WAIVERS

• Permission of author and publisher must be obtained for the direct use of material (text, photos, drawings) under copyright that does not belong to the author.
• Waivers must be obtained for photographs showing persons. When such waivers are not supplied, faces will be masked to prevent identification. For clinical studies the approval of the ethics committee must be presented.

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