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PERFORMANCE IN VITRO E IN VIVO DE SISTEMAS ADESIVOS UNIVERSAIS EM DIFERENTES ESTRATÉGIAS DE CONDICIONAMENTO DA DENTINA

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 Materiais Dentários, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Ciências Odontológicas.**

Orientador: Prof. Dr. Fabio Zovico Maxnuck Soares

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RESUMO

PERFORMANCE IN VITRO E IN VIVO DE SISTEMAS ADESIVOS UNIVERSAIS EM DIFERENTES ESTRATÉGIAS DE CONDICIONAMENTO DA DENTINA

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A última geração de sistemas adesivos, os “adesivos universais” ou “multi-modo”, são adesivos que podem ser utilizados tanto na estratégia autocondicionante (SE) como com condicionamento ácido prévio (ER). No presente trabalho são apresentados dois artigos acerca destes materiais. O objetivo do primeiro artigo foi avaliar a resistência de união imediata e após 1 ano de adesivos universais em dentina hígida e afetada, usando duas estratégias de condicionamento – ER e SE. Os adesivos universais testados foram: Scotchbond Universal, All-Bond Universal e Prime&Bond Elect. Os controles foram os adesivos Adper Single Bond 2 e Clearfil SE Bond, para as estratégias ER e SE respectivamente. Foram aplicados seguindo as recomendações dos fabricantes e um bloco de resina composta (Z250) foi construído sobre a dentina. A dentina afetada foi obtida artificialmente através de ciclagem de pH por 14 dias. Espécimes em forma de palitos foram obtidos (0,8 mm²) para serem submetidos ao teste de microtração imediatamente ou após 1 ano de armazenamento. Os dados obtidos em MPa foram analisados por ANOVA de 3 fatores para dados repetidos e post-hoc Tukey (α= 5%). Significativa interação entre os fatores (material x estratégia x tempo) foi encontrada tanto para dentina hígida (p=0,002) como para dentina afetada (p=0,009). Pronunciada degradação foi observada para todos os grupos após um ano em dentina afetada. Em dentina hígida, somente os adesivos universais foram capazes de manter estabilidade da união após 1 ano somente no modo autocondicionante assim como os adesivos controle. Em dentina afetada, os adesivos universais não foram capazes de manter a estabilidade de união. Em dentina hígida, a estratégia autocondicionante parece ser a melhor maneira para manter a estabilidade da união. O segundo artigo é um estudo clínico randomizado que teve como o objetivo avaliar a longevidade de restaurações de lesões cervicais não-cariosas (LCNC), utilizando dois sistemas adesivos universais: Prime&Bond Elect (PB) e Scotchbond Universal (SBU) sob diferentes estratégias de aplicação (ER e SE). Foram selecionados 54 participantes conforme critérios de eligibilidade (possuir no mínimo 1 dente com LCNC, livre de cárie, não-retentiva, profundidade maior que 1mm). Foram excluídos os indivíduos que apresentaram dentes sem vitalidade pulpar, sem presença de dente antagonista, relato de restauração prévia, em tratamento ortodôntico, fumantes, portadores de bruxismo severo, higiene oral extremamente pobre e/ou com doença periodontal severa ou crônica. Duzentos e onze dentes foram restaurados conforme os grupos experimentais: PB-ER, PB-SE, SBU-ER e SBU-SE, com resina composta, por 2 operadores previamente treinados. As restaurações foram avaliadas por um examinador treinado e calibrado após 1 semana (*baseline)* e 6, meses, utilizando os critérios de avaliação USPHS modficado e FDI. Os dados obtidos foram submetidos aos testes de Kruskal-Wallis e Mann-Whitney para comparar as falhas entre os grupos experimentais. Diferença estatística foi encontrada entre os grupos em relação as falhas para os dois critérios de avaliação. O grupo PBSE apresentou significativamente mais falhas que os outros grupos experimentais. Dessa forma, a performance clínica do Prime & Bond Elect foi dependente da estratégia de aplicação, sendo que piores resultados foram encontrados para o modo autocondicionante.

Palavras-chave: Adesivos Dentinários. Dentina. Ensaio Clínico. Resistência à Tração.

ABSTRACT

IN VITRO AND IN VIVO PERFORMANCE OF UNIVERSAL ADHESIVE SYSTEM ON DENTIN WITH DIFFERENT ETCHING STRATEGIES

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The new generation of adhesive systems, called as "universal adhesives" or "multi-mode", are adhesives which can be used in both self-etch (SE) or etch-and-rinse (ER) strategies. Two studies related to these materials are presented. The aim of the first paper was to evaluate immediate and 1 year bond strength of universal adhesives to sound and caries-affected dentin, using both SE and ER strategies. The universal adhesives tested were: Scotchbond Universal, All - Bond Universal, Prime & Bond Elect. As controls were used the adhesives Adper Single Bond 2 and Clearfil SE Bond, for ER and SE strategies respectively. They were applied following the manufacturers' instructions and a composite resin (Z250) block was built on dentin. Caries-affected dentin was artificially obtained by pH cycling for 14 days. Stick-shaped specimens were obtained (0.8 mm^2) and submitted to microtensile test immediately or after 1 year of water storage. The data obtained in MPa were submitted to 3-way ANOVA for repeated measures and Post-hoc Tukey's test ($\alpha = 5\%$). Significant cross-interaction among the three factors (material x strategy x time) was found on both sound ($p=0.002$) and caries-affected dentin ($p=0.009$) analyses. Pronounced degradation was observed for all groups after one year on caries-affected dentin. In sound dentin, only the universal adhesives used in SE mode and the control adhesives were able to maintain bond stability after 1 year. Thus, the universal adhesives were not able to maintain bond stability in caries-affected dentin. On sound dentin, the self-etching strategy seems to be the best approach to maintain bond stability. The second paper is a randomized clinical trial that aimed to evaluate the longevity of non-carious cervical lesions restorations (NCCL) using two universal adhesive systems: Prime&Bond Elect (PB) and Scotchbond Universal (SBU) under different application strategies (ER and SE). Fifty-four participants who fit into the eligibility criteria (at least 1 tooth with LCNC, caries-free, non-retentive, with a depth greater than 1mm) were selected. Individuals who presented teeth without pulp vitality, without presence of an antagonistic tooth, previous restoration report, orthodontic treatment, smokers, patients with severe bruxism, extremely poor oral hygiene and / or with severe or chronic periodontal disease were excluded. Two hundred and eleven teeth were restored according to the experimental groups: PB-ER, PB-SE, SBU-ER and SBU-SE, with composite resin, by 2 previously trained operators. A single trained and calibrated examiner evaluated restorations after 1 week (baseline) and 6 months, using the modified USPHS and FDI evaluation criteria. The obtained data were tabulated and submitted to Kruskal-Wallis and Mann-Whitney tests to compare the failures among experimental groups. Statistical difference was found between the groups in regard to failures for both evaluation criteria. The PB-SE group had significantly more failures than the other experimental groups. Thus, the clinical performance of Prime & Bond Elect was dependent on the application strategy, and a poorer performance was found for the self-etch strategy.

Key-words: Clinical trial. Dentin. Dentin-bonding agents. Tensile Strength.

SUMÁRIO

1. INTRODUÇÃO

Atualmente, o maior desafio da odontologia adesiva é prover união efetiva, homogênea e duradoura aos tecidos dentários (CARDOSO et al., 2011). A adesão à dentina é mais complexa quando comparada à adesão em esmalte e, dessa forma, demanda procedimentos mais criteriosos, como por exemplo a manutenção de umidade adequada, consumindo maior tempo clínico de aplicação do sistema adesivo. Dessa maneira, a evolução dos sistemas adesivos procura oferecer materiais simplificados e menos sensíveis à técnica de aplicação (VAN MEERBEEK et al., 2011).

Os sistemas adesivos atualmente disponíveis dividem-se em sistemas de condicionamento ácido prévio (*etch-and-rinse*) e autocondicionantes (*self-etch*), que diferem significativamente na maneira em que atuam no substrato dental. Os adesivos autocondicionantes contém monômeros acídicos capazes de desmineralizar o substrato, não necessitando de um passo separado para condicionamento ácido, tornando a técnica mais simplificada (VAN MEERBEEK et al., 2011). Assim, os sistemas adesivos autocondicionantes não removem a *smear layer* (camada de lama dentinária) e sim, a incorporam à interface hibridizada (TAY; PASHLEY, 2001).

O interesse por sistemas adesivos mais simplificados e menos sensíveis a técnica levou ao desenvolvimento de materiais mais versáteis, disponíveis atualmente no mercado, chamados “adesivos universais” ou “multi-modo”, que são basicamente adesivos autocondicionantes de passo único, mas que podem ser utilizados tanto com condicionamento ácido prévio como na técnica autocondicionante (HANABUSA et al., 2012; PERDIGÃO; LOGUERCIO, 2014). Adicionalmente, os fabricantes recomendam a técnica de condicionamento seletivo do esmalte, que combina as vantagens do condicionamento ácido em esmalte, com a simplificada técnica autocondicionante em dentina (MARCHESI et al., 2014; PERDIGÃO; LOGUERCIO, 2014).

Estudos recentes avaliaram em laboratório diferentes sistemas adesivos universais, comparando também as estratégias de aplicação (autocondicionante e com condicionamento ácido prévio) em diferentes tipos de substrato (CHEN et al., 2015; LUQUE-MARTINEZ et al., 2014; WAGNER et al., 2014; MUÑOZ et al., 2015; MUÑOZ et al., 2013; VERMELHO et al., 2017; NICOLOSO et al., 2017; FOLLAK et

al, 2018). Rosa e colaboradores (2015), em uma revisão sistemática sobre a performance *in vitro* de adesivos universais, encontraram que o condicionamento ácido prévio melhorou a resistência de união em esmalte. Já em dentina, a estratégia de condicionamento parece não ter influência quando se utilizam adesivos com pH suave.

Recentemente uma atualização da revisão sistemática previamente citada demostrou que o comportamento dos sistemas adesivos universais foi dependente do pH, do substrato (esmalte ou dentina) e da estratégia utilizada (com condicionamento ácido prévio ou autocondicionante) (CUEVAS-SUÁREZ et al., 2019). A nova meta-análise considerou o envelhecimento dos espécimes, diferentemente do trabalho publicado anteriormente, e demonstrou que a estabilidade da união em dentina foi dependente do pH do sistema adesivo utilizado. Portanto, para adesivos classificados como pH suave (pH ≈ 2), a estratégia de condicionamento não teve influência sobre a estabilidade de união após envelhecimento. Já para adesivos ultra-suaves (pH ≥ 2.5), o condicionamento ácido prévio melhorou a performance imediata, porém após o envelhecimento, apenas a estratégia autocondicionante apresentou estabilidade de união à dentina. No entanto, para adesivos com pH mais baixos (pH ≈ 1.5), considerados intermediários, houve prejuízo na estabilidade da união a dentina para ambas as estratégias (CUEVAS-SUÁREZ et al., 2019).

Considerando isso, o comportamento desses adesivos universais necessita ser mais estudado em relação à estabilidade da adesão, mas parece haver influência tanto da composição como da estratégia de aplicação. Muñoz e colaboradores (2015), compararam a resistência de união à dentina de diferentes adesivos universais e, após 6 meses de armazenamento em água, aqueles que continham o monômero metacriloxiloxidecil dihidrogenio fosfato (MDP) em sua composição mostraram-se mais estáveis. A estratégia de aplicação também se mostrou um fator a ser considerado, pois Marchesi e colaboradores (2014) também encontraram melhor resultado (maiores valores de resistência de união) para um sistema que também continha MDP em sua composição, porém utilizado na estratégia autocondicionante, após 6 meses e 1 ano de armazenamento.

Yoshida e colaboradores (2012) observaram interação química entre o MDP presente no sistema adesivo e a hidroxiapatita do substrato dental formando uma nano-camada estável, aumentando a resistência mecânica dessa interface adesiva.

Ainda, a deposição de sal MDP-Ca ao longo de nano-camadas parece explicar a alta estabilidade da adesão (YOSHIDA et al., 2012). Apesar desses produtos alegarem versatilidade em relação a estratégia de aplicação, as diferenças na composição de cada adesivo podem ser o motivo dos diferentes resultados encontradas nos estudos até o momento. Dessa forma, é importante a realização de estudos laboratoriais para testar o comportamento desses novos adesivos, principalmente em relação à sua longevidade e em diferentes condições de substrato relevantes clinicamente, como dentina hígida e afetada.

A tendência atual da odontologia restauradora preconiza tratamentos mais conservadores, embasados em um número significativo de evidências clínicas que suportam tratamentos como a remoção seletiva do tecido cariado. Uma revisão sistemática com meta-análise demostrou que essa abordagem pode diminuir o risco de exposição pulpar e sintomas pós-operatórios, sendo vantajoso para o tratamento de lesões de cárie profundas (SCHWENDICKE; DÖRFER; PARIS, 2013). Porém, a dentina afetada por cárie que permanece após a remoção seletiva de tecido cariado tem características e composição diferentes comparadas à dentina hígida. Devido à perda mineral, a dentina intertubular afetada por cárie é mais porosa do que a dentina intertubular hígida (YOSHIYAMA et al., 2002). Sendo assim, a resistência de união ao substrato afetado por cárie tem mostrado valores mais baixos do que aqueles ao substrato hígido (ERHARDT et al., 2014; NAKAJIMA et al., 2005; PEREIRA et al., 2006; SCHOLTANUS et al., 2010; YOSHIYAMA et al., 2002).

Considerando a performance de sistemas adesivos universais em dentina afetada, Nicoloso e colaboradores (2017) demostraram que não houve influência da estratégia de condicionamento na resistência de união imediata, porém os resultados obtidos em dentina afetada foram significativamente inferiores quando comparados ao substrato hígido. O mesmo comportamento para as diferentes estratégias também pode ser observado em outro estudo, todavia, significante degradação na resistência de união à dentina afetada já pode ser observada precocemente após 6 meses de envelhecimento dos espécimes (FOLLAK et.al., 2018).

A grande maioria do conhecimento gerado sobre os sistemas adesivos universais é originado de estudos laboratoriais (CUEVAS-SUÁREZ et al., 2019; CHEN et al., 2015; HANABUSA et al., 2012; LUQUE-MARTINEZ et al., 2014; MARCHESI et al., 2014; MUÑOZ et al., 2015; MUNOZ et al., 2013; WAGNER et al.,

2014; ZHANG et al., 2016), no entanto, o quanto os resultados desses estudos *in vitro* podem estar relacionados ou o quanto podem predizer o comportamento desses materiais *in vivo* ainda permanece incerto (BAYNE, 2011; CARVALHO et al., 2011; VAN MEERBEEK et al., 2010). Apesar das pesquisas *in vitro* apresentarem a vantagem de avaliar fatores isolados, relacionadas diretamente com as propriedades do material, in vivo esses fatores ocorrem simultaneamente levando à degradação da interface ao longo tempo (DE MUNCK et al., 2005). Assim, os estudos clínicos representam uma metodologia mais apropriada para avaliar-se a eficácia clínica de sistemas adesivos (MUNCK; LANDUYT; PEUMANS, 2005).

Segundo as recomendações da American Dental Association (ADA), para se avaliar a eficácia clínica de um sistema adesivo, em um estudo clínico, as restaurações devem ser realizadas em lesões cervicais não-cariosas. ("American Dental Association Council on Scientific Affairs, Acceptance Program Guidelines: Dentin and Enamel Adhesive Materials", 2001). Tais lesões são preferíveis pois não proporcionam macro-retenção da restauração, ou seja, a retenção da restauração se dará exclusivamente pela adesão propiciada pelo sistema adesivo, e ainda, essas lesões tem alta prevalência e o procedimento restaurador é relativamente simples, sem a necessidade de preparo cavitário (CARVALHO et al., 2011).

No entanto, poucos estudos clínicos estão disponíveis sobre os sistemas adesivos universais (LAWSON et al., 2015; LOGUERCIO et al., 2015; LOPES et al., 2016; MENA-SERRANO et al., 2013; PERDIGÃO et al., 2014; PERDIGÃO et al., 2019; RUSCHEL et al., 2018), sendo três desses estudos referentes a mesma amostra, em diferentes tempos de acompanhamento (MENA-SERRANO et al., 2013; PERDIGÃO et al., 2014; LOGUERCIO et al., 2015). Lawson e colaboradores (2015) avaliaram um adesivo universal (Scotchbond Universal, 3M ESPE) nas duas estratégias de aplicação (com condicionamento ácido prévio e autocondicionante) comparado a um adesivo convencional de três passos, considerado como padrão-ouro (Scotchbond Multi Purpose, 3M ESPE). Em um acompanhamento de 24 meses, o adesivo universal no modo autocondicionante obteve performance similar ao adesivo padrão-ouro. Para a estratégia com condicionamento ácido prévio a performance foi superior ao do adesivo controle, apresentando menor descoloração marginal (LAWSON et al., 2015).

Em outro estudo clínico randomizado duplo-cego, o mesmo adesivo universal foi avaliado na estratégia de condicionamento ácido prévio com diferentes umidades

do substrato (dentina seca ou úmida), e com e sem condicionamento seletivo do esmalte no modo autocondicionante. Em um acompanhamento longitudinal de 36 meses, não houve diferença significativa entre as estratégias adesivas utilizadas, no entanto, sinais de degradação, como manchamento marginal, foram encontrados quando o adesivo foi utilizado na estratégia autocondicionante (LOGUÉRCIO et al., 2015).

Lopes e colaboradores (2016) avaliaram em um ensaio clínico randomizado um outro adesivo universal, Xeno Select (Denstply), em ambas estratégias adesivas e ainda a condição de umidade do substrato na estratégia de condicionamento ácido prévio (dentina seca e úmida) e o uso ou não de condicionamento seletivo do esmalte na estratégia autocondicionante. Em seis meses de acompanhamento, diferença na taxa de retenção das restaurações foi encontrada entre as diferentes estratégias de condicionamento, sendo que o pior comportamento do adesivo se deu na estratégia autocondicionante. Dessa forma, o adesivo Xeno Select na estratégia autocondicionante não preencheu os critérios estabelecidos para total aprovação pela American Dental Association (menos de 5% de falha das restaurações em 6 meses) (LOPES et al., 2016).

Tendo em vista que esses materiais são relativamente novos no mercado e que há uma grande variabilidade de produtos disponíveis, mais estudos devem ser conduzidos para que se possa conhecer melhor o comportamento desses sistemas adesivos em diferentes substratos, como dentina afetada e lesões não-cariosas, principalmente em razão de estudos prévios terem demostrado resultados material-dependente.

A partir do contexto exposto, a presente tese de doutorado está dividida em dois artigos:

O primeiro deles, um estudo *in vitro* intitulado **"*Self-etch approach of multi-mode adhesives as an alternative to minimize bond degradation on dentin over time*"**, avaliou a influência da estratégia de adesivos universais na longevidade da resistência de união à dentina hígida e artificialmente afetada por cárie.

O segundo artigo é um estudo clinico, intitulado "***Six-month behavior of universal adhesives in non-carious cervical lesions: a randomized clinical trial***", que teve como objetivo avaliar o desempenho de dois sistemas adesivos universais utilizados em diferentes estratégias na longevidade de restaurações de resina composta em lesões cervicais não-cariosas.

2. ARTIGO 1 – SELF-ETCH APPROACH OF MULTI-MODE ADHESIVES AS AN ALTERNATIVE TO MINIMIZE BOND DEGRADATION ON DENTIN OVER TIME

Este artigo será submetido ao periódico *The Journal of Adhesive Dentistry*, *Quintessence*, ISSN: 1461-5185, Fator de impacto = 1.311; Qualis A2. As normas para publicação estão descritas no Anexo A.

Self-etch approach of multi-mode adhesives as an alternative to minimize bond degradation on dentin over time

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Self-etch approach of multi-mode adhesives as an alternative to minimize bond degradation on dentin over time

Abstract

Purpose: To investigate the influence of etching strategy of multi-mode adhesive systems on bond degradation to sound and artificially-induced caries-affected dentin.

Materials and Methods: The universal adhesive systems (Scotchbond Universal Adhesive; All-Bond Universal; Prime & Bond Elect) and adhesives used as controls (Adper Single Bond 2 and Clearfil SE Bond) were applied to sound and artificially-induced caries-affected (pH cycling for 14 days) bovine dentin in the self-etch and etch-and-rinse strategies. Microtensile bond strength was evaluated immediately (24h), and after one year of water storage (1yr). Representative specimens were also prepared to assess the nanoleakage with SEM. Bond strength data (MPa) were analyzed using three-way repeated-measures ANOVA and post-hoc Tukey test (α= 5%), considering each substrate separately.

Results: Bonding degradation was observed for all multi-mode adhesive systems on caries-affected dentin, irrespective of the etching strategy. On sound dentin, bonding degradation was observed when adhesives were used on the etch-and-rinse strategy.

Conclusion: The multi-mode adhesive systems were not capable of maintaining bond stability over time on caries-affected dentin. The self-etch strategy seems to be better to maintain the durability of adhesive interfaces created on sound dentin.

Keywords: Multi-mode adhesive; Dentin; Caries-affected dentin; Microtensile bond strength; Longevity; Nanoleakage.

Introduction

The longevity of dentin bonding is currently the primary goal of adhesive dentistry. It is known that a relative durable hybrid layer is achieved with 3-step etch-and-rinse and 2-step self-etch adhesives.[22,38] However, the pursuit for more user-friendly materials and less technique-sensitive resulted in the development of simplified adhesive systems. The hydrophilic nature of these adhesives leads to some problems that could reduce the durability of adhesive-dentin interface by hydrolytic degradation of the hybrid layer. [33]

Bonding approach decision with adhesive systems was extended by the introduction of so-called universal or multi-mode adhesive systems. They usually consist in one-step self-etch adhesives, that can either be used on etch-and-rinse or self-etch strategies. This versatility claimed by the manufacturers allows the clinician to opt for the more suitable etching protocol for each clinical situation. [11,23]

The bond strength of multi-mode adhesives has been assessed in several studies. [4,11,14–16,30,35,39,46] The etch-and-rinse strategy seems to improve their bond strength to enamel.[26] On the other hand, dentin bonding is apparently not affected by the etching strategy,[26] but a consensus is not established on literature especially concerning bond stability. Several multi-mode adhesives also contain a functional monomer, as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which interacts chemically with ions calcium of hydroxyapatite.[33,34,41,44] The presence of 10-MDP on multi-mode adhesives seems to produce a stable chemical bond that improves the durability of the adhesive-dentin interface.[41]

The majority of the evaluation of multi-mode adhesive systems is performed in sound substrate. [4,11,14–16,30,39,46] Nevertheless, substrates as caries-affected dentin and caries-infected dentin are currently clinically relevant substrates. It is known that sound dentin and caries-affected dentin differs on microstructure and composition,[45] and inferior performance of adhesive systems was observed on caries-affected dentin.[3,6,7,18,24,28,45] Regarding multi-mode adhesives, only few studies so far assessed the bond strength to permanent caries-affected dentin.[9,19,20] The studies demonstrated that the etching strategy did not influence the immediate bond strength.[9,19,20] However, a question remains about the long-term performance of multi-mode adhesives on caries-affected dentin.

Therefore, the aim of this *in vitro* study was to evaluate the influence of etching strategy of different multi-mode adhesives on the bond degradation to sound and caries-affected dentin after 1 year of water storage. The null hypotheses tested were: 1) multi-mode adhesives bond strength is similarly using either etch-and-rinse or self-etch strategy, in both dentin condition; 2) water-aging does not influence on bond strength of multimode adhesives systems to sound and artificially-induced caries-affected dentin, irrespective of the etching strategies.

Materials and Methods

Study design

One hundred and twelve teeth were first divided into substrates – sound dentin and artificially induced caries-affected dentin. Thus, half of the selected teeth were randomly designated to sound dentin and the other half to artificially induced caries-affected dentin. For each substrate, the fifty-six teeth were allocated into eight groups according to the adhesive system and bonding strategy (n=7).

Tooth selection and preparation

Freshly extracted bovine mandibular incisors were stored in 0.5% aqueous chloramine T at 4°C for a maximum of thirty days and used in this study. The root portion was removed using a diamond disc in a low-speed handpiece. The buccal surfaces were ground under water-cooling using a 100-grit SiC paper in a polishing machine (EcoMet 250, Buehler, Illinois, USA) to expose flat dentin surfaces. Further, for both substrates (sound dentin and artificially induced caries-affected dentin), buccal surfaces were ground manually under water-cooling using 600-grit SiC paper for 60s to create a standardized smear layer.[1,37]

Artificial caries induction

Half of teeth were submitted to artificial caries induction by pH-cycling model[12,20,32]. Teeth were individually submitted to immersion for 8 hours in 10 milliliters of demineralizing solution (2.2 mM CaCl2, 2.2 mM NaH2PO4, 50 mM acetic acid, adjusted pH of 4.8 with 1M KOH) and for 16 hours in the same volume of remineralizing solution (1.5 mM CaCl2, 0.9 mM NaH2PO4, 0.15 mM KCl with adjusted pH of 7.0). Solutions were changed at every cycle for 14 days and the

solutions' pH was confirmed on each cycle using a digital pH-meter (Digimed, DM22; ServMed Analítica, Guarulhos, SP, Brazil).

Experimental design

Teeth from each dentin substrate (sound and caries-affected) were randomly reassigned using a website (www.sealedenvelope.com) into 8 subgroups according to the adhesive system and etching strategy (n=7). Three multi-mode adhesives systems were evaluated: Scotchbond Universal - SU (3M ESPE; St Paul, MN, USA), All-Bond Universal - AB (Bisco; Schaumburg, IL, USA) and Prime & Bond Elect - PB (Dentsply Caulk; Milford, DE, USA). All materials were applied on dentin surfaces in either a self-etch (SE) or etch-and-rinse (ER) protocol. As control groups for each strategy, a two-step etch-and-rinse Adper Single Bond Plus - SB (3M ESPE; St Paul, MN, USA) and a two-step self-etch Clearfil SE Bond – CS (Kuraray Noritake Dental; Tokyo, Japan) were used.

Bonding and restorative procedures

A single trained operator applied the adhesive systems on dentin surfaces strictly under manufacturers' instructions (Table 1). After hybridization, a block (10 mm x 7 mm x 5 mm approximately) of resin composite (Filtek Z250, shade A2; 3M ESPE, St. Paul, MN, USA) was build up in three increments on dentin surfaces. Each increment was light-cured for 20s using a light-emitting diode curing unit (Emitter B, Schuster; Santa Maria, RS, Brazil) delivering 800 mW/cm^2, checked with a radiometer (Demetron Research Corp, Danbury, CT, USA) every three blocks. All specimens were stored in distilled water at 37ºC for 24 h.

Microtensile Bond Strength (μTBS)

Specimens were sectioned in two perpendicular axes under water cooling with a diamond saw in a cutting machine (Labcut 1010, Extec Co, Enfield, CT, USA) obtaining stick-shaped specimens with a cross-sectional area of approximately 0.8 mm^2 measured individually with a digital caliper (Carbografite, Petrópolis, RJ, Brazil). Then specimens from each tooth were randomly assigned to be tested immediately (24h) and after one year (1yr) of water storage.

For microtensile testing, specimens were fixed to a testing jig (Odeme Medical and Dental; Joaçaba, SC) with cyanoacrylate glue (Three Bond Super Gel,

ThreeBond, Diadema, SP, Brazil) and submitted to microtensile test by a single and blinded operator in a universal testing machine (EMIC DL in 1000, Equipment and Systems Ltda; São José dos Pinhais, PR, Brazil) with a load cell of 10 N at a crosshead speed of 1 mm/min until fracture. Specimens that failed before the test, during cutting or fixing procedures, were recorded as pre-testing failures (PTF) and included in the bond strength means. All fractured specimens were observed under a 40x magnifying stereoscope (Discovery.v20, Zeiss; Oberkochen, Germany) to identify and classify the type of failure as adhesive/mixed (failure at the resin–dentin interface or mixed with cohesive failure of the neighboring substrate) or cohesive (dentin or resin). Cohesive failures were excluded from the bond strength analysis.

Nanoleakage evaluation

Two specimens from each subgroup (adhesive and etching strategy) and 24h and 1 year of water storage were randomly selected for nanoleakage evaluation. The selected specimens were coated with two layers of nail varnish applied up to 1 mm of the bonded interfaces. The specimens were then rehydrated in distilled water for 10 min before immersion in the tracer solution for 24 h. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al.[31] The sticks were placed in ammoniacal silver nitrate in darkness for 24 h, rinsed thoroughly in distilled water and immersed in photo-developing solution for 8 h under fluorescent light to reduce silver ions to metallic silver grains in voids along the bonded interface. All specimens were wet polished with 600-grit silicon carbide abrasive paper to remove the nail varnish. Specimens were subsequently placed parallel to each other on double-sided adhesive tape, inside an acrylic ring and then embedded in epoxy resin (Redelease; São Paulo, Brazil). The specimens were polished down to 2500-grit SiC paper and 0.25 μm diamond paste (Risitec; São Paulo, Brazil) using a polishing cloth. The specimens were ultrasonically cleaned with distilled water after each polishing procedure. Finally, the specimens were air-dried, gold coated and analyzed in a scanning electron microscope (Vega 3, Tescan; Czech republic) operated in backscattered electron mode with 20 kV voltage. Images of each specimen interface were obtained with 1000X magnification and analyzed qualitatively by one blinded examiner.

Statistical analysis

The experimental unit in the study was the tooth. Thus, the means of μTBS (MPa) values of specimens tested at 24h or 1yr were averaged for statistical purposes. The sample size had been determined considering that a mean difference of 20% among groups, and expecting a variation coefficient of 20%, a minimum of 7 teeth per group was required to achieve a power of 0.8 and an α-error probability of 5%.

Analyses were performed separately for each substrate condition and three factors were considered in statistical analysis: adhesive system, etching strategy and storage time (24h and 1yr).

Normal distribution of the data was confirmed by the Kolmogorov-Smirnov test so the bond strength means were submitted to three-way repeated-measures Analysis of Variance (ANOVA) and post-hoc Tukey tests at a significance level of 0.05, using a statistical software package (Minitab, Minitab Inc., State College, PA, USA). Pre-testing failures were included in the bond strength means with a value of 0 (zero).[25,36]

Results

Significant cross-interaction among the three factors (material x strategy x time) was found on both sound (p=0.002) and caries-affected dentin (p=0.009) analyses. Table 2 presents the μTBS values (means and standard deviation) and contrasts found in the interactions separately to sound and caries-affected dentin.

On sound dentin no differences were found between etching strategies in each evaluation time (24h or 1 year) separately. For the control adhesives, the etch-and-rinse system (Adper Single Bond 2) showed significantly higher values compared to the self-etch adhesive system (Clearfil SE Bond). Significant bond strength reduction was observed for all multi-mode adhesives tested on etch-and-rinse strategy after one year of storage, on the other hand, control adhesives maintained stable.

On caries-affected dentin, also there was no difference between etching strategies considering the values obtained after 24h and 1 year, separately. Degradation over time was pronounced and significant for all groups, except for

Prime & Bond Elect on self-etch strategy, where no significant reduction in bond strength values was observed after 1 year of storage.

Interfacial failure pattern (failure at the resin/dentin interface) was predominant for all experimental groups, except for Adper Single Bond 2 on sound dentin after 1 year of water storage. Cohesive failures (resin or dentin) were more evident in sound dentin and increased in 1-year groups (Figure1). Pre-testing failures were more frequent on caries-affected dentin compared to sound dentin.

For the nano-leakage evaluation, representative SEM images of resin/dentin interfaces of each experimental group are illustrated in Figure 2. The nanoleakage test was performed for both dentin substrates. However, was not possible to obtain SEM images of experimental groups on caries-affected dentin, due to the weak resin/dentin interface of aged specimens, which failed during the preparation for nano-leakage test.

Qualitative analysis of nanoleakage on sound dentin showed silver nitrate infiltration for all experimental groups already on immediate assessment (Fig 2.a, c, e, g, i, k, m, o). The amount of silver nitrate uptake on 1-year evaluation was higher than the immediate (24h) for all adhesive systems on etch-and-rinse strategy (Fig 2.b, f, j, n). For Scotchbond Universal Adhesive on self-etch strategy (Fig 2.l) and Clearfil SE Bond (Fig 2.p), an increase of silver nitrate infiltration was observed after 1 year and the presence of water-trees protruding to the adhesive layer. The experimental groups All Bond Universal and Prime & Bond Elect, both on self-etch strategy (Fig 2.d and 2.h), seemed to maintain the amount of silver nitrate infiltration after 1 year of water aging.

Discussion

Multi-mode adhesive systems were developed in the concept of versatility for etching strategy and for different substrates. Recently, a study evaluated the performance of multi-mode adhesives on various substrates and demonstrated that the bond durability was dependent both on the type of substrate and adhesive system.[35] Thus, assessment of multi-mode adhesives on different clinical relevant substrates remains imperative, as only a few studies are available on literature regarding these adhesive systems and efficiency of bonding on different substrates.[9,19,35]

The current study assessed the bond strength of three multi-mode adhesives on sound and artificially induced caries-affected dentin. Bonding to caries-affected dentin is a pertinent topic that has been evaluated since the concept of minimally invasive dentistry was introduced.[3,8,18,24,28,29,45] Although multi-mode adhesive systems could be recommended for bonding on different substrates,[35] when evaluated on artificially induced caries-affected dentin in both etching strategies, the adhesives were not able to maintain bond stability over time. The multi-mode adhesives demonstrated accelerated degradation on this substrate, as bond strength significantly decreased after 1 year of water storage.

Caries-affected dentin showed to be an adverse substrate for bonding, even with newer developed adhesive systems. Several studies with different adhesive systems have reported lower bond strength values to caries affected dentin when compared to sound dentin.[3,6,7,18,24,28,45] This behavior could be explained by characteristics intrinsic to the substrate, as less mineral content and other morphological and chemical characteristics, which possibly affected the adhesive-dentin bond longevity.[18,43,45] A thicker and more porous hybrid layer has been found when bonding to caries-affected dentin,[18,45] which could explain the susceptibility to degradation on the interfaces created on this kind of substrate.

Regarding the etching strategy of multi-mode adhesives on caries-affected dentin, no influence on bond strength was found. Such behavior was also observed in previous studies, both on primary[13,20] and permanent artificially induced caries-affected dentin[9,19,20] at immediate evaluation. Besides, our study demonstrated that the etching strategy also had no influence on bond strength at long-term evaluation.

Considering the performance of multi-mode adhesives on sound dentin, the etching strategy had no influence on bond strength irrespective of storage time, which is in agreement with the previous studies[4,10,11,15,17,40]. Concerning degradation over time, significantly decrease on bond strength was found for all multi-mode adhesives used in the etch-and-rinse strategy on sound dentin, which means that exclusively the adhesives applied in the self-etch strategy and adhesive systems used as control were capable of maintaining bond stability over time. Some studies have also reported improved bonding effectiveness for multi-mode adhesives used in the self-etch approach.[15,16,46] Zhang and others showed that all universal adhesive systems tested in etch-and-rinse mode showed a significant decrease in bond strength values after 1 year of water storage.[46] Such behavior could be due to

incomplete penetration of resin monomers and consequent exposed collagen remains, possibly leading to degradation of the bonded interface.[2,42,46]

The presence of functional monomers, such as 10-MDP, in some multi-mode adhesive systems have been suggested to be capable of improving dentin bonding durability.[16,41,46] Recently, an *in vitro* study supported the concept of chemical stability of 10-MDP in self-etch adhesives, improving the durability of adhesive-dentin bonding.[41] However, the different compositions and concentrations of functional monomers of adhesive systems could lead to conflicting performances and bonding effectiveness.[41,45] In this study, the presence of 10-MDP seems to be advantageous for bonding durability only when the multi-mode adhesives (Scotchbond Universal Adhesive and All Bond Universal) were used in self-etch approach and for the 2-step self-etch gold-standard adhesive (Clearfil SE Bond). We hypothesized that the removal of available calcium by acid etching (etch-and-rinse strategy) might have prevented any potential chemical bonding to hydroxyapatite with 10-MDP.

Prime & Bond Elect was the only multi-mode adhesive tested that does not contain 10-MDP in composition. Although, this system is an acetone solvated HEMA free adhesive, unlike Scotchbond Universal Adhesive and All Bond Universal, which are ethanol-based adhesives. A recent study evaluated the bonded interface between Prime & Bond Elect and sound dentin observed no degradation on the hybrid layer after one-year of ageing when the system was applied in self-etch mode.[46] The authors speculated that higher vapour pressure of acetone might result in rapid solvent evaporation and less retention of residual water when compared to ethanol.[46] These findings are in agreement with the present results, as Prime & Bond Elect used in self-etch strategy presented no significant degradation after water storage.

The nanoleakage evaluation allows foreseeing possible defects at the resin-dentin interface, which might lead to bond degradation over time.[27] In the present study, the multi-mode adhesives used in etch-and-rinse strategy demonstrated an increase in silver nitrate infiltration after 1 year of water storage, what is in accordance to the bond strength values for these groups, since significant lower values were found at 1 year evaluation. However, for the multi-mode adhesives in self-etch strategy and the adhesives used as controls (Adper Single Bond 2 and Clearfil SE Bond) this pattern was not observed. Scotchbond Universal on self-etch approach, Adper Single Bond 2 and Clearfil SE Bond presented a higher amount of

silver nitrate uptake, but no statistical difference on bond strength was found between immediate (24h) and 1 year assessment. All Bond Universal and Prime & Bond Elect, both used in self-etch strategy, seemed to maintain the amount of silver nitrate infiltration after water storage, and concerning the bond strength values no difference was found for these groups after one-year evaluation. Considering that, it was not possible to associate the nanoleakage pattern and the bond strength values for some groups. These findings are following previous literature, which has shown a lack of correlation between nano-leakage expression and bond strength.[5,21]

Within the limitations of this *in vitro* study, the first null hypothesis can be accepted, since the etching strategy of multi-mode adhesives had no influence on the bond strength on sound or caries-affected dentin. Considering water aging, pronounced degradation was found for multi-mode adhesives in artificially induced caries-affected dentin. On sound dentin, the multi-mode adhesives presented degradation only when used in etch-and-rinse strategy. Thus, the second null hypothesis was partially rejected.

Conclusions

Multi-mode adhesives applied in artificially induced caries-affected dentin did not maintain bond stability over time. On sound dentin, the self-etch strategy is an approach to maintain the durability of adhesive-dentin interfaces over time.

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Clinical significance

When bonding caries-affected dentin with multi-mode adhesive systems, none etching mode was able to prevent degradation of the resin-dentin interface. However, when bonding to sound dentin, clinicians could opt to follow the self-etch strategy in order to minimize bond degradation over time.

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TABLES AND ILLUSTRATIONS

Table 1: Adhesive systems (manufacturers and batch number), composition and application mode*:

Adhesive system/ Batch	Composition	Application mode
All Bond Universal (Bisco Inc, Schaumburg, IL, USA) (1500000055)	Bis-GMA, 10-MDP, HEMA, ethanol, initiators, water	**SE:** 1. Dispense 1-2 drops into a clean well. 2. Apply two separate coats, scrubbing the preparation with a microbrush for 10-15 seconds per coat. Do not light cure between coats. 3.Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 seconds, there should be no visible movement of the adhesive. The surface should have a uniform glossy appearance. 4. Light cure for 10 seconds. **ER:** 1. Etch enamel and dentin using an etchant for 15 seconds. Rinse thoroughly. Remove excess water by blotting the surface with an absorbent pellet or high volume evacuation for 1-2 seconds, leaving the preparation visibly moist. 2. Apply adhesive as self-etch technique.
Prime Bond Elect (Dentsply Caulk, Milford, DE, USA) (140304)	Mono-, di- and trimethacrylate resins, PENTA, diketone, organic phosphine oxide, stabilizers, cetylamine hydrofluoride, acetone, water	**SE:** 1. Place 2-3 drops into a clean well. Immediately apply generous amounts of adhesive to thoroughly wet all the tooth surfaces. Agitate the applied adhesive for 20 seconds. Re-wetting of the microbrush may be required in order to coat the preparation for the full 20 seconds. 2. Remove excess solvent by gently drying with clean, dry air from a dental syringe for at least 5 seconds. Surface should have a uniform glossy appearance. 3. Light cure for 10 seconds. **ER:** 1. Apply Caulk 34% tooth conditioner gel. Condition enamel for at least 15 seconds and dentin for 15 seconds or less. Remove gel with aspirator and/or vigorous water spray and rinse conditioned areas thoroughly for at least 15 seconds. Remove rinsing water completely by blowing gently with an air syringe or by blot drying with a cotton pellet. 2. Apply adhesive as self-etch strategy.
Scotchbond Universal (3M-ESPE, St. Paul, MN, USA) (509806)	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler, Ethanol, Water, Initiators, Silane	**SE:** 1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 seconds. 2. Direct a gentle stream of air over the liquid for about 5 seconds until it no longer moves and the solvent is evaporated completely.3. Light-cure for 10 seconds. **ER**: 1. Apply etchant for 15 seconds. Rinse thoroughly and air dry or cotton pellet. Do not overdry! 2. Apply adhesive as in the self-etch strategy.
Adper Single Bond 2 (3M-ESPE, St. Paul, MN, USA) (N520165)	Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler, Ethanol, Water, Initiators	1. Apply etchant for 15 seconds. Rinse for 10 seconds. Blot excess water using a cotton pellet or mini-sponge. The surface should appear glistening without pooling of water. 2. Immediately after blotting, apply 2-3 consecutive coats of adhesive for 15 seconds with gentle agitation using a fully saturated applicator. Gently air thin for five seconds to evaporate solvents. 3. Light cure for 10 seconds.
Clearfil SE Bond (Kuraray Noritake Dental Inc., Tokyo, Japan) (Primer: 01233A Bond: 01865A)	PRIMER: 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, Water BOND: 10-MDP, Bis-GMA, HEMA Hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, Colloidal silica	**PRIMER:** 1. Dispense the necessary amount of PRIMER into a well of the mixing dish immediately before application. 2. Apply PRIMER to the entire cavity wall with a sponge or a disposable brush tip. Leave it in place for 20 seconds. Use caution not to allow saliva or exudate to contact the treated surfaces for at least 20 seconds. 3. After conditioning the tooth surface for 20 seconds, evaporate the volatile ingredients with a mild oil-free air stream. **BOND:** 1. Dispense the necessary amount of BOND into a well of the mixing dish. 2. Apply BOND to the entire surface of the cavity with a sponge or a disposable brush tip. 3. After application, make the bond film as uniform as possible using a gentle oil-free air stream. 4. Light-cure the BOND for 10 seconds with a dental curing light.

*According information provided by manufacturers; MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate; bis-GMA: bisphenyl-glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; PENTA: dipentaerytritol-penta-acrylate-monophosphate

Table 2. Bond strength mean values in MPa (standard deviation) for experimental groups* in Sound Dentin and Artificially Induced Caries-Affected Dentin [tested sps/pre-test failures]

		SOUND DENTIN (SND)		ARTIFICIALLY INDUCED CARIES-AFFECTED DENTIN (CAD)	
Material	Strategy	24h	1 yr	24h	1yr
AB	ER	50,3 (12,4)^{A,B} [52/0]	28,1 (13,6)^{C,D,E,F} [59/0]	13,1 (2,7)^{b,c,d} [46/14]	2,0 (0,1)^{e} [53/0]
	SE	34,7 (7,5)^{A,B,C,D,E,F} [53/2]	29,5 (8,1)^{B,C,D,E,F} [55/0]	22,5 (4,4)^{a,b} [55/5]	4,4 (3,2)^{d,e} [58/0]
PB	ER	42,3 (8,3)^{A,B,C,D} [62/2]	16,0 (8,5)^{F} [69/0]	19,5 (6,1)^{a,b} [49/10]	3,8 (1,8)^{d,e} [64/0]
	SE	26,2 (17,1)^{D,E,F} [48/14]	19,7 (9,7)^{E,F} [54/0]	16,3 (10,9)^{b,c} [41/22]	7,7 (6,4)^{c,d,e} [56/0]
SU	ER	48,0 (14,2)^{A,B,C} [53/4]	24,8 (15,1)^{D,E,F} [63/0]	21,3 (6,1)^{a,b} [56/5]	2,0 (0,1)^{e} [60/0]
	SE	40,1 (8,9)^{A,B,C,D,E} [55/2]	30,9 (9,9)^{B,C,D,E,F} [58/0]	17,7 (4,4)^{b,c} [55/5]	3,2 (1,4)^{d,e} [58/0]
SB		52,6 (11,8)^{A} [51/1]	44,4 (13,6)^{A,B,C,D} [62/0]	22,4 (6,4)^{a,b} [60/6]	3,6 (2,6)^{d,e} [65/0]
CS		26,5 (9,6)^{D,E,F} [52/18]	19,2 (3,9)^{E,F} [56/0]	29,2 (7,3)^{a} [58/4]	12,94 (11,4)^{b,c,d} [58/0]

*Different letters indicate statistically significant differences ($p<0.05$). Uppercase letters for comparison in SND and lowercase letters for comparison on CAD.

Figure 1: Fracture type distribution per experimental group

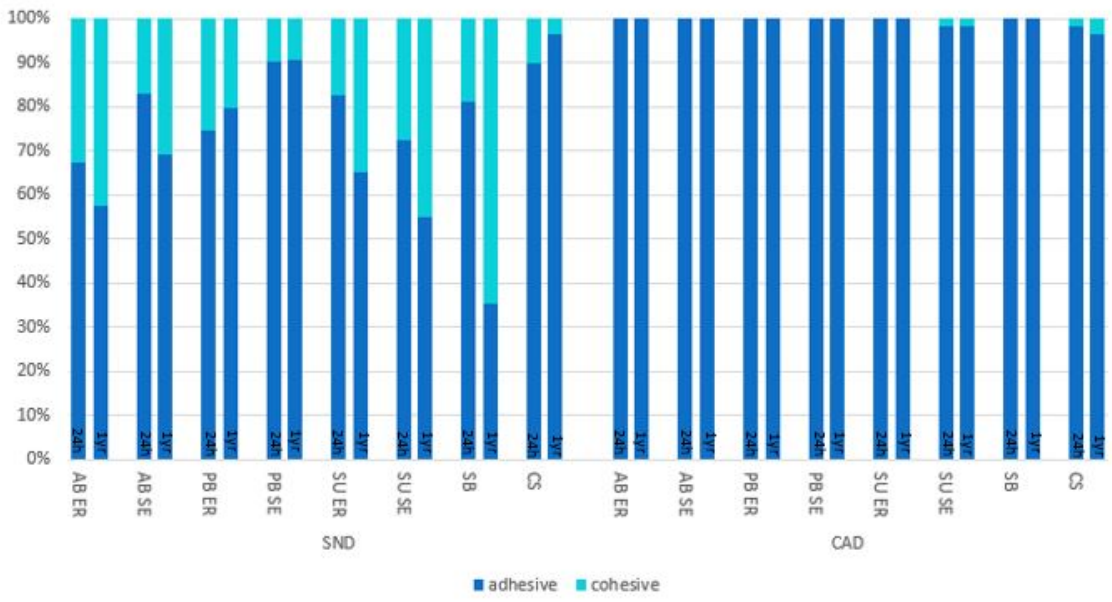
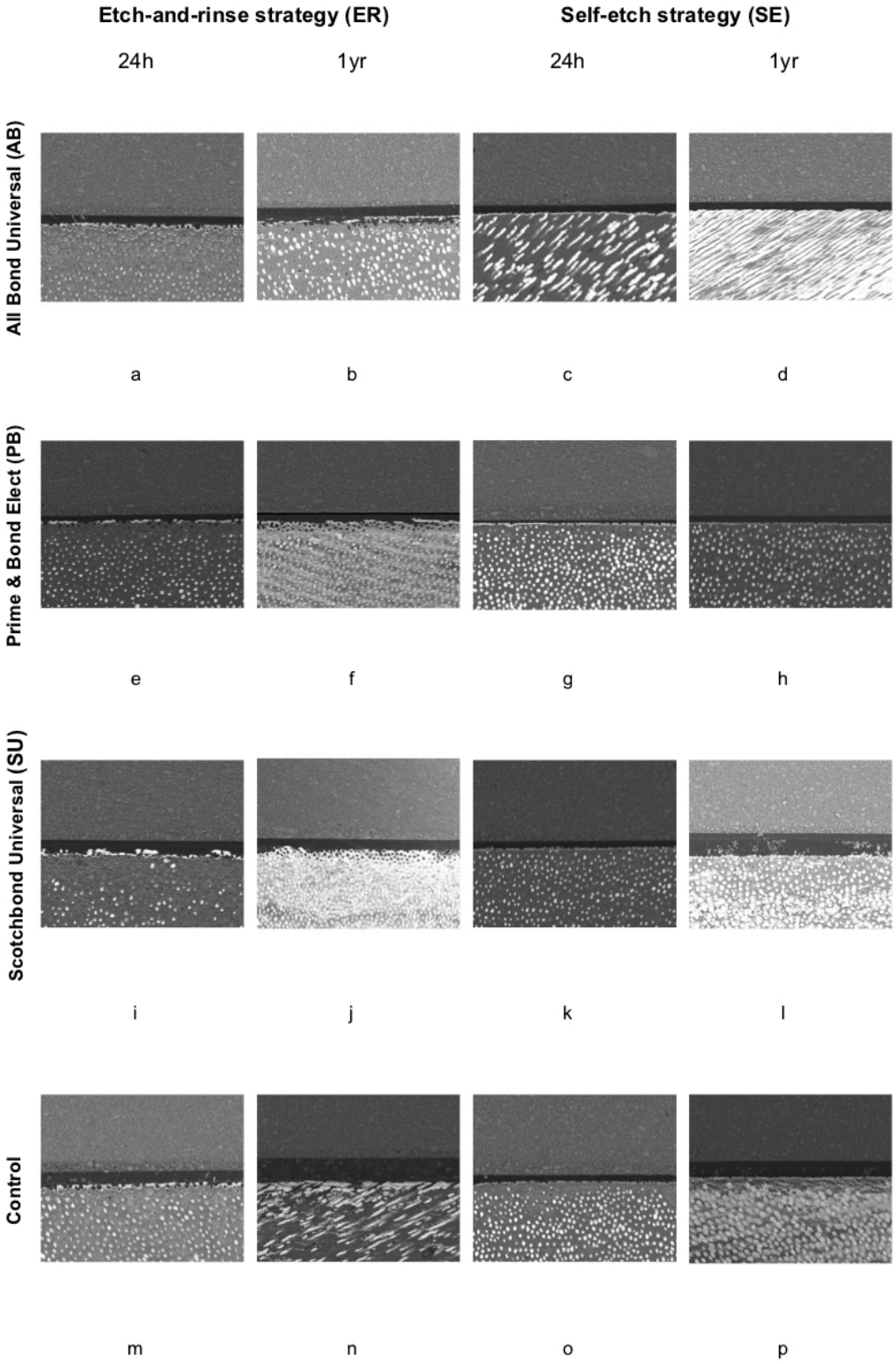


Figure 2. Representative SEM images (1000X magnification) of Nanoleakage expression on resin/dentin interfaces of each experimental group on Sound Dentin evaluated immediately (24h) and after 1 year of water storage.



3. ARTIGO 2 – SIX-MONTH BEHAVIOR OF UNIVERSAL ADHESIVES IN NON-CARIOUS CERVICAL LESIONS: A RANDOMIZED CLINICAL TRIAL

Este artigo será submetido a publicação no periódico Journal of Operative Dentistry, ISSN 0361-7734, fato de impacto 2.390, Qualis CAPES A1. As normas para publicação estão descritas no Anexo B.

SIX-MONTH BEHAVIOR OF UNIVERSAL ADHESIVES IN NON-CARIOUS CERVICAL LESIONS: A RANDOMIZED CLINICAL TRIAL

Running title: Six-month follow up of two universal adhesives on NCCLs

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Running title: Six-month follow up of two universal adhesives on NCCLs

SUMMARY

Objectives: This randomized double-blind clinical trial, parallel design, evaluated the behavior of two universal adhesives - used in etch-and-rinse (ER) or self-etch (SE) approach in non-carious cervical lesions (NCCLs).

Methods and Materials: The study was registered in the Brazilian Clinical Trial Registry (RBR-2GQMVF) and financed by CAPES (code-001). Two hundred and eleven NCCLs were selected in 54 participants and randomly allocated to one of four experimental groups: Scotchbond Universal Adhesive – SBU (3M Oral Care) and Prime & Bond Elect – PB (Denstply Sirona) used in both etching strategies (ER or SE mode). All resin composite restorations (Filtek Z250, 3M Oral Care) were placed by two trained operators. Restorations were evaluated at baseline and after 6 months using FDI and USPHS modified criteria, by a blinded calibrated examiner. Kruskal-Wallis and Mann-Whitney U non-parametric tests were used to compare the restoration failures among experimental groups.

Results: Statistical significant differences were found among groups regarding failures ($p=0.000$ for both FDI and USPHS criteria). PBSE presented more failures ($p<0.05$) than other experimental groups. No significant difference was found between any other pair of groups ($p>0.05$).

Conclusions: Prime & Bond Elect clinical performance on NCCLs depends on the etching strategy used, as self-etch mode presented a significant inferior performance after six-month follow-up.

Clinical relevance: The clinical behavior of mild universal adhesive Prime & Bond Elect depends on the bonding strategy used.

Keywords: Universal adhesive; Non-carious cervical lesions; FDI criteria; USPHS criteria.

Introduction

The interest for more simplified and less technique sensitive adhesive systems led to the development of more versatile materials, called "universal adhesives" or "multi-mode adhesives", which are basically single-step self-etching adhesives that can be used in both etch-and-rinse and self-etch strategies.[1,2] Additionally, manufacturers recommend the technique of selective enamel etching, which combines the advantages of acid etching in enamel, with the simplified self-etching technique in dentin.[2,3]

A great amount of in vitro studies of universal adhesives' performance on different substrates are available on literature.[4-12] A recent updated systematic review with meta-analysis showed that the enamel bond strength of universal adhesives was improved by the etch-and-rinse approach. Concerning bonding to dentin, prior acid etching showed to increase the bond strength when using ultra-mild or intermediately strong universal adhesives. Although, this behavior was not observed for mild universal adhesives, that showed bond strength stability for both strategies.[13]

According to American Dental Association, clinical trials of adhesive systems should be conducted in non-carious cervical lesions (NCCLs).[14] Such lesions are preferable due to the lack of macro-retention of the restoration, so the maintenance of restoration will be exclusively due to the performance of the adhesive system. Furthermore, these lesions have high prevalence in populations and the restorative procedure is relatively simple as there is no need to cavity preparation.[15]

Considering that universal adhesives have been recently introduced to the market, only a few clinical trials are available in the literature. Trials that evaluated performance of Scotchbond Universal showed no significant differences between bonding strategies after 24[16] and 36[17] months of clinical service. However, another trial of the same adhesive demonstrated that in 36-month evaluation the etch-and-rinse strategy improved the retention rate of the NCCLs' restorations.[18]

Studies that evaluated adhesives containing functional monomers other than MDP showed controversial results. Ruschel and others demonstrated similar clinical performance of Scotchbond Universal and Prime & Bond Elect on both etching strategies after 18 months of clinical service.[19] Other clinical trial demonstrated that the 6-month clinical behavior of Xeno Select was dependent of the bonding strategy, and when used on self-etch approach, the adhesive did not fulfill the ADA criteria for

full approval.[20]

Considering the heterogeneity of results so far available on literature, more clinical studies of these materials should be conducted, in order to elucidate their clinical performance. Therefore, the objective of this randomized, double-blind clinical trial, parallel design, was to evaluate the behavior of two universal adhesives with different functional monomers, applied in etch-and-rinse and self-etch strategies, on non-carious cervical lesions. The primary null hypothesis was that there was no difference in clinical behavior between the two universal adhesives, and secondary, no difference between etch-and-rinse and self-etch strategies in NCCLs restorations.

Materials and Methods

Ethical approval

The local Ethics Committee on Investigations Involving Human Subjects reviewed and approved this study (CAE 63280216.0.0000.5346). All participants were informed about the nature and objectives of the study and signed a statement of informed consent if they agreed to participate. The study was registered in the Brazilian Clinical Trial Registry (ReBEC; RBR-2GQMVF) and is reported following the recommendations of the CONSORT statement.[21]

Study design

This was a double-blind (evaluator and participants), parallel design, randomized multi-arm clinical trial, with four groups (two universal adhesives - Prime & Bond Elect and Scotchbond Universal Adhesive, in both etch-and-rinse and self-etch strategies).

Settings and Participants' recruitment

The study was conducted from August of 2017 to June of 2019, in a university setting at the School of Dentistry from Federal University of Santa Maria, Rio Grande do Sul, Brazil. Two trained examiners recruited the patients referred from de triage center if they met the eligibility criteria. Those who qualified for the study were invited to participate.

Eligibility criteria

The patients had to be in good general health (ASA I and II in classification by American Society of Anesthesiologists),[22] at least 18 years old, at least 20 teeth present in the mouth and capable of understanding and sign the written informed consent. The inclusion criteria were as the following: patients with 1) at least one non-carious cervical lesion (NCCL) in the buccal surface of incisors, canines or premolars, 2) lesions with a minimum of 1mm of depth, 3) absence of caries and previous restorations in the selected NCCL. Patients with NCCL in the non-vital tooth or a tooth without the antagonist, patients under active orthodontic treatment, with smoking habits, heavy bruxism, poor oral hygiene (gingival bleeding index exceeding 20%) and severe or chronic periodontitis were excluded.

Sample size calculation

The success rates of a previous study[16] that evaluated a multi-mode adhesive system in non-carious cervical lesions over 24 months was considered as a parameter for the sample size calculation. A sample size calculation was performed using a freely available online website (www.sealedenvelope.com) with the parameters: $\alpha = 5\%$, power of 80%, considering the outcome binary (success/failure) and equivalence study, with a limit of 10%. Considering the probability of losing some participant, the minimal sample sized was 55 restorations in each group.

Randomization and allocation concealment

The randomization process was performed using a website (www.sealedenvelope.com) by a researcher who was not involved in any other procedure of the study. Allocation concealment was maintained using opaque, sealed, and serially numbered envelopes. NCCLs were randomized in four experimental groups, according to the adhesive system and etching strategy. The group assignment sequence was unknown until the moment of the restorative procedure. The operators who performed the restorations were not blinded to group assignment, although participants and examiner were blinded to the experimental group.

Restorative procedures

Two trained operators performed the restorative procedures (A.F. and B.I.). Previously, the operators were trained by the study director (F.S.), who presented all restorative procedures followed by the restoration of some cavities with each adhesive system, in both etching strategy, under supervision. Any failure in the restorative procedure was pointed out and discussed before starting the study. All materials were used strictly according to the respective manufacturer's instructions (Table 1).

Following the composite resin shade selection, the participant had the tooth anesthetized (lidocaine 2% with epinephrine 1:100:000), and received the rubber dam isolation, except in cases where it was not possible to place due to the cavity margin location or dental anatomy aspects. In these cases, the restorations were performed using cotton rolls, high-power suckers, lip retractor, and gingival retraction wire. No additional retention or beveling was conducted, as recommended by the ADA.[14] At this point, the assistant (L.R.) defined the adhesive system and etching strategy to be used by opening the allocation envelope. After the adhesive procedures, composite resin (Filtek Z250, 3M Oral Care, St. Paul, MN, USA) was applied in at least two increments (not exceeding 2 mm thick). Each increment was light-cured for 20 seconds with a LED light curing-unit (Radii-cal, SDI, Bayswater, VI, AUS) at an irradiance of 800 mW/cm^2. All restorations were finished with diamond burs (3195FF, All Prime, Capanema, PR, BR) under water-cooling and polished with rubber points (SH3122, SH3123, SH3124, American Burrs, Palhoça, SC, BR) after seven days of restoration placement, by the same operator. Baseline for clinical assessment was considered after finishing and polishing procedures (seven days after restoration placement).

Clinical Assessment

The following information regarding individual and tooth-level variables were registered at the first appointment: 1) demographic data (age, sex, educational level, family income, skin color, dental service, parafunctional, dietary and oral hygiene habits); 2) degree of dentin sclerosis, according to the criteria described by Swift et al.[23] (Table 2); 3) cavity dimensions (height, width and depth), measured with a millimeter periodontal probe; 4) cavity shape, identified and classified as wedge or saucer-shaped;[24] 5) restoration margin, classified as supra-gingival,

gingival level or sub-gingival; 6) preoperative sensitivity, measured by blowing air from a dental syringe for 5 seconds at a 2 cm from the tooth surface, and identified by the patient in a scale ranged from 0 to 4.[24] All data were collected by two trained operators (A.F. and B.I.), calibrated for the degree of dentin sclerosis criteria (kappa = 0.89).

Outcomes

The primary outcome was the success of restoration. The World Dental Federation (FDI) [25] and modified US Public Health Service (USPHS) [26] criteria, as described in Tables 3 and 4, were used to define the restorations success.

A single trained and calibrated examiner (R.R.), blinded for the experimental groups and not involved in any other procedure of the study, performed the evaluations after seven days (baseline) and six months follow up. The training was based on the oral presentation of the criteria, followed by the discussion of photographic images. Subsequently, the examiner evaluated individually ten composite resin restorations of individuals treated at the dental clinics. The calibration was finished when the intra-examiner agreement Kappa value of at least 0.75 was obtained.[27]

Statistical analysis

Descriptive statistics were used to describe the demographic data of subjects, tooth, and NCCL. Restoration failure, according to the adhesive system and etching strategy after 6-month follow-up, was considered as the primary outcome. The restorations with FDI scores 4 and 5 and USPHS score C (Charlie) were considered as failure in the survival analysis. An additional analysis was carried out considering only the FDI score 5 as a failure, considering score 4 as a possibility of repair and not as a failure (repair analysis). Frequency distribution and cross-tabulation of each evaluation criteria were constructed. Kruskal-Wallis and Mann-Whitney tests were used to compare the restoration failure among experimental groups. Analyses were performed using Minitab 18 statistical software (Minitab Inc., State College, PA, USA) with a significance level of 5%.

The restoration retention rates were calculated according to the ADA guidelines.[14] Cumulative failure percentage = [(PF + NF)/(PF + RR)] x 100% (PF- number of previous failures before de current recall; NF- number of new failures at the current recall; RR- number of currently recalled restorations).[20]

RESULTS

Fifty-four subjects participated in the study (27 men and 27 women), mean age of 52.3 ± 11.5 (range 22 to 70 years). A total of 211 NCCLs were restored (mean of 3.9 NCCLs per subject) and evaluated at baseline and after six months. All demographic characteristics are displayed in Table 5.

The characteristics of NCCLs and their distribution per experimental group are described in Table 6. The majority of teeth restored were premolars, with a degree of dentin sclerosis 2 or 3. Almost 80% of NCCLs had 1 to 1.5 mm of depth, cervical-incisal height ranging from 2 to 4 mm and width of 3 to 4 mm. Ninety-two lesions were classified as wedge-shaped and 119 as a saucer-shaped lesion. Only few restorations had their margin at the supra-gingival level, and most of the teeth were restored with a rubber dam.

The retention rate after 6-months was 96% (98% for Prime & Bond Elect on etch-and-rinse mode; 86% for Prime & Bond Elect on self-etch mode; 100% for Scotchbond Universal Adhesive on etch-and-rinse mode and 100% for Scotchbond Universal Adhesive on self-etch mode) for both FDI and USPHS criteria. Considering all failures, 92,9% and 95,3% of restorations were considered as success for FDI and USPHS criteria respectively. When FDI score 4 was not considered as a failure (repair analysis), 95,3% of restorations were considered as success. The main reason for failure was retention (8 restorations failed), and failures on marginal adaptation (3), marginal pigmentation (1), surface gloss and roughness (1), occurrence of caries (1) and color match (1) were also observed. The number of evaluated restorations for each experimental group classified according to FDI and USPHS criteria are described in Table 7 and 8, respectively.

Statistical significant differences were found among groups regarding failure ($p=0.000$ for both FDI and USPHS criteria). Prime & Bond Elect on self-etch mode presented more failures ($p<0.05$) than other experimental groups. No significant difference was found between any other pair of groups ($p>0.05$). The number of accepted and failed restorations per group according to the FDI and USPHS criteria is reported in Table 9 and the number and percentage of restorations retention for each variable is described in Table 10.

DISCUSSION

Since the development of the universal adhesive systems, there are several studies regarding their performance. However, they are mainly in vitro research.[1,3-12] A recent updated systematic review of in vitro studies demonstrated that the enamel bond strength of universal adhesives is improved by previous acid etching and when bonding to dentin, the performance and stability were dependent of adhesives' pH.[13] These findings contradicts the manufacturers' statement on the use of universal adhesives, that is the adhesive system could be used in the etch-and-rinse or self-etch approach without jeopardizing the performance.[1,2]

Clinical studies in non-carious cervical lesions are recommended by the American Dental Association, to evaluate the performance of adhesive systems, mainly because these lesions are non-retentive and the restoration retention depends primarily on the adhesive performance.[14,15] Some clinical trials of universal adhesives in NCCLs have been published of many materials that differ from one another in aspects such as composition, pH, and presence of functional monomers.[16-20] However, there is still a lack of information on the long-term clinical performance of universal adhesives.

The present randomized clinical trial evaluated the performance of two different adhesive systems Prime & Bond Elect and Scotchbond Universal Adhesive after six months of clinical service in NCCLs. Fifteen restorations failed when evaluated with FDI criteria and 10 failed when USPHS criteria was used. When score 4 of FDI criteria was not considered as failure (possible repair), just ten restorations failed in both adhesive systems' group. The difference in the behavior of adhesives was found only for Prime & Bond Elect on self-etch approach, presenting poor bonding behavior among of the experimental groups. Thus, the null hypothesis of no difference between etch-and-rinse and self-etch strategies in NCCLs restorations could not be accepted.

The presence of functional monomers in adhesive systems, such as 10-MDP, could improve the bonding performance, since a chemical bond occurs to the calcium of hydroxyapatite present on enamel and dentin.[28] A systematic review showed that the chemical bonding potential of adhesive systems is important for the quality and durability of the bond in NCCLs, as better effectiveness of bonding were obtained with mild self-etching adhesives.[29]

Prime & Bond Elect contain in composition the phosphate ester monomer called dipentaerythritol penta acrylate monophosphate (PENTA). PENTA has a spatial molecular structure different from the molecule of MDP, being the first a linear molecule with the main chain with five vinyl groups, which turn the molecule more viscous.[30] Different in vitro studies showed similar performance of adhesives containing PENTA and those based on MDP. [4,12,31] Nonetheless, in the present study, the clinical behavior of the adhesives was different since PB in SE mode was significantly inferior to the other groups. There is evidence that PENTA can also chemically bond to the calcium of dental substrate; [32] however, this chemical interaction should be stable in an aqueous environment.[33] Also, the PENTA ester group is considered a weak acid. [20] Therefore, it can be hypothesized that the weaker behavior of Prime & Bond Elect on self-etch approach is due to a less stable chemical bond with dentin and insufficient acidity to achieve a durable bonding to NCCLs.

In the etch-and-rinse approach, Prime & Bond Elect performed similarly to the Scotchbond Universal Adhesive in both strategies. This behavior could be related to the fact during phosphoric acid etching, the calcium of the substrate is partially removed, and the bonding relies on micromechanical retention.[34] The comparable performance was found in another study, which evaluated six-month' performance of an adhesive with similar composition, and by the same manufacturer of Prime & Bond Elect. The authors showed an inferior performance of the adhesive tested on SE mode after six months of clinical service in NCCLs, concluding that the adhesive performance was dependent on the etching strategy used.[20]

On the other hand, Ruschel and collaborators demonstrated similar clinical performance between Prime & Bond Elect and Scotchbond Universal Adhesive, in both etch-and-rinse and self-etch approach, after 18-month follow-up in non-carious cervical lesions.[19] The different result from the present study could be because they roughened the lesions with diamond burs. A systematic review with meta-analysis showed that roughening enamel and dentin improve the durability of cervical restorations.[35] Our study followed ADA recommendations of not execute additional retention or bevel to lesions.[14]

Regarding the performance of Scotchbond Universal Adhesive, good clinical behavior was expected, since other studies with longer follow-up periods already demonstrated similar retention rates for both etching strategies.[16,17,19] Scotchbond

Universal Adhesive contains the functional monomer 10-MDP, which has been shown a chemical bond to hydroxyapatite.[33] Yoshida et al. observed an effective chemical interaction between the MDP present in the adhesive system and hydroxyapatite of dental substrate, forming a stable nanolayer and increasing the mechanical strength of this adhesive interface. Furthermore, deposition of MDP-Calcium salt along nanolayers seems to explain the high stability of adhesion.[28]

In the present study, both FDI and USPHS evaluation criteria were used. Recent publications that used both FDI and USPHS modified criteria concluded that FDI is more sensitive to small variations of clinical outcomes than USPHS when evaluating restorations in NCCLs.[17,36,37] Therefore, both criteria were used in order to allow future comparisons with other studies since FDI criteria were introduced recently, and the majority of published studies used USPHS as evaluation criteria.

Considering the six-month' clinical behavior of Prime & Bond Elect on self-etch approach, that was significantly inferior to the other experimental groups and did not fulfill to the ADA criteria to have less than 5% failure rate after six months of clinical performance.

CONCLUSIONS

The clinical behavior of universal adhesives with different functional monomers on non-carious cervical lesions can be depending on the bonding strategy.

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TABLES

Table 1. Adhesive systems (manufacturers and batch number), composition and application mode*

Adhesive system/ Batch	Composition	Application mode
Prime & Bond Elect (Dentsply Sirona, Milford, DE, USA) (140304)	Mono-, di- and trimethacrylate resins, PENTA, diketone, organic phosphine oxide, stabilizers, cetylamine hydrofluoride, acetone, water	**SE:** 1. Place 2-3 drops into a clean well. Immediately apply generous amounts of adhesive to thoroughly wet all the tooth surfaces. Agitate the applied adhesive for 20 seconds. Re-wetting of the microbrush may be required in order to coat the preparation for the full 20 seconds. 2. Remove excess solvent by gently drying with clean, dry air from a dental syringe for at least 5 seconds. Surface should have a uniform glossy appearance. 3. Light cure for 10 seconds. **ER:** 1. Apply Caulk 34% tooth conditioner gel. Condition enamel for at least 15 seconds and dentin for 15 seconds or less. Remove gel with aspirator and/or vigorous water spray and rinse conditioned areas thoroughly for at least 15 seconds. Remove rinsing water completely by blowing gently with an air syringe or by blot drying with a cotton pellet. 2. Apply adhesive as self-etch strategy.
Scotchbond Universal Adhesive (3M Oral Care, St. Paul, MN, USA) (509806)	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler, Ethanol, Water, Initiators, Silane	**SE:** 1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 seconds. 2. Direct a gentle stream of air over the liquid for about 5 seconds until it no longer moves and the solvent is evaporated completely. 3. Light-cure for 10 seconds. **ER**: 1. Apply etchant for 15 seconds. Rinse thoroughly and air dry or cotton pellet. Do not overdry! 2. Apply adhesive as in the self-etch strategy.
Filtek Z250 Universal Restorative System (3M Oral Care, St. Paul, MN, USA)	Bis-GMA, Bis-EMA, UDMA, TEGDMA, particles of zirconia and silica	Introduce the material into the cavity in increments up to 2mm thick, and light-cure for 20s, with light curing unit of at least 400mW/cm^2 intensity.

*According information provided by manufacturers; PENTA: dipentaerythritol penta acrylate monophosphate; MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate; BIS-GMA: bisphenyl-glycidyl methacrylate; Bis-EMA: Bisphenol A polyetheylene glycol diether dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; UDMA: urethane dimethacrylate; TEGDMA: tri[ethylene glycol] dimethacrylate

Table 2. Dentin Sclerosis Scale*

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

* adapted from Swift and others

Table 3. World Dental Federation (FDI) criteria used for clinical evaluation

	ESTHETIC PROPERTIES				
Score	**1.Surface gloss/luster and roughness**	**2. Surface staining**	**3. Marginal Staining**	**4. Color match/stability and translucency**	**5. Anatomic form**
1. Clinically very good (VG)	1.1 luster comparable to enamel	2.1 No surface staining	3.1 No marginal staining	4.1 Good color match. No difference in shade and translucency	5.1 Form is ideal
2. Clinically good (GO) -after correction very good	1.2 Slightly dull, not noticeable from speaking distance	2.2 Minor staining, easily removable	3.2 Minor marginal staining, easily removable by polishing	4.2 Minor deviations	5.2 Form is only affected
3. Clinically sufficient/satisfactory (SS)- (minor shortcomings, no unacceptable effects but not adjustable w/o damage to the tooth)	1.3 Dull surface but acceptable if covered with film of saliva	2.3 Moderate surface staining, also present on other teeth, not esthetically unacceptable	3.3 Moderate marginal staining, not esthetically unacceptable	4.3 Clear deviation but acceptable. Does not affect esthetics	5.3 Form differs but is not esthetically displeasing
4. Clinically unsatisfactory (UN)- but reparable	1.4 Rough surface, cannot be masked by saliva film, simple polishing is not sufficient. Further polishing is necessary	2.4 Surface staining present on the restoration and is unacceptable; major intervention necessary for improvement	3.4 Pronounced marginal staining; major intervention necessary for improvement	4.4 (Localized) clinically unsatisfactory but can be corrected by repair.	5.4 Form is affected and unacceptable esthetically. Intervention (correction) necessary
5. Clinically poor (PO)- replacement necessary	1.5 Quite rough, unacceptable plaque, retentive surface	2.5 Severe staining and/or subsurface staining (generalized or localized); not accessible for intervention	3.5 Deep marginal staining not accessible for intervention	4.5 Unacceptable. Replacement necessary.	5.5 Form is completely unsatisfactory and/or lost. Repair not feasible/reasonable, Replacement needed

Table 3. Continued.

	FUNCTIONAL PROPERTIES			BIOLOGICAL PROPERTIES		
Score	**6. Fracture/retention**	**7. Marginal adaptation**	**8. Patient's view**	**9. Postoperative sensitivity**	**10. Recurrence of pathology**	**11. Tooth integrity**
1. Clinically very good (VG)	6.1 Restoration retained, no fractures/crack	7.1 Harmonious outline, no gaps, no discoloration	8.1 Entirely satisfied	9.1 No hypersenstivity	10.1 No secondary or primary caries	11.1 Complete integrity
2. Clinically good (GO) -after correction very good	6.2 small hairline crack	7.2.1 Marginal gap (<150 µm) 7.2.2 Small marginal fracture removable by polishing	8.2 Satisfied	9.2 Low hypersensitivity for a limited period of time	10.2 Very small and localized demineralization; no operative treatment required	11.2.1 Small margin enamel split (<150 µm) 11.2.2 Hairline crack in enamel (<150 µm not probable)
3. Clinically sufficient/satisfactory (SS)- (minor shortcomings, no unacceptable effects but not adjustable w/o damage to the tooth)	6.3 Two or more or larger hairline cracks and/or chipping (not affecting the marginal integrity or proximal contact)	7.3.1 Gap < 250 µm not removable 7.3.2 Several small enamel or dentin fractures	8.3. Minor criticism of esthetics	9.3.1 Premature/slightly more intense 9.3.2 Delayed/weak sensitivity; no subjective complaints, no treatment needed	10.3 Larger areas of demineralization, but only preventive measures necessary (dentin not exposed)	11.3.1 Enamel split < 250 µm 11.3.2 Crack <250 µm; no adverse effects
4. Clinically unsatisfactory (UN)- but reparable	6.4 Chipping fractures with damage marginal quality or proximal contacts; bulk fractures with or without partial loss (less than half of the restoration)	7.4.1 Gap > 250 µm or dentin/base exposed 7.4.2 Chip/fracture damaging margins 7.4.3 Notable enamel or dentin wall fracture	8.4 Desire for improvement (reshaping of anatomic form or refurbishing etc.)	9.4.1 Premature/very intense 9.4.2 Extremely delayed/weak with subjective complaints 9.4.3 Negative sensitivity intervention necessary but not replacement	10.4 Caries with cavitation (localized and accessible and can be repaired)	11.4.1 Major enamel split (gap > 250 µm or dentin or base exposed) 11.4.2 Crack >250 µm; probe penetrates
5. Clinically poor (PO)- replacement necessary	6.5 (Partial or complete) loss of restoration	7.5 Filling is loose but in situ	8.5 Completed dissatisfied and/or adverse effects.	9.5 Very intense, acute pulpitis or nonvital; endodontic treatment is necessary and restoration has to be replaced	10.5 Deep secondary caries or exposed dentine that is not accessible for repair of restoration	11.5 Cusp or tooth fracture

Table 4. Modified USPHS criteria used for clinical evaluation

Score	1. Marginal staining	2. Anatomic form	3. Surface gloss/luster and roughness	4. Color match
Alfa (A)	No discoloration along the margin	The restoration is continuous with existing anatomic form	Smooth and glossy surface, similar to enamel	No color mismatch between restoration and tooth
Bravo (B)	Slight and superficial staining (removable, usually localized)	Generalized wear but clinically acceptable	Slight roughness, reverted by polishing	Slight mismatch
Charlie (C)	Deep staining cannot be polished away	Wear beyond the dentin-enamel junction	Rough and irregular surface, pitted or fractured	Obvious or gross mismatch

Table 4. Continued.

Score	5. Marginal integrity	6. Marginal adaptation	7. Retention	8. Recurrence of caries	9. Postoperative sensitivity
Alfa (A)	The explorer does not catch when drawn across the surface of the restoration across the tooth; or if the explorer does catch, there is no visible crevice along the periphery of the restoration	Restoration is continuous with existing anatomic form	Retained	No evidence of caries contiguous with the margin	No postoperative sensitivity directly after the restorative process and during the study period
Bravo (B)	The explore catches and there is visible evidence of a crevice, which the explore penetrates, indicating that the edge of the restoration; the dentin or base is not exposed and the restoration is not mobile	Detectable V-shaped defect in enamel only catches explorer going both ways	Partially retained	----------	----------
Charlie (C)	The explorer penetrate crevice defect extended to the dentin-enamel junction	Detectable V-shaped defect to dentin-enamel junction	Missing	Evidence of presence of caries	Sensitivity present at any time during the study period

Table 5. Characteristics of the subjects included into the study groups

Characteristics of research participants		Frequency - Nº (%)
Sex	Male	27 (50)
	Female	27 (50)
Age (years)	20-40	10 (18.5)
	41-60	31 (57.4)
	>60	13 (24.1)
Educational Level	Primary school	19 (35.2)
	High school	19 (35.2)
	Graduate	16 (29.6)
Family Income (BMW)*	<2 BMW	25 (46.3)
	2-4 BMW	16 (29.6)
	>4 BMW	13 (24.1)
Skin Color	White	45 (83.3)
	Black/brown	9 (16.7)
Dental Service	Out-of-pocket	11 (20.4)
	Private health insurance	3 (5.6)
	Public free	40 (74.1)
Parafunctional Habits	Yes	35 (64.8)
	No	19 (35.2)
Ingestion Of Staining Beverages	Yes	42 (77.8)
	No	12 (22.2)
Type Of Toothbrush	Soft	40 (74.1)
	Medium	13 (24.1)
	Firm	1 (1.9)
Toothbrushing Frequency Per Day	<2 times	10 (18.6)
	3 times	37 (68.5)
	>4 times	7 (13.0)
*BMW- Brazilian minimum monthly wage (± U$ 500)		

Table 6. Characteristics of NCCLs distributed in experimental groups

Characteristics of NCCL's		PBER	PBSE	SBUER	SBUSE
Tooth Distribution	Incisors	6 (2.8)	6 (2.8)	6 (2.8)	8 (3.8)
	Canines	5 (2.4)	6 (2.8)	6 (2.8)	12 (5.7)
	Premolars	42 (19.9)	38 (18.0)	42 (19.9)	34 (16.1)
Arc Distribution	Maxillary	17 (8.1)	25 (11.8)	24 (11.4)	33 (15.6)
	Mandibular	36 (17.1)	25 (11.8)	30 (14.2)	21 (10.0)
Degree of dentin sclerosis	1	1 (0.5)	5 (2.4)	6 (2.8)	3 (1.4)
	2	21 (10.0)	18 (8.5)	30 (14.2)	33 (15.6)
	3	23 (10.9)	21 (10.0)	16 (7.6)	17 (8.1)
	4	8 (3.8)	6 (2.8)	2 (0.9)	1 (0.5)
Degree of preoperative sensitivity	0-absent	32 (25.2)	33 (15.6)	27 (12.8)	27 (12.8)
	1-slight	12 (5.7)	9 (4.3)	15 (7.1)	12 (5.7)
	2- moderate	6 (2.8)	3 (1.4)	8 (3.8)	5 (2.4)
	3-considerable	3 (1.4)	4 (1.9)	2 (0.9)	7 (3.3)
	4-severe	0 (0)	1 (0.5)	2 (0.9)	3 (1.4)
Depth (mm)	1.0-1.5 -Flat	41 (19.4)	39 (18.5)	44 (20.9)	42 (19.9)
	1.5-2.0-Medium	10 (4.7)	8 (3.8)	10 (4.7)	10 (4.7)
	>2-Deep	2 (0.9)	3 (1.4)	0 (0)	2 (0.9)
Cervico-incisal height (mm)	<2	9 (4.3)	7 (3.3)	13 (6.2)	16 (7.6)
	2.0-4.0	42 (19.9)	40 (19.0)	40 (19.0)	36 (17.1)
	>4	2 (0.9)	3 (1.4)	1 (0.5)	2 (0.9)
Widht (mm)	<3	3 (1.4)	4 (1.9)	6 (2.8)	6 (2.8)
	3.0-4.0	45 (21.3)	39 (18.5)	43 (20.4)	40 (19.0)
	>4	5 (2.4)	7 (3.3)	5 (2.4)	8 (3.8)
Shape of lesion	U-Saucer shaped	32 (15.2)	28 (13.3)	34 (16.1)	25 (11.8)
	V-Wedge shaped	21 (10.0)	22 (10.4)	20 (9.5)	29 (13.7)
Restoration margin	Supra-gingival	5 (2.4)	4 (1.9)	8 (3.8)	8 (3.8)
	Gingival level	29 (13.7)	31 (14.7)	23 (10.9)	24 (11.4)
	Subgingival	19 (9.0)	15 (7.1)	23 (10.9)	22 (10.4)
Isolation method	Rubber dam	50 (23.7)	46 (21.8)	47 (22.3)	47 (22.3)
	Cotton rolls/ retraction cord	3 (1.4)	4 (1.9)	7 (3.3)	7 (3.3)

Table 7. Number of evaluated restorations for experimental group classified according to FDI criteria

Time		Baseline				6 months			
FDI criteria		PB-ER	PB-SE	SBU-ER	SBU-SE	PB-ER	PB-SE	SBU-ER	SBU-SE
1. Surface gloss/luster and roughness	VG	52	48	51	52	41	27	43	37
	GO	1	2	3	2	3	6	9	11
	SS	-	-	-	-	8	9	2	6
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	-	1	-	-
2. Surface staining	VG	53	50	54	54	50	41	53	54
	GO	-	-	-	-	1	-	-	-
	SS	-	-	-	-	1	2	1	-
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-				
3. Marginal staining	VG	53	50	54	54	51	34	52	52
	GO	-	-	-	-	1	6	2	2
	SS	-	-	-	-	-	3	-	-
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	-	-	-	-
4.Color match/ stability and translucency	VG	46	47	50	50	37	33	45	43
	GO	7	1	4	4	5	2	7	5
	SS	0	2	0	0	10	7	2	6
	UN	-	-	-	-	-	1	-	-
	PO	-	-	-	-	-	-	-	-
5. Anatomic form	VG	51	46	53	52	48	37	51	45
	GO	2	4	1	2	3	4	3	7
	SS	-	-	-	-	1	2	-	2
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	-	-	-	-
6. Fracture/ retention	VG	53	50	54	54	52	43	54	54
	GO	-	-	-	-	-	-	-	-
	SS	-	-	-	-	-	-	-	-
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	1	7	-	-
7. Marginal adaptation	VG	51	46	51	49	47	32	50	42
	GO	1	1	3	4	2	9	3	9
	SS	1	3	-	1	1	2	1	1
	UN	-	-	-	-	2	-	-	2
	PO	-	-	-	-	-	-	-	-
8. Patient's view	VG	53	50	54	54	52	43	53	54
	GO	-	-	-	-	-	-	1	-
	SS	-	-	-	-	-	-	-	-
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	-	-	-	-
9. Postoperative sensitivity	VG	52	50	54	53	52	43	54	52
	GO	1	-	-	-	-	-	-	1
	SS	-	-	-	1	-	-	-	1
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	-	-	-	-
10. Recurrence of pathology	VG	53	50	54	54	50	43	54	52
	GO	-	-	-	-	1	-	-	1
	SS	-	-	-	-	1	-	-	-
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	-	-	-	1
11. Tooth integrity	VG	53	50	54	54	52	43	54	54
	GO	-	-	-	-	-	-	-	-
	SS	-	-	-	-	-	-	-	-
	UN	-	-	-	-	-	-	-	-
	PO	-	-	-	-	-	-	-	-

Table 8. Number of evaluated restorations for experimental group classified according to USPHS criteria

Time		Baseline				6 months			
USPHS criteria		PB-ER	PB-SE	SBU-ER	SBU-SE	PB-ER	PB-SE	SBU-ER	SBU-SE
Marginal staining	A	53	50	54	54	49	31	53	50
	B	-	-	-	-	3	11	1	4
	C	-	-	-	-	-	1	-	-
Anatomic form	A	51	45	52	48	48	37	50	43
	B	2	5	2	6	4	6	4	11
	C	-	-	-	-	-	-	-	-
Surface gloss/luster and roughness	A	51	49	52	54	42	32	43	41
	B	2	1	2	-	10	11	11	13
	C	-	-	-	-	-	-	-	-
Color match	A	48	48	52	51	36	35	46	41
	B	5	2	2	3	16	8	8	13
	C	-	-	-	-	-	-	-	-
Marginal integrity	A	51	47	52	53	48	35	51	47
	B	2	3	2	1	4	8	3	7
	C	-	-	-	-	-	-	-	-
Marginal adaptation	A	53	50	54	53	52	42	53	50
	B	-	-	-	1	-	1	1	4
	C	-	-	-	-	-	-	-	-
Retention	A	53	50	54	54	52	43	54	54
	C	-	-	-	-	1	7	-	-
Recurrence of caries	A	53	50	54	54	52	43	54	53
	C	-	-	-	-	-	-	-	1
Postoperative sensitivity	A	53	50	54	54	52	43	54	54
	C	-	-	-	-	-	-	-	-

Table 9. Statistical analysis* and the number of acceptable (failed) restorations according to the FDI, USPHS and FDI (repair) after 6-month evaluation.**

Experimental group #	Criteria		
	FDI	USPHS	FDI (repair)
PB-ER	50 (3) A	52 (1) A	52 (1) A
PB-SE	41 (9) B	42 (8) B	42 (8) B
SBU-ER	54 (0) A	54 (0) A	54 (0) A
SBU-SE	51 (3) A	53 (1) A	53 (1) A
	p=0.004	p=0.000	p=0.000

* Kruskal-Wallis p-value. ** Different letter indicated significant differences between groups (Mann-Whitney, p<0.05).
# PB-ER = Prime & Bond Elect Etch-and-rinse; PB-SE = Prime & Bond Elect Self-etch; SBU-ER = Scotchbond Universal Etch-and-rinse; SBU-SE = Scotchbond Universal Self-etch

Table 10. Number and percentage of retention for each variable

Variable		With retention	No retention
Educational Level	Primary school	18 (94.7)	1 (5.3)
	High school	17 (89.5)	2 (10.5)
	Graduate	12 (75.0)	4 (25.0)
Family Income (BMW)*	<2 BMW	22 (88.0)	3 (12.0)
	2-4 BMW	15 (93.7)	1 (6.3)
	>4 BMW	10 (76.9)	3 (23.1)
Dental Service	Out-of-pocket	11 (100)	0 (0)
	Private health insurance	2 (66.7)	1 (33.3)
	Public free	34 (85.0)	6 (15.0)
Parafunctional Habits	Yes	32 (91.4)	3 (8.6)
	No	15 (78.9)	4 (21.1)
Tooth Distribution	Incisors	24 (92.3)	2 (7.7)
	Canines	29 (100)	0 (0)
	Premolars	150 (96.1)	6 (3.9)
Arc Distribution	Maxillary	98 (99.0)	1 (1.0)
	Mandibular	105 (93.8)	7 (6.2)
Degree of dentin sclerosis	1	14 (93.3)	1 (6.7)
	2	99 (97.0)	3 (3.0)
	3	74 (96.1)	3 (3.9)
	4	16 (94.1)	1 (5.9)
Degree of preoperative sensitivity	0-absent	116 (97.5)	3 (2.5)
	1-slight	46 (95.8)	2 (4.2)
	2- moderate	21 (95.4)	1 (4.6)
	3-considerable	14 (87.5)	2 (12.5)
	4-severe	6 (100)	0 (0)
Depth (mm)	1.0-1.5 -Flat	159 (95.8)	7 (4.2)
	1.5-2.0-Medium	37 (97.4)	1 (2.6)
	>2-Deep	7 (100)	0 (0)
Cervico-incisal height (mm)	<2	43 (95.5)	2 (4.5)
	2.0-4.0	152 (96.2)	6 (3.8)
	>4	8 (100)	0 (0)
Widht (mm)	<3	18 (94.7)	1 (5.3)
	3.0-4.0	160 (95.8)	7 (4.2)
	>4	25 (100)	0 (0)
Shape of lesion	U-Saucer shaped	114 (95.8)	5 (4.2)
	V-Wedge shaped	89 (96.7)	3 (3.3)
Restoration margin	Supra-gingival	25 (100)	0 (0)
	Gingival level	103 (96.3)	4 (3.7)
	Subgingival	75 (94.9)	4 (5.1)
Isolation method	Rubber dam	184 (96.8)	6 (3.2)
	Cotton rolls/ retraction cord	19 (90.5)	2 (9.5)

4. DISCUSSÃO

Ambos estudos apresentados anteriormente avaliaram a performance de adesivos universais utilizados nas diferentes estratégias de condicionamento recomendadas pelos fabricantes. Podemos considerar que esses materiais são basicamente adesivos autocondicionantes de um frasco que podem também ser utilizados com a estratégia de condicionamento ácido prévio (HANABUSA et al., 2012; PERDIGÃO; LOGUERCIO, 2014).

O primeiro artigo avaliou o comportamento *in vitro* de três diferentes sistemas adesivos universais (AllBond Universal, Prime & Bond Elect e Scotchbond Universal) em dois tipos de substrato, dentina hígida e dentina afetada. Já o segundo estudo avaliou a performance clínica de dois dos adesivos pesquisados no primeiro artigo (Prime & Bond Elect e Scotchbond Universal) após 6 meses, através de restaurações de lesões cervicais não-cariosas.

O estudo *in vitro* demonstrou que não houve influência do tipo de estratégia utilizada, independentemente do substrato e do tempo avaliado. Esse resultado está de acordo com estudos prévios, tanto para dentina afetada (LENZI et al. , 2015; NICOLOSO et al., 2016; NICOLOSO et al., 2017; FOLLAK et al., 2018) como para dentina hígida (CHEN et al., 2015; FOLLAK et al., 2018; HANABUSA et al., 2012; MARCHESI et al., 2014; MUÑOZ et al., 2013; WAGNER et al., 2014). Sendo assim, considerando o comportamento *in vitro* dos adesivos universais avaliados esses materiais podem de fato ser utilizados em ambas estratégias de condicionamento em dentina sem prejuízo nos valores de resistência de união, conforme recomendado pelos fabricantes.

Levando-se em conta o comportamento desses materiais após o envelhecimento em água, apenas os adesivos universais utilizados na estratégia autocondicionante e em dentina hígida mantiveram estabilidade nos valores de resistência de união. Esses resultados estão de acordo com o apresentado por uma recente revisão sistemática com meta-análise de 59 artigos de estudos *in vitro* com adesivos universais (CUEVAS-SUÁREZ et al., 2019). Os autores demonstraram que adesivos com pH ultra-suaves e suaves quando utilizados na estratégia autocondicionante são capazes de manter a estabilidade da união mesmo após envelhecimento (CUEVAS-SUÁREZ et al., 2019). O adesivo AllBond Universal é um

adesivo ultra-suave, e os adesivos Prime & Bond Elect e Scotchbond Universal são considerados adesivos de pH suave (CUEVAS-SUÁREZ et al., 2019).

No entanto, quando avaliada a performance dos adesivos universais em dentina afetada após 1 ano de envelhecimento *in vitro*, esses materiais não foram capazes de manter estabilidade da resistência de união em nenhuma das estratégias utilizadas. Pronunciada degradação da união desse substrato foi encontrada para todos os materiais avaliados, incluindo os sistemas adesivos utilizados como controle. Esse comportamento já foi bastante relatado em estudos anteriores (NAKAJIMA et al., 2005; WANG; SPENCER, 2007; YOSHYIAMA et al., 2002) e ocorre devido as características intrínsecas a esse substrato, como menor conteúdo mineral e outras características químicas e morfológicas. Adicionalmente, uma camada híbrida mais espessa e mais porosa foi exibida quando avaliada a união de sistemas adesivos à dentina afetada, o que pode explicar a suscetibilidade desse substrato à degradação (WANG; SPENCER, 2007; YOSHYIAMA et al., 2002).

Apesar dos resultados *in vitro* encontrados para esses materiais, o comportamento clínico dos mesmos ainda não está bem estabelecido, pois por serem materiais relativamente novos, poucos estudos clínicos estão disponíveis. Esses estudos tem demostrado resultados material-dependente (LAWSON et al., 2015; LOGUERCIO et al., 2015; LOPES et al., 2016; PERDIGÃO et al., 2019; RUSCHEL et al., 2018;), justificando assim a execução de novos estudos clínicos com diferentes materiais.

No segundo estudo, foram avaliados dois adesivos universais nas duas estratégias de condicionamento em restaurações de lesões cervicais não-cariosas. A justificativa da escolha desses dois materiais (PB e SBU) baseou-se na diferença de composição entre eles, principalmente em relação a presença de monômeros funcionais. O adesivo Prime & Bond Elect possui em sua composição o monômero PENTA (dipentaeritritol-penta acrilato fosfato) e o Scotchbond Universal apresenta o monômero MDP (metacriloxiloxidecil dihidrogenio fosfato). Alguns estudos *in vitro* têm demostrado que pode haver influência da presença do MDP na composição de alguns adesivos universais, melhorando a durabilidade da adesão dos mesmos à dentina (MUÑOZ et al., 2015; WANG et al., 2016; ZHANG et al., 2016). No entanto, as diferentes concentrações do monômero e a presença de outros componentes podem levar a resultados divergentes (WANG et al., 2017).

Diferentemente dos resultados encontrados no primeiro artigo, no estudo *in vivo* houve diferença entre as estratégias em 6 meses de acompanhamento das restaurações. O adesivo Prime & Bond Elect, quando utilizado na estratégia autocondicionante em lesões cervicais não-cariosas, obteve a pior performance, com significativa diferença na taxa de falha das restaurações após 6 meses de acompanhamento.

Como já citado anteriormente, esse sistema adesivo possui em sua composição o monômero PENTA, diferentemente do Scotchbond Universal que apresenta o MDP em sua composição. Há evidências de que o PENTA também possa interagir quimicamente com o substrato (LATTA et al., 2007), porém pode-se especular que talvez essa interação não seja tão estável em meio aquoso (YOSHIDA et al., 2004). A performance desse adesivo na estratégia de condicionamento ácido prévio pode ser explicada pelo fato de que quando realizamos o condicionamento com ácido fosfórico no substrato, menos mineral ficará disponível para uma possível adesão química, e assim a adesão formada será predominantemente micromecânica (VAN MEERBEEK et al., 2011).

O comportamento encontrado para o adesivo Scotchbond Universal nos 6 meses de avaliação já era esperado, uma vez que outros estudos clínicos com tempos mais longos de avaliação demostraram uma boa performance em lesões cervicais não-cariosas para esse adesivo, sem diferença entre as duas estratégias de condicionamento (LAWSON et al., 2015; LOGUERCIO et al., 2015; RUSCHEL et al., 2018;). O bom comportamento desse adesivo em estudos *in vitro* e *in vivo* vem sendo atrelado à presença do monômero MDP em sua composição (LAWSON et al., 2015; LOGUERCIO et al., 2015; MARCHESI et al., 2014; MENA-SERRANO et al., 2013; MUÑOZ et al. 2015; PERDIGÃO et al., 2014; RUSCHEL et al., 2018; WANG et al., 2016; ZHANG et al., 2016).

Considerando os resultados dos dois artigos, diferentes comportamentos foram encontrados para os sistemas adesivos quando testados *in vitro* e *in vivo*. Assim, o estudo *in vitro* não foi capaz de predizer o comportamento de todos os materiais testados clinicamente, corroborando o fato de que ainda é limitada a correlação entre os dois tipos de estudo (BAYNE, 2011; CARVALHO et al., 2011; VAN MEERBEEK et al., 2010).

5. CONCLUSÃO

Com base nos achados dos artigos que compõem a presente tese, pode-se concluir que há diferença na performance dos sistemas adesivos universais quando aplicados em diferentes substratos. No estudo *in vitro*, não houve diferença de comportamento quando utilizadas as diferentes estratégias de condicionamento independentemente do tempo e do substrato. Apesar disso, quando consideramos a degradação através do tempo, apenas os adesivos universais utilizados no modo autocondicionante e em dentina hígida foram capazes de manter a estabilidade de união após um ano de armazenamento em água. No entanto, diferença entre a estratégia de aplicação foi encontrada para um dos adesivos testados clinicamente em restaurações de lesões cervicais não-cariosas, na qual o modo autocondicionante do adesivo Prime & Bond Elect teve a pior performance após 6 meses de serviço clínico. Dessa forma, podemos concluir que o comportamento dos adesivos universais é material-dependente.

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ANEXO A – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *THE 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:

1***. Clinical and basic science research reports*** – based on original research in adhesive dentistry and related topics.
2. ***Reviews topics*** – on topics related to adhesive dentistry
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4a. ***Invited focus articles*** – presenting a position or hypothesis on a basic science or clinical subject of relevant related topics. These articles are not intended for the presentation of original results, and the authors of the articles are selected by the Editorial Board.
4b. ***Invited commentaries*** – critiquing a focus article by addressing the strong and weak points of the focus article. These are selected by the Editorial Board in consultation with the focus article author, and the focus article and the commentaries on it are published in sequence in the same issue of the Journal.
5. ***Invited guest editorials*** – may periodically be solicited by the Editorial Board.
6. ***Proceedings of symposia, workshops, or conferences*** – covering topics of relevance to adhesive dentistry and related topics.
7. ***Letters to the Editor*** – may be submitted to the editor-in-chief; these should normally be no more than 500 words in length.

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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 PCword 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)

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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.

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• **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.
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Journal reference style:
1. Turp JC, Kowalski CJ, Stohler CS. Treatment- seeking patters of facial pain patients: Many possibilities, limited satisfaction. J Orofacial Pain 1998;12:61-66.
Book reference style:
1. Hannam AG, Langenbach GEJ, Peck CC. Computer simulations of jaw biomechanics. In: McNeill C (ed). Science and Practice of Occlusion. Chicago: Quintessence, 1997:187-194.

ILLUSTRATIONS

• All illustrations must be numbered and cited in the text in order of appearance.
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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.

PAGE CHARGE

The first 8 printed pages in an article are free of charge. For excess pages, the charge is €140 per printed page. The approximate number of characters on a printed page is approximately 6,800. Please also consider the number and size of illustrations.

ANEXO B – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *JOURNAL OF OPERATIVE DENTISTRY*

Manuscript Submission (Author Guidelines)

General Requirements

Operative Dentistry requires electronic submission of all manuscripts. All submissions must be sent to Operative Dentistry using the Allen Track upload site. A mandatory and nonrefundable $50.00 fee is required at submission. Your manuscript will only be considered officially submitted after it has been approved through our initial quality control check, and any quality problems have been resolved. You will have 6 days from when you start the process to submit and approve the manuscript. After the 6 day limit, if you have not finished the submission, your submission may be removed from the server. You are still able to submit the manuscript, but you must start from the beginning. Be prepared to submit the following manuscript files in your upload:

- A Laboratory or Clinical Research Manuscript file must include: o a title

  o a running (short) title
  o a clinical relevance statement
  o a concise summary (abstract)
  o introduction, methods & materials, results, discussion and conclusion
  o references (see Below)

- The manuscript body **MUST NOT** include any:

  o Author identifying information such as:

  ▪ Authors names or titles

  - ▪ Acknowledgements
  - ▪ Correspondence information
  - ▪ Response to reviewer files should also NOT include any author identifying information, such as a signature at the end, etc.

    o Figures

    o Graphs

    o Tables

- An acknowledgement, disclaimer and/or recognition of support (if applicable) must be uploaded as a separate file and uploaded as

miscellaneous material.

- Appendix material that you would like us to publish electronically

with your article, but not as part of your printed manuscript (such as indices, supplemental tables, etc.), should be submitted as ***supplemental material***. It will not be typeset, and will appear exactly as you provide to Operative Dentistry. References submitted as part of supplemental material should appear in our preferred reference format. Supplemental material is viewable by the reviewers, and so SHOULD NOT contain any author identifiable information.

- All figures, illustrations, graphs and tables must also be provided as individual files. Figures should be submitted without figure letters or numbers within the image itself, these designations will be added by the journal staff as needed. All Figures should be high-resolution images, which are used by the editor in the actual typesetting of your manuscript. Please refer to the instructions below for acceptable formats and sizes.
- All other manuscript types use this template, with the appropriate changes as listed below.
- When figures of identifiable individuals are submitted, the author must verify that they have received releases from the individual or guardian to use said figure. Eye blocks are no longer sufficient to anonymize an individual. Eye blocks may still be used, but a release will still be required.
- ALL studies using human tissue must have an accompanying Institutional Review Board (IRB) statement – it must indicate that either the board has approved the study, or that the study is exempted from approval. There are no exceptions to this policy.
- All studies using animal tissue must have an accompanying approval from the appropriate ethics board.
- All manuscripts reporting on a Clinical Trial must indicate that the trial information was submitted to a public Clinical Trial Registry. A URL of where the trial appears in a registry is required to be submitted with the manuscript.

Complete the online form (which includes complete author information, copyright release and conflict of interest), and select the files you would like to send to Operative Dentistry. Manuscripts that do not meet our formatting and data requirements listed below will be sent back to the corresponding author for correction.

Important Information

- All materials submitted for publication must be submitted exclusively to Operative Dentistry.
- The editor reserves the right to make literary corrections.
- Currently, color will be provided at no cost to the author if the editor deems it essential to the manuscript. However, we reserve the right to convert to gray scale if color does not contribute significantly to the quality and/or information content of the paper.
- The author(s) retain(s) the right to formally withdraw the paper from consideration and/or publication for any reason up to the submission of the final paper to our press vendor for publication.
- International authors whose native language is not English must have their work reviewed by a native English speaker prior to submission.

o Manuscripts that are rejected before peer-review for English correction should be entered as a new manuscript upon resubmission. In the manuscript comments box the comment, “this is a resubmission of manuscript number XX-XXX” should be noted.

o Manuscripts that are rejected after peer-review are not eligible for resubmission.

o Manuscripts that have major revisions requested (i.e. For English correction) are entered as a resubmission of the original article.

- Spelling must conform to the American Heritage Dictionary of the English Language, and SI units for scientific measurement are preferred.
- While we do not currently have limitations on the length of manuscripts, we expect papers to be concise; authors are also encouraged to be selective in their use of figures and tables, using only those that contribute significantly to the understanding of the research.
- Acknowledgement of receipt is sent automatically upon acceptance through quality control. This may take up to 7 days. If you do not receive such an acknowledgement, please check your author homepage at http://jopdent.allentrack.net if the paper does not appear there please resend your paper.

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Manuscript Type Requirements All Manuscripts

CORRESPONDING AUTHOR must provide a WORKING / VALID e-mail address which will be used for all communication with the journal. ***NOTE:** Corresponding authors MUST update their profile if their e-mail or postal address changes. If we cannot contact authors within seven days, their manuscript will be removed from our publication queue.

AUTHOR INFORMATION must include: • full name of all authors

• complete mailing address for each author
• valid email address for each author
• degrees (e.g. DDS, DMD, PhD)
• affiliation (e.g. Department of Dental Materials, School of Dentistry, University of Michigan)

MENTION OF COMMERCIAL PRODUCTS/EQUIPMENT must include:

• full name of product
• full name of manufacturer
• city, state and country of manufacturer

MANUSCRIPTS must be provided as Word for Windows files. Files with the .doc and .docx extensions are accepted.

TABLES may be submitted as either Word (.doc and .docx) or Excel (.xls and

.xlsx) files. All tables must be legible, with fonts being no smaller than 7 points. Tables have the following size limitations: In profile view a table must be no larger than 7 x 9 inches; landscape tables should be no wider than 7 inches. It is the Editor's preference that tables not need to be rotated in order to be printed, as it interrupts the reader's flow.

ILLUSTRATIONS, GRAPHS AND FIGURES must be provided as **TIFF** or high resolution **JPEG** files with the following parameters:
• **line art** (and tables that are submitted as a graphic) must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 600 dpi and a maximum resolution of 1200 dpi. This means the shortest side should be no smaller than 3000 pixels.

• **gray scale/black & white figures** must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means the shortest side should be no smaller than 1500 pixels.

• **color figures and photographs** must be sized with the short edge being no shorter than 3.5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means that the shortest side should be no smaller than 1050 pixels.

Other Manuscript Type – Additional Requirements

CLINICAL TECHNIQUE/CASE STUDY MANUSCRIPTS must include as part of the narrative:

• a running (short) title
• purpose
• description of technique
• list of materials used
• potential problems
• summary of advantages and disadvantages • references (see below)

LITERATURE AND BOOK REVIEW MANUSCRIPTS must include as part of the narrative:

• a running (short) title

• a clinical relevance statement based on the conclusions of the review • conclusions based on the literature review...without this, the review is just an exercise and will not be published • references (see below)

References

REFERENCES must be numbered (superscripted numbers) consecutively as they appear in the text and, where applicable, they should appear after punctuation. The reference list should be arranged in numeric sequence at the end of the manuscript and should include:

1. Author(s) last name(s) and initial (ALL AUTHORS must be listed) followed by the date of publication in parentheses.

2. Full article title.

3. Full journal name in italics (**no** abbreviations), volume and issue numbers and first and last page numbers complete (i.e. 163-168 NOT attenuated 163-68).

4. Abstracts should be avoided when possible but, if used, must include the above plus the abstract number and page number.

5. Book chapters must include chapter title, book title in italics, editors' names (if appropriate), name of publisher and publishing address.

6. Websites may be used as references, but must include the date (day, month and year) accessed for the information.

7. Papers in the course of publication should only be entered in the references if they have been accepted for publication by a journal and then given in the standard manner with "In press" following the journal name.

8. **DO NOT** include unpublished data or personal communications in the reference list. Cite such references parenthetically in the text and include a date.

9. References that contain Crossref.org's DOIs (Digital Object Identifiers) should always be displayed at the end of the reference as permanent URLs. The prefix http://dx.doi.org/ can be appended to the listed DOI to create this URL. i.e. http://dx.doi.org/10.1006/jmbi.1995.0238 **Reference Style Guide**

- Journal article-two authors: Evans DB & Neme AM (1999) Shear bond strength of composite resin and amalgam adhesive systems to dentin *American Journal of Dentistry* **12(1)** 19-25.
- Journal article-multiple authors: Eick JD, Gwinnett AJ, Pashley DH & Robinson SJ (1997) Current concepts on adhesion to dentin *Critical Review of Oral and Biological Medicine* **8(3)** 306-335.
- Journal article: special issue/supplement: Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **(Supplement 6)** 119-144.

- Abstract: Yoshida Y, Van Meerbeek B, Okazaki M, Shintani H & Suzuki K (2003) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **82(Special Issue B)** Abstract #0051 p B-19.
- Corporate publication: ISO-Standards (1997) ISO 4287 Geometrical Product Specifications Surface texture: Profile method – Terms, definitions and surface texture parameters *Geneve: International Organization for Standardization* **1st edition** 1-25.
- Book-single author: Mount GJ (1990) *An Atlas of Glass-ionomer Cements* Martin Duntz Ltd, London.
- Book-two authors: Nakabayashi N & Pashley DH (1998) *Hybridization of Dental Hard Tissues* Quintessence Publishing, Tokyo.
- Book-chapter: Hilton TJ (1996) Direct posterior composite restorations In: Schwarts RS, Summitt JB, Robbins JW (eds) *Fundamentals of Operative Dentistry* Quintessence, Chicago 207-228.
- Website-single author: Carlson L (2003) Web site evolution; Retrieved online July 23, 2003 from: http://www.d.umn.edu/~lcarlson/cms/evolution.html
- Website-corporate publication: National Association of Social Workers (2000) NASW Practice research survey 2000. NASW Practice Research Network, 1. 3. Retrieved online September 8, 2003 from: http://www.socialworkers.org/naswprn/default

• Journal Article with DOI: SA Feierabend, J Matt & B Klaiber (2011) A Comparison of Conventional and New Rubber Dam Systems in Dental Practice. *Operative Dentistry* **36(3)** 243-250, http://dx.doi.org/10.2341/09-283-C

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