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LONGEVIDADE DA UNIÃO DE SISTEMAS ADESIVOS UNIVERSAIS EM DENTINA HÍGIDA E AFETADA

Santa Maria, RS
2016

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Dissertação apresentada ao Curso de Mestrado 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 título de **Mestre em Ciências Odontológicas**.

Orientador: Prof. Dr. Fábio Zovico Maxnuck Soares

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RESUMO

LONGEVIDADE DA UNIÃO DE SISTEMAS ADESIVOS UNIVERSAIS EM DENTINA HÍGIDA E AFETADA

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Recentemente foram introduzidos no mercado uma nova geração de adesivos, reportados pela literatura como “adesivos universais” ou “multi-modo”. Esses adesivos são materiais versáteis, podendo ser utilizados tanto na técnica autocondicionante como na técnica com condicionamento ácido prévio. No entanto, pouco sabe-se ainda sobre o comportamento desses adesivos universais em relação à longevidade da adesão. Então, o objetivo deste estudo foi avaliar a resistência de união imediata e após 6 meses de diferentes adesivos universais em dentina hígida e afetada, usando as estratégias de condicionamento ácido prévio e autocondicionante. Os sistemas adesivos testados foram: Scotchbond Universal (3M ESPE), All-Bond Universal (Bisco), Prime & Bond Elect (Denstply Caulk), Adper Single Bond 2 (3M ESPE) e Clearfil SE Bond (Kuraray Noritake Dental). Todos os adesivos foram aplicados seguindo as recomendações dos fabricantes e um bloco de resina composta foi construído sobre a dentina. A dentina afetada foi obtida artificialmente através de ciclagem de pH (8 horas em solução desmineralizante e 16 horas em solução remineralizante, por 14 dias). Espécimes em forma de palitos foram obtidos (0,8 mm^2) para serem submetidos ao teste de microtração imediatamente ou após 6 meses de armazenamento. Os dados obtidos em MPa foram analisados por análise de variância de 3 fatores e teste de contraste de Tukey (α= 5%). A análise foi realizada separadamente para cada substrato. Em dentina hígida, a estratégia de condicionamento não teve influência na resistência de união dos adesivos universais e não houve diferença estatisticamente significante entre a resistência de união imediata e após 6 meses. Em dentina afetada, os sistemas adesivos apresentaram resultados similares, independente da estratégia de condicionamento. No entanto, houve uma significativa redução na resistência de união após 6 meses. Assim, a estratégia de condicionamento não influencia a resistência de união dos adesivos universais em dentina, porém a resistência de união a longo prazo é reduzida em dentina afetada.

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

ABSTRACT

BONDING LONGEVITY OF UNIVERSAL ADHESIVE SYSTEMS ON SOUND AND CARIES-AFFECTED DENTIN

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A new generation of adhesive systems are currently available on market. They were reported on the literature as “universal adhesives” or “multimode adhesives”. These adhesives are versatile, as they can be use in either etch-and-rinse or self-etch strategy. However, only a few data is available about long-term bonding behavior of universal adhesive systems. Then, the aim of this study is to evaluate immediate and six months bond strength of different universal adhesive systems on sound and caries-affected dentin, either on etch-and-rinse or self-etch strategies. The adhesive systems tested were: Scotchbond Universal (3M ESPE), All-Bond Universal (Bisco), Prime & Bond Elect (Denstply Caulk), Adper Single Bond 2 (3M ESPE) and Clearfil SE Bond (Kuraray Noritake Dental). All adhesive systems were applied under manufacturer’s instructions and a composite block was build up on dentin surfaces. Caries-affected dentin was artificially induced by pH-cycling (8h immersed on demineralizing solution and 16h on remineralizing solution, for 14 days). Stick shaped specimens (0,8 mm^2) were obtained to be submitted to microtensile test at immediate time or after 6 months of storage. Bond strength data (MPa) were analyzed by three-way repeated measures ANOVA and post-hoc Tukey test (α= 5%). Analysis were performed for each substrate separately. On sound dentin, the etching strategy did not influence bond strength of universal adhesives and no significant statistical differences were found for bond strength values after 6 months of water storage. On caries-affected dentin, adhesive systems also showed similar results regardless etching strategies. However, significant reduction in bond strength values was found after six months. The etching strategy did not influence bond strength performance of universal adhesives on dentin substrate, although the long-term bond strength is decreased on caries-affected dentin.

Keywords: Dentin. Dentin-Bonding Agents. Tensile Strength.

SUMÁRIO

1. INTRODUÇÃO

Atualmente, o maior desafio em relação a adesão dentária é prover união efetiva, homogênea e duradoura a ambos tecidos dentários, esmalte e dentina, ainda que em possível condições de depleção mineral parcial, decorrente da abordagem operatória e do processo de progressão das lesões cariosas (CARDOSO et al., 2011). A adesão à dentina é complexa e demanda procedimentos mais criteriosos, consumindo maior tempo clínico de aplicação. Dessa maneira, a evolução dos sistemas adesivos procura oferecer materiais simplificados e com menor sensibilidade à técnica (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 novos materiais, mais versáteis, que estão disponíveis atualmente no mercado. A literatura se reporta a eles como “adesivos universais” ou “multi-modo”, pois podem ser utilizados tanto na técnica de condicionamento ácido prévio como na técnica autocondicionante, possibilitando ao profissional optar a cada caso ou condição, o protocolo adesivo mais adequado (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 diferentes sistemas adesivos universais, comparando também as estratégias de aplicação (autocondicionante e com condicionamento ácido prévio) (CHEN et al., 2015; LUQUE-MARTINEZ et al., 2014; WAGNER et al., 2014), obtendo resultados diversos, e em geral, material dependente

(MUÑOZ et al., 2015, 2013). Pouco se sabe sobre o comportamento desses adesivos universais em relação à longevidade da adesão, mas parece haver influência tanto da composição como da estratégia de aplicação. Muñoz e colaboradores (2015), compararam diferentes adesivos universais e, após 6 meses, aqueles que continham o monômero metacriloiloxidecil 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) encontraram melhor resultado para um sistema que continha MDP em sua composição e na estratégia de aplicação autocondicionante após 6 meses e 1 ano de armazenamento. Yoshida e colaboradores (2012) observaram uma efetiva 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 da nano-camada pode explicar a alta estabilidade da adesão (YOSHIDA et al., 2012).

Apesar desses novos produtos alegarem versatilidade em relação a estratégia de aplicação, as diferenças na composição de cada adesivo podem ser o motivo das diferentes performances encontradas nos estudos até o momento. Dessa forma, é importante a realização de testes 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.

Os novos conceitos da odontologia restauradora preconizam tratamentos mais conservadores. Houve um aumento significativo nas evidências clínicas que suportam tratamentos como a remoção parcial 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). A dentina afetada por cárie que permanece após a remoção parcial 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).

Tendo em vista as considerações apresentadas, o objetivo deste trabalho foi avaliar a resistência de união imediata e após 6 meses de armazenamento de diferentes sistemas adesivos universais, utilizando duas estratégias de condicionamento (condicionamento ácido prévio e autocondicionante) em dois tipos de substrato (dentina hígida e dentina afetada por cárie).

2. ARTIGO – ETCHING STRATEGY OF UNIVERSAL ADHESIVES DOES NOT INFLUENCE THE LONGEVITY OF BONDING TO SOUND OR ARTIFICIALLY CARIES-AFFECTED DENTIN

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Etching strategy of universal adhesives does not influence the longevity of bonding to sound or artificially caries-affected dentin

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Etching strategy of universal adhesives does not influence the longevity of bonding to sound or artificially caries-affected dentin

Abstract

Purpose: To evaluate the immediate and six-months bond strength of different universal adhesive systems to sound and artificially-induced caries-affected dentin using either an etch-and-rinse and self-etch strategy.

Materials and Methods: Buccal flat dentin surfaces of one hundred and twelve sound bovine incisors were randomly assigned to 16 groups (n=7) according to: substrate condition (sound dentin - SND and artificially-induced caries-affected dentin - CAD – pH-cycling for 14 days); adhesive systems (Scotchbond Universal Adhesive - SU, All-Bond Universal - AB, Prime & Bond Elect – PB, Adper Single Bond 2 - SB and Clearfil SE Bond - CS) and etching strategies (etch-and-rinse and self-etch). All adhesive systems were applied under manufacturer's instructions and a composite block was build up on each dentin surfaces. After storage in distilled water at 37ºC for 24h, specimens were sectioned into stick shaped specimens (0.8 mm^2) to be submitted to microtensile test at immediate (24h) or after six-months of water storage (6Mos). Bond strength data (MPa) were analyzed by three-way repeated measures ANOVA and post-hoc Tukey test (α= 5%), considering each substrate separately (SND and CAD).

Results: On SND, the etching strategy did not influence the bond strength of universal adhesives and no significantly statistical differences were found between 24h and 6Mos values. On CAD, adhesive systems also showed similar results regardless of the etching strategies, but significant reduction in bond strength values was found after 6Mos.

Conclusion: The etching strategy does not influence the bond strength of universal adhesives to dentin, although the long-term bond strength is decreased on artificially-induced caries-affected dentin.

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

Introduction

Adhesive systems can be classified according their etching and interaction with dental substrate during adhesive protocol as etch-and-rinse (ER) or self-etch (SE).[20,27] While etch-and-rinse systems removes the smear layer with a prior application of phosphoric acid[27], self-etch systems incorporates a modified smear layer to hybrid layer due to acidic monomers that simultaneously act as conditioner and primer agent.[20] The interest for more simplified and less technique sensitive adhesives resulted in development of versatile materials that can be adapted to different clinical situations and different substrate conditions.

In order to simplify adhesive technique and improve clinical versatility, new materials referred as universal or multimode adhesives have been developed. They consist generally in one-bottle adhesives that can either be use on etch-and-rinse or self-etch strategy. Moreover, manufacturers and previous studies recommend an alternative protocol: the enamel selective etching technique, which combines the advantages of enamel etching with simplified self-etching technique in dentin.[28,29] In addition, most of universal adhesives incorporated in composition a phosphate monomer that chemically bonds with the calcium in hydroxyapatite, as methacryloyloxydecyl dihydrogen phosphate (10-MDP)[44], in attempt to achieve a durable bond strength. As hybrid layer is prone to both collagen and resin matrix hydrolytic degradation[27,39,40], it is extremely important evaluate the long-term performance of the universal adhesive systems on different substrates as they are recently available on the market.

Recent studies have evaluated different universal adhesive systems, comparing the performance between etch-and-rinse and self-etch strategies.[6,11,17–19,23,24,37,42,46] A systematic review with meta-analysis demonstrated that the performance of these adhesive systems was dependent on the approach strategy for enamel and for one adhesive system in dentin.[33] Although these studies presented satisfying immediate bond strength values for universal adhesives[6,11,17,19,24,37,42], the question about the long-term bonding effectiveness to dentin remains, as the results of the available in vitro studies are still controversial.[6,18,19,23,37,42,46] Moreover, only one study have reported the behavior of a universal adhesive system to caries-affected dentin and also found that etching strategy was substrate dependent.[15]

Caries-affected dentin has been a common substrate on restorative dentistry as result of the partial caries removal technique. This approach could reduce significantly the risk of pulp exposure and post-operative pulp symptoms, which seems advantageous for deep caries treatment.[36] However, caries-affected dentin differs in composition and microstructure compared to sound dentin,[45] and lower bond strength have been reported on literature for this substrate, regardless of the adhesive system tested.[5,7,8,25,31,35,45] It is also known that hybrid layer degradation is more pronounced on caries-affected dentin due to substrate intrinsic characteristics.[31] Based on this, laboratory assessments of bonding to caries-affected dentin is relevant,[45] including the performance of universal adhesive systems on this kind of substrate.

Therefore, the aim of this in vitro study was to evaluate immediate and six months bond strength of three universal adhesive systems to sound and artificially-induced caries-affected dentin, using either an etch-and-rinse or self-etch strategy. The null hypotheses tested were: 1) the universal adhesives present similar bonding independent of etching strategies on either sound or artificially-induced caries-affected dentin, and 2) water-aging has no effect on bond strength of universal adhesives to sound and artificially-induced caries-affected dentin, irrespective of the etching strategies.

Materials and methods

Tooth selection and preparation

One hundred and twelve freshly extracted bovine incisors were stored in 0.5% aqueous chloramine T at 4°C for a maximum of thirty days and used in this study. The teeth were divided into dentin substrates (sound and artificially-induced caries-affected) and for each substrate, teeth were allocated into eight groups (n=7). The root portion was removed using a diamond disc in a low-speed hand piece. 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 and obtain flat dentin surfaces. Further, for both substrates (sound dentin and artificially-induced caries-affected dentin), buccal surfaces were ground manually using 600-grit SiC paper for 60s to create a standardized smear layer.

Artificial caries induction

The teeth were randomly assigned in two groups according to the substrate condition: sound (SND) and artificially-induced caries-affected dentin (CAD). After procedures to obtain a standardized smear layer, teeth allocated to sound dentin group were immersed in distilled water only and those of artificially-induced caries-affected dentin group were submitted to artificial caries induction by pH-cycling model. [4,14] 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 pHmeter (Digimed, DM22; ServMed Analítica, Guarulhos, SP, Brazil).

Bonding and restorative procedures

Teeth from each dentin substrate (sound dentin and artificially-induced caries-affected dentin) were randomly reallocated into 8 groups according to the adhesive system and etching strategy (n=7). Three universal adhesives systems were evaluated: Scotchbond Universal - SU (3M ESPE; St Paul, MN, USA), All-Bond Universal - AB (Bisco; Shaumburg, 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 2 - SB (3M ESPE; St Paul, MN, USA) and a two-step self-etch Clearfil SE Bond – CS (Kuraray Noritake Dental; Tokyo, Japan) were used. A single trained operator applied the adhesive systems on dentin surfaces according to 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 incrementally build up on dentin surfaces, and each increment was light cured for 20 s using a light emitting diode curing unit (Emitter B, Schuster; Santa Maria, RS, Brazil). All specimens were stored in distilled water at 37ºC for 24 h.

Microtensile Bond Strength (µTBS)

Specimens were sectioned in two perpendicular axes with under water cooled diamond saw in a cutting machine (Labcut 1010, Extec Co, Enfield, CT, USA) by a single and blinded operator, obtaining stick shaped specimens with cross-sectional area of approximately 0.8 mm^2 measured individually with a digital caliper (Carbografite, Petrópolis, RJ, Brazil). Then, half of the specimens obtained from each tooth were randomly assigned to be tested immediately (24h) and the other half after six months (6Mos) of water storage.

For microtensile testing, specimens were fixed to metallic devices (Odeme Medical and Dental; Joaçaba, SC) with cyanoacrylate glue (Three Bond Super Gel, ThreeBond, Diadema, SP, Brazil) and submitted to microtensile test in a universal testing machine (EMIC DL in 1000, Equipment and Systems Ltda; Sao Jose dos Pinhais, PR, Brazil) at a crosshead speed of 1 mm/min until fracture. Specimens that failed prior to the test, during cutting or fixing procedures, were recorded as pre-testing failures (PTF) and included in the bond strength means. A single and blinded operator performed the microtensile test. All fractured specimens were observed under 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).

Statistical Analysis

The experimental unit in the study was the tooth. Thus, the means of µTBS (MPa) values of specimens tested at 24h or 6Mos were average 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%.

Three variation factors were considered for statistical analysis: adhesive system (AB, PB, SU, SB and CS), etching strategy (ER and SE) and evaluation time (24h and 6Mos). Analyses were performed for each substrate separately (SND and CAD).

A normal distribution of the data was confirmed by Kolmogorov-Smirnov test. Data were analyzed by a 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).

Results

Statistical analyses were performed separately for each substrate (SND and CAD). Significant cross-interaction among the three factors (material x strategy x time) was found on both SDN ($p=0.005$) and CAD ($p=0.012$) analyses. Table 2 presents the µTBS values (means and standard deviation) and contrasts found in the interactions separately to each substrate.

On SND, etching strategy did not influence on bond strength of all tested universal adhesives. However, significant differences were found between adhesives used as control, since etch-and-rinse system (SB) presented higher values compared to self-etch adhesive (CSE). After 6Mos of water storage, all materials showed a trend of reducing numerical values, but with no significant differences, except the AB on ER strategy, that showed bond strength values significantly lower than immediate ones.

On CAD, adhesive systems also showed similar results regardless etching strategy, including the control adhesive. Considering degradation over time on the CAD, different behavior was observed since significant reduction in bond strength values was found, except for AB on ER strategy and PB on SE strategy.

Adhesive/mixed failure pattern was predominant for all experimental groups, except for SB ER on SND after 6Mos of water storage. Cohesive failures (resin or dentin) seemed to be more frequent in sound dentin, and increased on 6Mos groups (Figure 1). Pre-testing failures were numerically more evident on CAD compared to SND (Table 2).

Discussion

Caries-affected dentin has been a common substrate through direct restoration procedures according to the Minimal Intervention on restorative dentistry. Several studies tested different adhesive systems, with different strategies in an attempt to achieve better performance on this kind of substrate.[5,7–9,15,22,25,26,35,45] Intrinsic characteristics of caries-affected dentin lead in general to lower bond strength when compared to sound dentin.[10,25,31,45] Thus, in our study, sound and artificially-induced caries-affected dentin were considered separately on the statistical analyses, enabling to evaluate three universal adhesive systems, discriminating the etching strategies and bond strength storage time for each substrate condition. This is the first study that assessed the long-term bond strength of different universal adhesives to caries-affected dentin.

On SND, the etching approach did not influence the immediate bond strength values for the universal adhesives. These findings are in accordance with other studies that also found no differences between etch-and-rinse or self-etch strategies on bond strength of different universal adhesives.[11,19,24,42] After 6Mos of water storage, the same behavior was observed, i.e., the bond strength values remained stable irrespective of the etching strategy, with exception of the All Bond Universal in ER strategy that presented significant bond degradation after 6Mos.

Considering the CAD, all adhesives tested presented similar results, regardless of the etching strategy. However, the bond strength values to CAD were clearly lower than the results found for SND. Carious dentin is more porous than sound dentin due to mineral loss in demineralization process.[45] Less content of minerals on dentin substrate could cause a decrease in bond strength of the adhesive systems.[31] A previous study reported that this decrease on bond strength could be consequence of a discrepancy between a deeper demineralized layer and resin monomers, as monomers may not penetrate so deeply into intertubular dentin.[34] This behavior on substrates such as caries-affected dentin is well stated on literature.[5,7,8,25,35,45] Other authors already reported similar results between etch-and-rinse and self-etch adhesive systems on caries-affected dentin.[7,8,26,35,45] Lenzi and others[15] also found no difference among the adhesives tested, which one was a universal adhesive (SU) – and between etching strategies when their performances were compared in CAD of primary teeth, what is in accordance to our findings.

After 6Mos of water storage, contrary to what happened on SND, bond strength degradation to CAD was observed. There was an evident numerical drop on bond strength values for all the adhesive systems tested on this substrate. Only AB on ER strategy and PB on SE strategy maintained statistically similar results after water ageing on CAD. The bond strength values for these two adhesives on respective strategies already showed the lowest immediate bond strength of adhesives tested to CAD. Less mineral content, wetness and other morphological and chemical characteristics apparently had strong influences on resin-cad bonds longevity due higher permeability and poor quality hybrid layer.[25,43,45] In addition, action of matrix metalloproteinase (MMPs) is more intense in caries-affected dentin.[31] The susceptibility to degradation of the interfaces created on CAD was already reported in others studies[10,22] and this was confirmed in our study, including universal adhesive systems.

The three universal adhesives tested in this study present differences in composition. Two of them (AB and SU) contain a monomer that chemically bonds to the dentin substrate (10-MDP). Also, SU incorporates the polyalkenoic acid copolymer (PAC) that also chemically interacts with the calcium of hidroxyapatite. With the years, studies presented that MDP-mediated chemical bond maintained stable adhesion and prevented bond degradation as component of a two-step self-etch adhesive Clearfil SE Bond, which was used as control in our study for SE strategy.[13,30,41] The presence of 10-MDP monomer resulted in stable long-term bond strength only for SU on ER and SE strategies and AB on SE strategy, considering the universal adhesives in our study. Yoshida and others[44] showed that MDP-containing adhesives form a nano-layer at the adhesive interface in different degrees, depending on the adhesive composition. They speculated that compositional differences and possibly different MDP concentrations could explain the distinct behavior of the MDP-containing adhesives tested. Moreover, they hypothesized that the presence of other components as PAC or 2-hydroxyethyl methacrylate (HEMA) may compete with MDP on bonding sites to the calcium of hydroxyapatite.[44] These characteristics could explain the behavior of MDP-containing adhesives tested in our study.

Similar performance was found for Muñoz and others[23], and they speculated that as only AB on ER strategy presented degradation of bond strength values after 6Mos, the presence of PAC is more important to etch-and-rinse adhesives than for self-etch. They indicated that PAC improves stability to humidity of the substrate, an important factor for etch-and-rinse adhesives. We hypothesized that the decreased performance of AB on ER after water storage could be explained by the influence of etching strategy. The removal of available calcium by acid etching might have prevented any potential chemical bonding mediated by the phosphate monomer, once

AB on SE strategy remained with stable bond strength values after long-term evaluation.

The other tested universal adhesive (Prime&Bond Elect) is a HEMA-free adhesive that presents acetone as solvent. Zhang and others[46] explained that no bond degradation observed for PB might be related to the fact that acetone has higher vapor pressure compared to ethanol, resulting in rapid solvent evaporation and less retention of residual water. A recent study concluded that entrapment of residual water on resin-dentin bonds could compromise the performance of universal adhesives.[17] Furthermore, PB does not contain HEMA in composition, making it a less hydrophilic adhesive. To avoid or reduce the hydrolytic degradation of hybrid layer, researches have been developing less hydrophilic adhesives, as HEMA-free adhesives.[3,40] Therefore, we hypothesized that these characteristics on composition of the PB may explain its performance on dentin after 6Mos of ageing.

The adhesive systems used as controls performed differently on SND. The etch-and-rinse control (SB) showed higher bond strength values than the self-etch control (CSE). Such behavior for these adhesive systems was already presented for other authors on SND.[1,12,38] We speculated that the decrease in self-etch gold standard performance when compared to the other adhesive used as control might be related to the fact that CSE was the only tested adhesive that the manufacturers does not recommend active application. Several studies demonstrated that active application enhance immediate and long-term bond strength of self-etch adhesives.[2,16,21,32] The fact that CSE is available on market for a long time could explain manufacturer's instructions without active application.[30]

Considering presented results, we accepted the first null hypothesis since the universal adhesives tested showed similar performance for both strategies on SND and CAD. Moreover, we partially accept the second null hypothesis as water aging had no effect only on SND for both strategies, and degradation over time was observed on CAD.

Conclusions

Within the limitations of this study, it was concluded that the etching strategy does not influence the performance of universal adhesives tested on both dentin substrates (sound dentin or artificially-induced caries-affected dentin). Six-month water storage does not affect universal adhesives bond strength to sound dentin, although the long-term bond strength is decreased on artificially-induced caries-affected dentin.

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Clinical significance

When bonding to dentin with universal adhesive systems, clinicians could opt to follow either etch-and-rinse or self-etch strategy without bonding disadvantage. In addition, adhesion to caries-affected dentin is more prone to degradation over time and none etching mode made the bond strength to caries-affected dentin as close as possible to that to sound dentin.

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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.

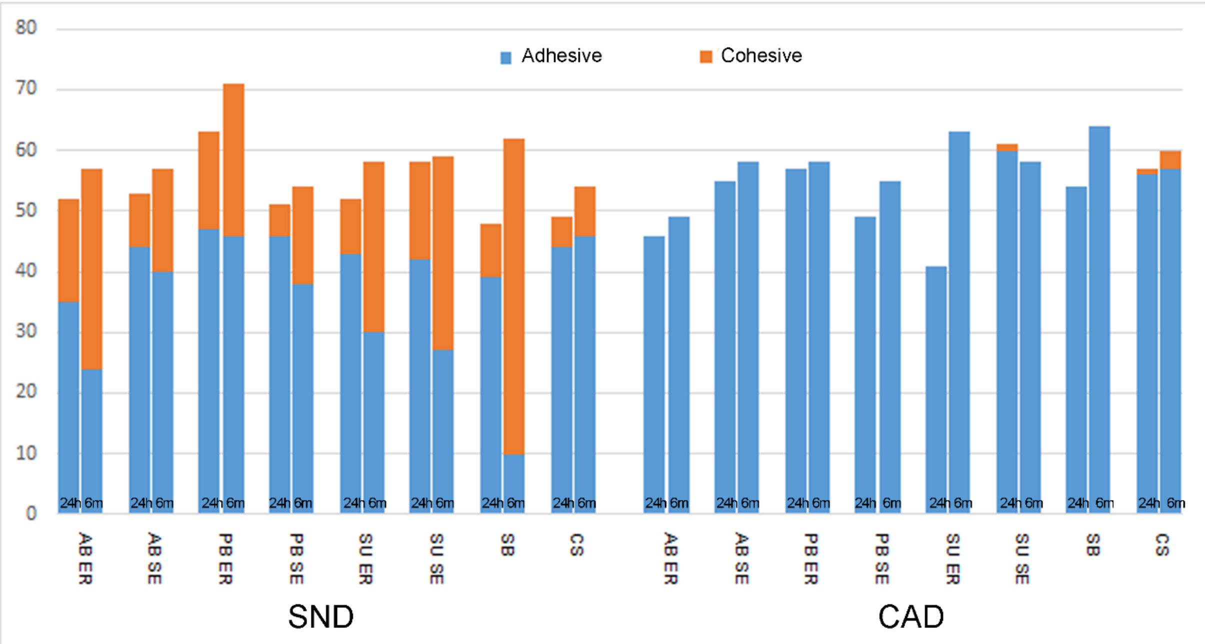
*According information provided by manufacturers; MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate; bis-GMA: bisphenyl-glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate

Table 2: Bond strength mean values in MPa (standard deviation) for experimental groups* [tested sps/pre-test failures]

Material	Strategy	SOUND DENTIN 24h	SOUND DENTIN 6Mos	CARIES-AFFECTED DENTIN 24h	CARIES-AFFECTED DENTIN 6Mos
AB	ER	50,3 (12,4)^{A} [52/0]	28,6 (11,3)^{B,C,D} [57/0]	13,1 (2,7)^{b,c,d} [46/14]	2,5 (0,3)^{d} [49/2]
	SE	34,7 (7,5)^{A,B,C,D} [53/2]	34,1 (10,8)^{A,B,C,D} [57/0]	22,5 (4,4)^{a,b} [55/5]	3,7 (2,4)^{d} [58/0]
PB	ER	42,3 (8,3)^{A,B,C} [62/2]	24,7 (8,1)^{C,D} [71/0]	19,5 (6,1)^{a,b,c} [49/10]	3,9 (1,6)^{d} [58/0]
	SE	26,2 (17,1)^{C,D} [48/14]	20,0 (5,7)^{D} [53/0]	16,3 (10,9)^{b,c} [41/22]	9,4 (7,9)^{c,d} [55/0]
SU	ER	48,0 (14,2)^{A,B} [53/4]	33,6 (15,4)^{A,B,C,D} [58/0]	21,3 (6,1)^{a,b} [56/5]	2,4 (0,2)^{d} [63/0]
	SE	40,1 (8,9)^{A,B,C,D} [55/2]	34,7 (8,8)^{A,B,C,D} [59/0]	17,7 (4,4)^{b,c} [55/5]	4,7 (3,2)^{d} [58/0]
SB		52,6 (11,8)^{A} [51/1]	47,3 (10,4)^{A,B} [61/0]	22,4 (6,4)^{a,b} [60/6]	3,4 (1,8)^{d} [64/0]
CSE		26,5 (9,6)^{C,D} [52/18]	23,2 (7,7)^{C,D} [54/0]	29,2 (7,3)^{a} [58/4]	12,5 (11,3)^{b,c,d} [60/0]

*Different letters indicate statistically significant differences ($p<0.05$). Uppercase letters for sound dentin and lowercase for artificially-induced caries-affected dentin)

Figure 1: Fracture type distribution per experimental group



3. CONCLUSÃO

Esta dissertação avaliou o comportamento de diferentes sistemas adesivos universais, usados em diferentes estratégias de condicionamento e em dois substratos, imediatamente e após 6 meses de armazenamento.

Os adesivos universais são materiais cuja proposta é a versatilidade quanto à estratégia de condicionamento, ou seja, podem ser utilizados tanto com condicionamento ácido prévio quanto no modo autocondicionante. No entanto, por estarem a pouco tempo disponíveis no mercado, a literatura disponível a respeito do comportamento desses novos adesivos em relação a longevidade da união ainda é escassa. Os resultados encontrados ainda parecem ser material-dependente e podem ser atribuídos ao fato de os materiais diferenciarem-se em relação a sua composição.

Em tempos de odontologia restauradora minimamente invasiva, tem-se deparado clinicamente com diferentes tipos de substrato. Procedimentos como remoção parcial de cárie têm permitido que substratos como dentina afetada por cárie sejam mantidos, uma vez que estudos mostraram que esse substrato é passível de remineralização. Entretanto, devido a características intrínsecas, a adesão a esse substrato afetado é diferente daquela obtida em substrato hígido. Assim, permanece a dúvida sobre o comportamento desses novos sistemas adesivos em substrato afetado, sendo que apenas um estudo até agora reportou sobre o assunto.

Apesar das limitações de um estudo *in vitro*, o presente trabalho prediz, com ressalvas, o comportamento dos novos sistemas adesivos testados, mostrando que o desempenho dos adesivos universais avaliados não depende da estratégia de condicionamento. Da mesma forma, a longevidade da união se manteve ao longo dos 6 meses de avaliação, porém esse comportamento foi válido apenas para o substrato hígido. Considerando a adesão ao substrato afetado, pôde-se observar degradação da união ao longo do tempo. Entende-se que mais estudos são necessários sobre esses materiais, principalmente estudos clínicos randomizados. Porém, os achados do nosso estudo são um importante passo para o entendimento do comportamento dos sistemas adesivos universais.

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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
3. ***Short communications*** – of original research in adhesive dentistry and related topics. Max. 4 printed pages, including figures and references (max. characters 18,000). High priority will be given to the review of these papers to speed publication.
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.

SUBMISSION INSTRUCTIONS

Submission of manuscripts in order of preference:

1. Submission via online submission service (www.manuscriptmanager.com/jadd). Manuscript texts should be uploaded as PC-word files with tables and figures preferably embedded within the PC-word document. A broad range of file formats are acceptable. No paper version required but high resolution photographs or illustrations should be sent to the editorial office (see below). Online submissions are automatically uploaded into the editorial office's reviewer assignment schedule and are therefore processed immediately upon upload.
2. Submission via e-mail as a PC-word document (wintonowycz@quintessenz.de). Illustrations can be attached in any format that can be opened using Adobe Photoshop, (TIF, GIF, JPG, PSD, EPS etc.) or as Microsoft PowerPoint Documents (ppt). No paper version required but high resolution photographs or illustrations should be sent to the editorial office.
3. One paper copy of the manuscript plus a floppy diskette or CD-ROM (mandatory) containing a 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)

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

Review/editing of manuscripts. Manuscripts will be reviewed by the editor-in-chief and at least two reviewers with expertise within the scope of the article. The publisher reserves the right to edit accepted manuscripts to fit the space available and to ensure conciseness, clarity, and stylistic consistency, subject to the author's final approval.
Adherence to guidelines. Manuscripts that are not prepared in accordance with these guidelines will be returned to the author before review.

MANUSCRIPT PREPARATION

• The Journal will follow as much as possible the recommendations of the International Committee of Medical Journal Editors (Vancouver Group) in regard to preparation of manuscripts and authorship (Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126: 36-47).
• **Title page.** The first page should include the title of the article (descriptive but as concise as possible) and the name, degrees, job title, professional affiliation, contribution to the paper (e.g., idea, hypothesis, experimental design, performed the experiments in partial fulfillment of requirements for a degree, wrote the manuscript, proofread the manuscript, performed a certain test, consulted on and performed statistical evaluation, contributed substantially to discussion, etc.) and full address of all authors. Phone, fax, and e-mail address must also be provided for the corresponding author, who will be assumed to be the first listed author unless otherwise noted. If the paper was presented before an organized group, the name of the organization, location, and date should be included.
• **3-8 keywords.**
• **Structured abstract.** Include a maximum 250-word structured abstract (with headings *Purpose, Materials and Methods, Results, Conclusion*).
• **Introduction.** Summarize the rationale and purpose of the study, giving only pertinent references. Clearly state the working hypothesis.
• **Materials and Methods.** Present materials and methods in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made. Indicate the statistical methods used, if applicable.
• **Results.** Present results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables or illustrations; emphasize only important observations.
• **Discussion.** Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given

in the Introduction or Results section. Relate observations to other relevant studies and point out the implications of the findings and their limitations.

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

• **Abbreviations.** The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

• **Trade names.** Generic terms are to be used when ever possible, but trade names and manufacturer should be included parenthetically at first mention.

• **Clinical Relevance.** Please include a very brief (2 sentences or 3 lines) clinical relevance statement.

REFERENCES

• **All references must be cited** in the text, according to the alphabetical and numerical reference list.

• **The reference list** should appear at the end of the article, in alphabetical and numerical sequence.

• **Do not include unpublished data** or personal communications in the reference list. Cite such references parenthetically in the text and include a date.

• **Avoid using abstracts** as references.

• **Provide complete information** for each reference, including names of all authors. If the reference is part of a book, also include title of the chapter and names of the book‘s editor(s).

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.

• Submitted figures should meet the following minimum requirements:

– High-resolution images should have a width of 83 mm and 300 dpi (for column size).

– Graphics (bar diagrams, schematic representations, drawings) wherever possible should be produced in Adobe Illustrator and saved as AI or EPS files.

– All figures and graphics should be separate files – not embedded in Word or Power Point documents.

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

1. As an e-mail attachment, if the files are not excessively large (not more than 10 MB), to our production department: Steinbrueck@quintessenz.de

2. Online File Exchange Tool: Please send your figures with our Online File Exchange Tool. This web tool allows you to upload large files (< 350.0 MB) to our server. Please archive your figures with a maximum size of 350 MB first. Then upload these archives

with the following link: http://files.qvnet.de/JAD/, password: IAAD. Please name the archive with your name and article number so we can identify the figures.

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

TABLES

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

MANDATORY SUBMISSION FORM

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

PERMISSIONS & WAIVERS

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

PAGE CHARGE

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