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EFEITO DE DIFERENTES PROTOCOLOS DE APLICAÇÃO DE ADESIVOS UNIVERSAIS NAS PROPRIEDADES E LONGEVIDADE DA UNIÃO À DENTINA

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Orientadora: Prof$^{\underline{a}}$. Dr$^{\underline{a}}$. Nara Hellen Campanha Bombarda
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Área de concentração: Clínica Integrada.

Ponta Grossa, 7 de Fevereiro de 2014.

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MUÑOZ MA. Efeito de diferentes protocolos de aplicação de adesivos universais nas propriedades e longevidade da união à dentina. [Tese – Doutorado em Clínica Integrada]. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2013.

RESUMO

O objetivo do presente trabalho foi avaliar *in vitro* as propriedades e longevidade da união à dentina dos novos sistemas adesivos universais aplicados segundo as estratégias adesivas convencional (CON) e autocondicionante (AC). Os objetivos específicos para cada um dos três estudos foram: avaliar a resistência de união à microtração (RU), a nanoinfiltração (NI) e o grau de conversão (GC) no tempo imediato - 24 h - (Experimento 1) e seis meses (Experimento 2) dos sistemas adesivos universais para as estratégias CON e AC; e o efeito de uma camada hidrofóbica adicional (Experimento 3, no tempo imediato) sobre essas mesmas propriedades. Cento e quarenta terceiros molares foram planificados na face oclusal até se obter dentina e alocados aleatoriamente em 28 grupos (n=5). Nos Experimentos 1 e 2, 40 dentes foram divididos em oito grupos de acordo com o adesivo (Peak Universal [Pk], Scotchbond Universal [Sc], Allbond Universal [Al]) e estratégia adesiva (CON e AC) tendo como controle, o sistema adesivo convencional Adper Single Bond 2 (SB) e o autocondicionante Clearfil SE Bond (CSE). Para o Experimento 3, 60 dentes foram divididos em 12 grupos de acordo com o adesivo (Sc, Al, G-Bond Plus [Gbp]), estratégia adesiva (CON e AC) e com a presença ou não de camada hidrofóbica (Heliobond, HE). Após aplicação das variáveis adesivas e do procedimento restaurador, os dentes foram armazenados em água destilada (37^{o}C/24 h) e cortados para obter palitos de dentina-resina (0,8 mm^{2} de secção transversal) sendo submetidos aos teste de RU e NI nos tempos imediato (Experimentos 1 e 3) e também seis meses (Experimento 2); e análise de GC no tempo imediato. Os dados do Experimento 1 foram submetidos à análise de variância de um fator (Estratégia Adesiva) e os dos Experimentos 2 e 3, à de dois fatores (Estratégia adesiva versus Tempos de análise; e Estratégia adesiva versus Camada hidrofóbica), respectivamente. As análises foram complementadas pelo teste de Tukey ($\alpha=0,05$). Os resultados de RU obtidos no Experimento 1 demonstraram que apenas PkCon e PkAc foram similares aos respectivos grupos controle ($p>0,05$), que os outros materiais universais resultaram em médias de RU inferiores aos seus controles CON e AC ($p<0,05$) e que AlAc apresentou as médias mais baixas de RU ($p<0,05$). Para NI, ScCon, ScAc, AlAc, e AlCon apresentaram baixos níveis de NI similares aos grupos controle ($p<0,05$). Para GC, unicamente ScAc demostrou baixo GC, quando comparado com os outros materiais ($p<0.05$). Segundo os resultados do Experimento 2, os sistemas Pk e AlCon apresentaram diminuição da RU ($p<0,05$) e apenas Pk teve degradação significativa (NI) após seis meses ($p<0,05$). De acordo com o Experimento 3, Al e Gbp obtiveram maiores resultados de RU na estratégia CON ($p<0,05$); o uso de HE aumentou a RU unicamente na estratégia AC ($p<0,05$); baixa NI foi observada somente em Sc e Al na estratégia CON+HE e em Gbp em AC+HE; Sc e Gbp apresentaram alto GC quando usados em estratégia CON, que foi aumentado com a aplicação de HE ($p<0,05$). O GC de Al foi similar em todas as condições. A partir dos três experimentos que compõem o presente trabalho, pôde-se concluir que: 1) os adesivos universais apresentaram um comportamento dependente do material em ambas estratégias (CON e AC) e foram inferiores, pelo menos em uma das propriedades avaliadas (RU, NI ou GC), quando comparados aos adesivos controle (CSE e SB); 2) Em geral, após seis meses os adesivos AC mantém mais estáveis os valores de RU e NI, o que parece estar relacionado principalmente à sua capacidade de união química ao substrato dental; 3) A utilização de uma camada hidrofóbica na estratégia AC pôde aumentar a RU e o GC de adesivos universais e a redução da NI foi mais dependente da composição do adesivo do que da estratégia adesiva.

Palavras-chave: dentina; sistemas adesivos; adesivos convencionais; adesivos autocondicionantes; resistência de união; grau de conversão; nanoinfiltração.

MUÑOZ MA. Effect of different universal adhesive application protocols on the properties and longevity of bond to dentin [Thesis – Doctorate in Integrated Clinic]. Ponta Grossa: State University of Ponta Grossa; 2013.

ABSTRACT

The aim of the present study was to make an *in vitro* evaluation of the properties and stability of the bond to dentin of new universal adhesive systems applied according to the etch-and-rinse (ER) and self-etching (SE) adhesive strategies. The specific objectives for each of the three studies were: To evaluate the microtensile bond strength (RU), nanoleakage (NI) and degree of conversion (DC) in the time intervals of 24 h (Experiment 1) and six months (Experiment 2) of the universal adhesive systems applied in accordance with the manufacturers' recommendations for strategies ER and SE; and the effect of an additional hydrophobic layer (Experiment 3, in the immediate time interval) on these same properties. The occlusal surfaces of 140 third molars were flattened until dentin was obtained, and then the teeth were randomly allocated to 28 groups (n=5). In Experiments 1 and 2, 40 teeth were divided into eight groups according to the adhesive used (Peak Universal [Pk], Scotchbond Universal [Sc], Allbond Universal [Al]) and adhesive strategy (ER and SE), with the ER adhesive system Adper Single Bond 2 (SB) and self-etching Clearfil SE Bond (CSE) used as controls. For Experiment 3, 60 teeth were divided into 12 groups according to the adhesive (Sc, Al, G-Bond Plus [Gbp]), adhesive strategy (ER and SE) and with or without hydrophobic layer (Heliobond, HE). After application of the variables *adhesives* and *restorative procedure*, the teeth were stored in distilled water (37°C/24 h) and cut to obtain dentin-resin sticks (0.8 mm^2 cross-section) which were submitted to the RU and NI tests in the following time intervals: immediate (Experiments 1 and 3) and six months (Experiment 2); and analysis of DC in the immediate time interval. The data of Experiment 1 were submitted to one-way analysis of variance (Adhesive Strategy) and those of Experiments 2 and 3, to two-way analysis of variance (Adhesive strategy vs. Time intervals; and Adhesive strategy vs. Hydrophobic layer) respectively. The analyses were complemented by the Tukey Test (α=0.05). The RU results obtained in Experiment 1 demonstrated that only PkEr and PkSe were similar to the respective control groups (p>0.05); that the other universal materials resulted in lower RU means than those of their controls ER and SE (p<0. 05) and that AlSe presented the lowest mean RU values (p<0.05). For NI, the Groups ScEr, ScSe, AlSe, and AlEr presented low levels of NI similar to those of the control groups (p<0.05). For DC, only Group ScSe demonstrated low DC when compared with the other materials (p<0.05). According to the results of Experiment 2, the systems Pk and AlEr presented a reduction in RU (p<0.05) and only Pk underwent significant degradation (NI) after six months (p<0.05). According to Experiment 3, Al and Gbp obtained higher RU results with the ER strategy (p<0.05); the use of HE increased the RU only in strategy SE (p<0.05); a low level of NI was observed only in Groups Sc and Al with the CON+HE strategy and in Group Gbp with SE+HE; Groups Sc and Gbp presented a high DC when used with the ER strategy, which was increased with the application of HE (p<0.05). The DC if Al was similar under all the conditions. From the three experiments of which this study was composed, it could be concluded that: 1) the universal adhesives presented a material-dependent behavior in both strategies (ER and SE) and were inferior, in at least one of the properties evaluated (RU, NI or DC), when compared with the control adhesives (CSE and SB); 2) In general, after six months the adhesives SE maintained the most stable RU and NI values, which appears to be related mainly to their capacity to form a chemical bond to dental substrate; 3) The use of a hydrophobic layer in strategy SE was able to increase the RU and DC of universal adhesives and the reduction in NI was more dependent on the composition of the adhesive than on the adhesive strategy.

Key Words: dentin; adhesive systems; etch-and-rinse; self-etch; bond strength; degree of conversion; nanoleakage.

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LISTA DE SIGLAS E ABREVIATURAS

A	Fratura adesiva
AC ou Ac	Estratégia Adesiva Autocondicionante
A/M	Fratura adesiva/mista
Al	Allbond Universal
Bis-GMA	Bisfenol Glicidil dimetacrilato
C	Fraturas coesivas
COEP	Comitê de Ética em Pesquisa
CON ou Con	Estratégia Adesiva Convencional
Cp	Corpo de prova
CSE	Clearfil SE Bond
FP	Número de falhas prematuras
Gbp	G-Bond Plus
GC	Grau de conversão
HE	Heliobond
HEMA	2-Hidroxi-etil-metacrilato
I	Integral
LED	Diodo emissor de luz, pela frase em inglês *Light Emitting Diode*
MDP	10-Metacriloiloxidecil dihidrogênio fosfato
MEV	Microscopia Eletrônica de Varredura
n^o	Número
NI	Nanoinfiltração
np	Não polimerizado

p	Polimerizado
PAC	Copolímero de ácido polialquenóico
pH	Potencial hidrogeniônico
Pk	Peak Universal
RU	Resistência de União à microtração
SB	Adper Single Bond 2
Sc	Scotchbond Universal
SiC	Carbeto de silício
TEGDMA	Trietilenoglicoldimetacrilato

Capítulos

ABU	Allbond Universal
DC	Degree of conversion
Er	Etch–and-rinse
GBP	G-Bond Plus
NL	Nanoleakage
PF	Premature failures
R	Ratio of aliphatic and aromatic peak areas
SBU	Scotchbond Universal
Se	Self-etch
SEM	Scanning electron microscope
µTBS	Microtensile bond strengths

LISTA DE SÍMBOLOS E UNIDADES

#	Número
%	Porcentagem
=	Igual
<	Menor que
>	Maior que
±	Mais ou menos
≈	Aproximadamente
µm	Micrometro
cm^{-1}	Centímetro(s) recíproco(s) ou centímetro(s) inverso(s)
s	Segundo(s)
min	Minuto(s)
MPa	Mega Pascal
mm	Milímetro(s)
mm^{2}	Milímetro(s) quadrado(s)
mm/min	Milímetro(s) por minuto
mW	MiliWatts
mW/cm^{2}	MiliWatts por centímetro(s) quadrado
ºC	Grau(s) Celsius
n	Número de corpos de prova
N	Newton
nm	Nanômetro
X	Número de vezes
rpm	Rotações por minuto

h	Hora(s)
α	Nível de significância
p	Valor de probabilidade

SUMÁRIO

1. INTRODUÇÃO

Vários fatores podem afetar a longevidade de restaurações, entretanto, a cárie adjacente às margens ainda é uma das maiores causas de sua substituição (Demarco et al.[1] 2012). Esta falha pode estar relacionada à falta de selamento da interface dente/restauração, o que faz com que haja falta de resistência desta interface aos agentes internos - enzimas proteolíticas - e externos - fluidos e microorganismos (Spencer et al.[2] 2010; Perdigao et al.[3] 2013; Tjaderhane et al.[4] 2013). Esta interface adesiva dente/restauração é produzida pela interação de um sistema adesivo sobre os tecidos dentários duros após a substituição de minerais removidos desses tecidos por monômeros resinosos, formando um polímero interligado micro-mecanicamente com os substratos dentais (Nakabayashi et al.[5] 1982).

Os atuais sistemas adesivos presentes no mercado podem ser classificados segundo sua estratégia de aplicação clínica em: convencional (CON) que inclui condicionamento ácido prévio (*etch-and-rinse*, no idioma Inglês); ou autocondicionante (AC) (*self-etch*, em inglês). Ambas são subdivididas segundo os passos de aplicação em versões de três (unicamente em CON), dois e um passo, este último unicamente em AC (Van Meerbeek et al.[6] 1998; De Munck et al.[7] 2005). As versões simplificadas de ambas categorias foram possíveis através da inclusão de monômeros hidrofílicos e do aumento da quantidade de solventes, para torná-los compatíveis com a umidade inerente da dentina (Van Meerbeek et al.[8] 2003; Yiu et al.[9] 2005).

Na estratégia CON, um gel de ácido fosfórico (20-40%) é aplicado no substrato dentário para remoção da *smear layer* e a exposição das fibrilas de colágeno na dentina. No esmalte, este ácido promove o aumento da área de superfície e da energia superficial. A seguir é aplicado um *primer* que é a mistura de solventes (acetona, etanol e/ou água) e monômeros resinosos hidrofílicos (por exemplo, HEMA) que infiltram o substrato para torná-lo adequado para receber uma mistura de monômeros resinosos relativamente mais hidrofóbicos denominado de *bond*. Estas etapas executadas separadamente (ácido, *primer* e *bond*) exemplificam os sistemas adesivos CON de 3-passos. Da mistura do *primer* e do *bond* numa mesma solução nasceram as versões simplificadas de 2-passos (Van Meerbeek et al.[6] 1998; De Munck et al.[7] 2005; Van Meerbeek et al.[10] 1992).

A principal desvantagem dos sistemas CON, principalmente nas versões de 2-passos, é o risco de as fibrilas colágenas colapsarem durante o processo de secagem da dentina desmineralizada, o que leva a uma diminuição da resistência de união (Spencer, Swafford[11] 1999; Tay et al.[12] 1996). O colapso das fibrilas colágenas é evitado mantendo-se a dentina desmineralizada úmida, tarefa difícil de ser realizada clinicamente. Isto porque a umidade adequada depende do tipo de solvente utilizado no material (Reis et al.[13] 2003) e da interpretação do clínico das instruções do fabricante. A infiltração incompleta das fibrilas de colágeno (Sano et al.[14] 1994) e a necessidade de protegê-las contra os mecanismos de degradação presentes no meio oral (Burrow et al.[15] 1996; Hashimoto et al.[16] 2000) levaram ao desenvolvimento da segunda categoria de sistemas adesivos denominados AC.

Na estratégia adesiva AC (de 1- ou 2-passos) não se indica aplicar previamente o ácido fosfórico nos substratos dentários. Nesta estratégia, utiliza-se uma solução que mistura solventes com monômeros ácidos não laváveis acontecendo simultaneamente desmineralização e infiltração da dentina (De Munck et al.[7] 2005; Miyazaki et al.[17] 2002), assim os cristais de hidroxiapatita dissolvidos e a *smear layer* residual são incorporados na camada híbrida (De Munck et al.[7] 2005; Van Meerbeek et al.[18] 2003). O procedimento de união com os adesivos autocondicionantes pode ser realizado em dois ou um passos, dependendo se o *primer*/condicionador encontra-se separado do *bond* (2-passos) ou combinado com ele (1-passo). Exceto para os sistemas AC com pH muito baixo (Carvalho et al.[19] 2005; Wang, Spencer[20] 2005), toda a extensão em profundidade da dentina desmineralizada é impregnada por monômeros de resina.

Assim, uma das vantagens desta técnica é que ela não é sensível à umidade, já que o adesivo é aplicado em substrato seco. Esta pode ser a razão pela qual os sistemas AC não estão associados com as características de sensibilidade da técnica de união à dentina condicionada úmida (Reis et al.[13] 2003; Tay et al.[21] 1998; Perdigao[22] 2010). Por outro lado, uma desvantagem evidente do protocolo AC é a redução da eficácia da união ao esmalte (Kanemura et al.[23] 1999; Pashley, Tay[24] 2001), devido à menor capacidade de remoção dos minerais desse tecido.

Consequentemente, o aumento na área de superfície do esmalte obtido com adesivos AC é menor do que o atingido com o ácido fosfórico, sendo esta capacidade dependente do pH do adesivo AC (Pashley, Tay[24] 2001).

Assim, alguns estudos têm investigado a utilização de ácido fosfórico antes da aplicação de adesivos AC (Van Meerbeek et al.[18] 2003; Rotta et al.[25] 2007; Frankenberger et al.[26] 2008), observando uma melhora no comportamento desses adesivos. No entanto, este procedimento tem-se mostrado inadequado na dentina (Van Landuyt et al.[27] 2006; Van Landuyt et al.[28] 2006; Hanabusa et al.[29] 2012) situação que poderia acontecer acidentalmente durante o condicionamento de esmalte por escoamento de ácido fosfórico de baixa viscosidade. O efeito do condicionamento intencional da dentina com ácido fosfórico prévio à aplicação dos adesivos AC tem sido estudado (Van Landuyt et al.[27] 2006; Hanabusa et al.[29] 2012; Erhardt et al.[30] 2008; Proenca et al.[31] 2007; Taschner et al.[32] 2012), com resultados controversos e material-dependentes.

Considerando as diferenças no julgamento profissional a respeito da seleção da estratégia adesiva e número de passos em diferentes situações clinicas, alguns fabricantes lançaram sistemas adesivos mais versáteis que incluem as opções convencional (de 2-passos) e autocondicionante (2- e 1-passo). Estes novos materiais são chamados de “Universais,” “Multimodo” ou “Multipropósito” (Hanabusa et al.[29] 2012; Perdigao et al.[33] 2012) mas ainda existe pouca informação acerca do comportamento desta nova classe de sistemas adesivos. Estes materiais, em geral, são adesivos simplificados na sua concepção e, portanto, presume-se com grande grau de hidrofilicidade, o que poderia ocasionar, em parte, os mesmos problemas de degradação da união dos adesivos anteriores.

Algumas pesquisas têm avaliado os mecanismos de envelhecimento envolvidos na degradação das interfaces de união (Liu et al.[34] 2011; Breschi et al.[35] 2008), porém este mecanismo não é claramente compreendido (Breschi et al.[35] 2008; Vaidyanathan, Vaidyanathan[36] 2009; Hashimoto[37] 2010). Acredita-se que a degradação se inicia com a extração da resina infiltrada na matriz dentinária através de nano-espaços cheios de água presentes na camada híbrida e pelo ataque enzimático das fibrilas colágenas expostas, levando à sua instabilidade (Spencer et al.[2] 2010; Sano[38] 2006).

Como relatado anteriormente, versões simplificadas foram possíveis através da inclusão de monômeros hidrofílicos, ácidos, e do aumento da quantidade de solventes (Van Meerbeek et al.[8] 2003; Yiu et al.[9] 2005). Porém, isto vai conduzir a uma maior quantidade de solvente residual retido na camada híbrida e camada de adesivo (Yiu et al.[9] 2005), impedindo a formação de um polímero com reticulado elevado (Ye et al.[39] 2007; Loguercio et al.[40] 2009; Paul et al.[41] 1999), diminuindo o grau de conversão (Cadenaro et al.[42] 2005), reduzindo a resistência de união resina-dentina (Hass et al.[43] 2011; Reis et al.[44] 2010; Takahashi et al.[45] 2002; Cho, Dickens[46] 2004) e aumentando a permeabilidade da camada adesiva, após polimerização (Malacarne et al.[47] 2006; Ito et al.[48] 2005). Consequentemente, os polímeros serão mais susceptíveis à degradação ao longo do tempo (De Munck et al.[7] 2005; Breschi et al.[49] 2008). Por outro lado, tem se demonstrado que a infiltração de monômeros dos sistemas adesivos CON é sempre menor do que a extensão da desmineralização da dentina (Wang, Spencer[50] 2003; Wang et al.[51] 2006; Santini, Miletic[52] 2008) e que isto também poderia acontecer com os sistemas adesivos AC (Carvalho et al.[19] 2005; Wang, Spencer[20] 2005), resultando em zonas incompletamente infiltradas ao longo da parte inferior da camada híbrida que contêm fibrilas de colágenas expostas (Sano et al.[53] 1995) e nanoespaços com água denominados de nanoinfiltração (Sano et al.[53] 1995). Assim, o sinergismo entre a falta de infiltração dos monômeros e a instabilidade do polímero permitiria uma exposição contínua de fibrilas colágenas as quais ficariam susceptíveis à atividade de enzimas proteolíticas como as metaloproteinases (MMPs) (De Munck et al.[54] 2003; Nishitani et al.[55] 2006; Pashley et al.[56] 2004) e cisteína catepsina (Tersariol et al.[57] 2010; Nascimento et al.[58] 2011).

Reis e colaboradores (Reis et al.[59] 2013) indicaram diferentes alternativas clínicas que podem ser usadas para diminuir a degradação da interface de união à dentina, e assim, aumentar a longevidade das restaurações. Dentre as técnicas discutidas se inclui a aplicação de uma camada adicional de resina hidrofóbica sobre o adesivo simplificado (King et al.[60] 2005). Esta camada extra de resina tem como objetivo aumentar a espessura e uniformidade da interface adesiva, bem como reduzir o fluxo de fluido através dela (King et al.[60] 2005; de Andrade e Silva et al.[61] 2009). Excelentes resultados *in vitro* e clínicos tem sido mostrados após a colocação de um revestimento de resina hidrofóbica sobre um adesivo AC de 1-passo (Reis et al.[62] 2008; Reis et al.[63] 2009), no entanto esta técnica não foi testada com os novos adesivos “universais”.

2. PROPOSIÇÃO

O objetivo deste estudo foi avaliar o efeito da aplicação das estratégias adesivas convencional ou autocondicionante, modificadas ou não pela aplicação de uma camada de resina hidrofóbica adicional, nas propriedades de união à dentina de diferentes sistemas adesivos universais.

2.1 Objetivos específicos

1. Avaliar resistência de união à microtração, nanoinfiltração e grau de conversão na camada híbrida de adesivos universais aplicados em dentina, de acordo com as estratégias adesivas convencional e autocondicionante, no tempo imediato (24 h).
2. Avaliar resistência de união à microtração e nanoinfiltração de adesivos universais aplicados em dentina de acordo as estratégias adesivas convencional e autocondicionante no tempo imediato (24 h) e após seis meses.
3. Avaliar a resistência de união à microtração, nanoinfiltração e grau de conversão na camada híbrida de adesivos universais aplicados com uma camada hidrofóbica em dentina para as estratégias adesivas convencional e autocondicionante, no tempo imediato (24 h).

3. MATERIAL E MÉTODOS

3.1. Discriminação dos experimentos constantes no presente trabalho

Os experimentos que compõem o presente trabalho são: Experimento 1. Avaliação das propriedades imediatas de união de adesivos universais à dentina (Capítulo 1: *Immediate bonding properties of universal adhesives to dentine*); Experimento 2: Avaliação da longevidade das propriedades de união de adesivos universais à dentina (Capítulo 2: *Longevity of bonding properties of universal adhesives to dentin*); e Experimento 3: Influência de uma camada de resina hidrofóbica na performance adesiva de adesivos universais à dentina (Capítulo 3: *Influence of a hydrophobic resin coat on the bonding efficacy of three universal adhesives*).

Foram utilizados sete tipos de materiais adesivos distribuídos nos três experimentos desenvolvidos **(Quadro 1)**.

Quadro 1 – Materiais adesivos, composição e fabricante

Material adesivo	Componente: Composição	Fabricante
Adper Single Bond 2	1. Condicionador: ácido fosfórico 35% (Scotchbond Etchant) 2. Adesivo: Bis-GMA, HEMA, dimetacrilato, etanol, água, fotoiniciator, metacrilato copolimérico funcional de ácido poliacrílico e politacônico, partículas de sílica esféricas de 5 nm de diâmetro (10% peso)	3M ESPE, Saint Paul, Minnesota, EUA
Clearfil SE Bond	1. *Primer*: água, MDP, HEMA, camforquinona, dimetacrilato hidrofílico 2. *Bond*: MDP, Bis-GMA, HEMA, canforquinona, dimetacrilato hidrofílico, N,N-dietanol p-toluidina bond, sílica coloidal	Kuraray, Minami-ku, Okayama, Japão
Peak Universal	1. Condicionador: ácido fosfórico (Ultraetch) 2. *Primer:* álcool etílico, ácido metacrílico , 2-hidroxietil metacrilato (Peak SE Primer) 3. Adesivo: álcool etílico, 2-hidroxietil metacrilato (Peak LC Bond)	Ultradent Products Inc., South Jordan, Utah, EUA
Scotchbond Universal *	1. Condicionador: ácido fosfórico 34%, água, sílica amorfa sintética, polietileno glicol, óxido de alumínio (Scotchbond Universal Etchant) 2. Adesivo: monômero de fosfato MDP, dimetacrilato, HEMA, copolímero de ácido polialquenóico metacrilato modificado, carga de partículas, etanol, água, iniciadores, silano	3M ESPE, Saint Paul, Minnesota, EUA
All-Bond Universal	1. Condicionador: ácido fosfórico 32%, cloreto de benzalcônio (Uni-Etch) 2. Adesivo: MDP, Bis-GMA, HEMA, etanol, água, iniciadores	Bisco Inc., Shaumburg, Illinois, EUA
G-Bond Plus**	Acetona, dimetacrilato, 4-metacrietil trimetílico anidrido, monômero éster de ácido fosfórico, dióxido de silício, fotoiniciador, água destilada.	GC Corporation Tokyo, Japão
Heliobond	Bis-GMA, TEGDMA	Ivoclar Vivadent, Schaan, Liechtenstein

* distribuído no Brasil como Single Bond Universal, **distribuído também como Gaenial Bond; Bis-GMA: bisfenol glicidil metacrilato; HEMA: metacrilato de 2-hidroxietila; MDP: 10-metacriloiloxidecil dihidrogênio fosfato; TEGDMA: trietileno glicol dimetacrilato; * distribuído no Brasil como Single Bond Universal,**distribuído também como Gaenial Bond

3.1.1 Obtenção e preparação dos dentes

Cento e quarenta molares humanos livres de cárie foram obtidos do Banco de Dentes da Universidade Estadual de Ponta Grossa sob o protocolo de pesquisa número 17878/11, aprovado pelo Comitê de Ética (Anexo A).

Os dentes foram desinfetados em cloramina 0,5% e armazenados em água destilada trocada semanalmente até sua utilização. Com o auxílio de uma politriz (Arotec E, Cotia, São Paulo, Brasil) e usando lixa de carbeto de silício de granulação #180, sob irrigação constante, foi retirado o esmalte oclusal e planificada a superfície dos molares até exposição da dentina. As bordas periféricas de esmalte foram totalmente desgastadas com pontas diamantadas n^{o} 4137 (KG Sorensen, Barueri, São Paulo, Brasil) em alta rotação e irrigação constante deixando unicamente tecido dentinário **(Figura 1).** A seguir, as superfícies de dentina foram polidas manualmente sob irrigação por 60 s para a padronização da *smear layer* utilizando lixas de carbeto de silício de granulação #600 (Extec, Enfield, Connecticut, EUA).

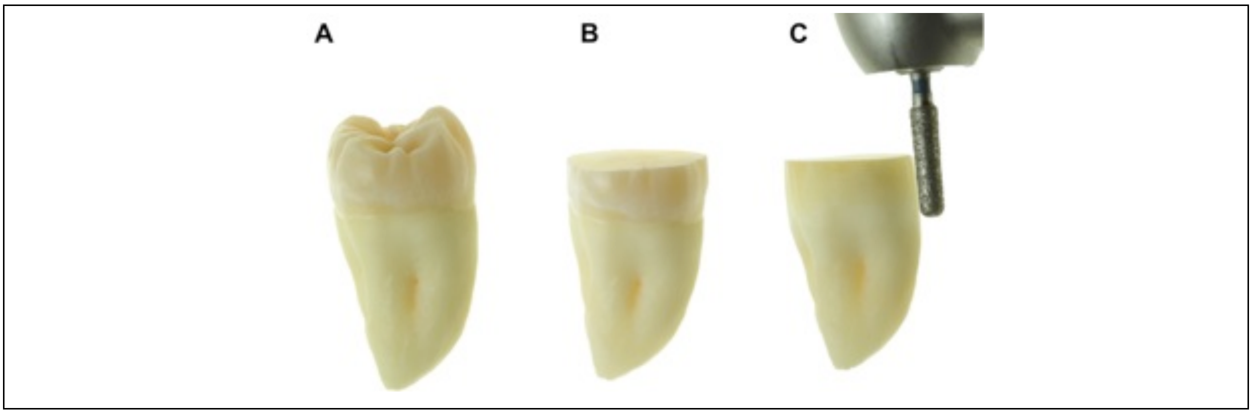

Figura 1 - Preparo da superfície de dentina. (A) dente hígido; (B) superfície oclusal planificada e em dentina; desgaste de todas as bordas periféricas de esmalte.

3.1.2 Desenho experimental e procedimentos adesivos e restaurador

Para o Experimento 1, bem como para o Experimento 2, 40 dentes preparados como descrito anteriormente foram distribuídos aleatoriamente em oito grupos experimentais (n=5), de acordo com o adesivo e as estratégias adesivas convencional (CON) ou autocondicionante (AC). A sequência técnica das estratégias adesivas está demonstrada nas **Figuras 2 e 3**, respectivamente. Como grupos controle foram selecionados os sistemas adesivos Adper Single Bond 2 (SB), segundo a estratégia CON de 2 passos; e Clearfil SE Bond (CSE), segundo a estratégia autocondicionante (AC), também de 2-passos. Os grupos experimentais do estudo foram constituídos por três adesivos universais aplicados com estratégias CON ou AC: Peak Universal (Peak SE Primer e Peak LC Bond), aplicado como 2-passos CON (PkCon) e 2-passos AC (PkAc); Scotchbond Universal, aplicado como 2-passos CON (ScCon) e 1-passo AC (ScAc); e All-Bond Universal aplicado como 2-passos CON (AlCon) e 1-passo AC (AlAc) **(Quadro 2).**

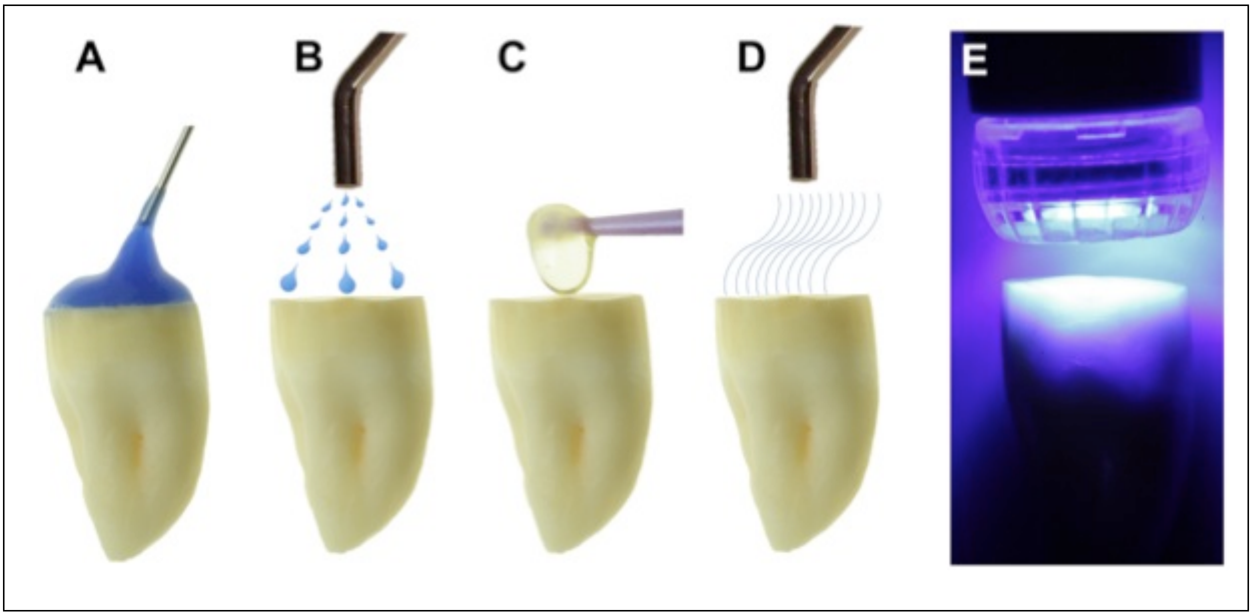

Figura 2 – Estratégia adesiva convencional. (A) condicionamento ácido; (B) lavagem e secagem; (C) Aplicação do adesivo; (D) secagem para evaporação do solvente; (E) fotoativação. Todos os procedimentos realizados segundo as indicações do fabricante, os quais estão discriminados no quadro 2.

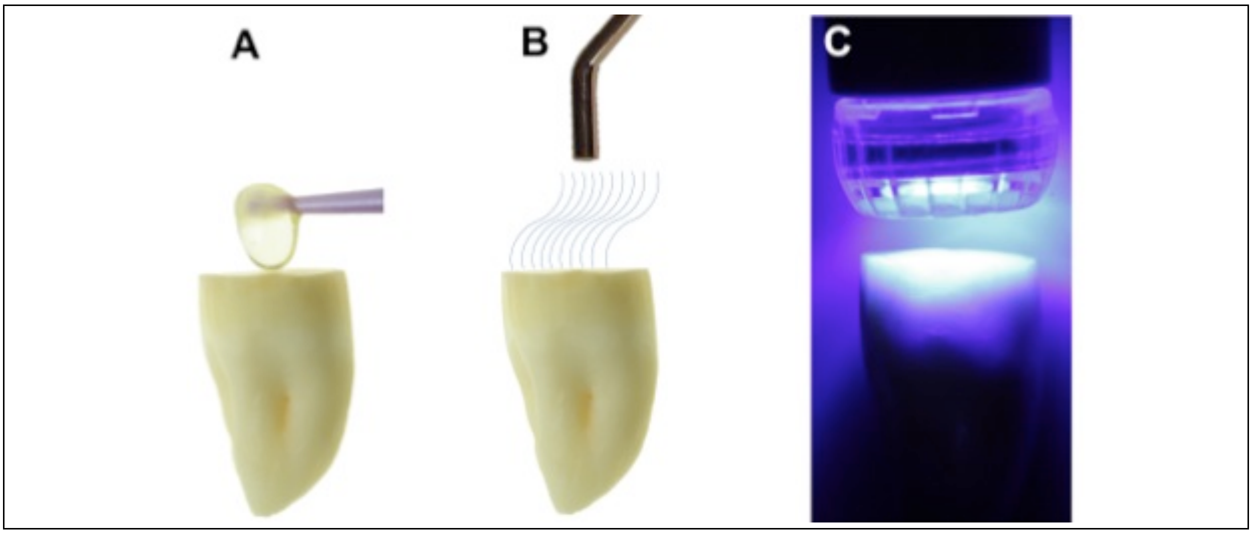

Figura 3 - Estratégia adesiva autocondicionante (dispensa a aplicação e a lavagem do ácido fosfórico). (A) Aplicação do adesivo; (B) secagem para evaporação do solvente; (C) fotoativação. Todos os procedimentos realizados segundo as indicações dos fabricantes, os quais estão discriminados no quadro 2.

Para o Experimento 3, 60 terceiros molares foram divididos em 12 grupos (n=5), de acordo com o adesivo e a estratégia adesiva **(Figuras 2 e 3)**, da mesma maneira como descrito para os Experimentos 1 e 2, porém variando-se alguns dos materiais selecionados. Além disso, este Experimento avaliou o efeito da adição **(Figuras 4 e 5)** ou não **(Figuras 2 e 3)** de uma camada final de adesivo hidrofóbico nas duas estratégias de união. Os grupos experimentais formados pelas combinações descritas anteriormente foram: Scotchbond Universal convencional (ScCon), convencional + camada hidrofóbica (ScCon-HE), autocondicionante (ScAc), autocondicionante + camada hidrofóbica (ScAc-HE); Allbond Universal convencional (AlCon), convencional + camada hidrofóbica (AlCon-HE), autocondicionante (AlAc), autocondicionante + camada hidrofóbica (AlAc-HE); G-Bond Plus convencional (GbpCon), convencional + camada hidrofóbica (GbpCon-HE), autocondicionante (GbpAc), autocondicionante + camada hidrofóbica (GbpAc-HE) **(Quadro 3).**

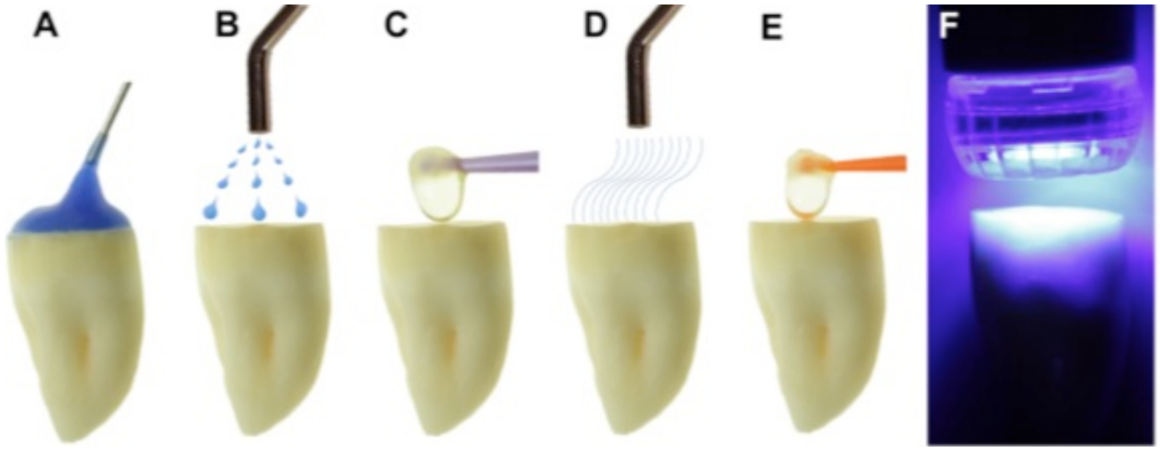

Figura 4 - Estratégia adesiva convencional com aplicação de camada hidrofóbica. (A) condicionamento ácido; (B) lavagem e secagem; (C) Aplicação do adesivo; (D) secagem para evaporação do solvente; (E) aplicação de uma camada de resina hidrofóbica; (F) fotoativação. Vide Quadro 3.

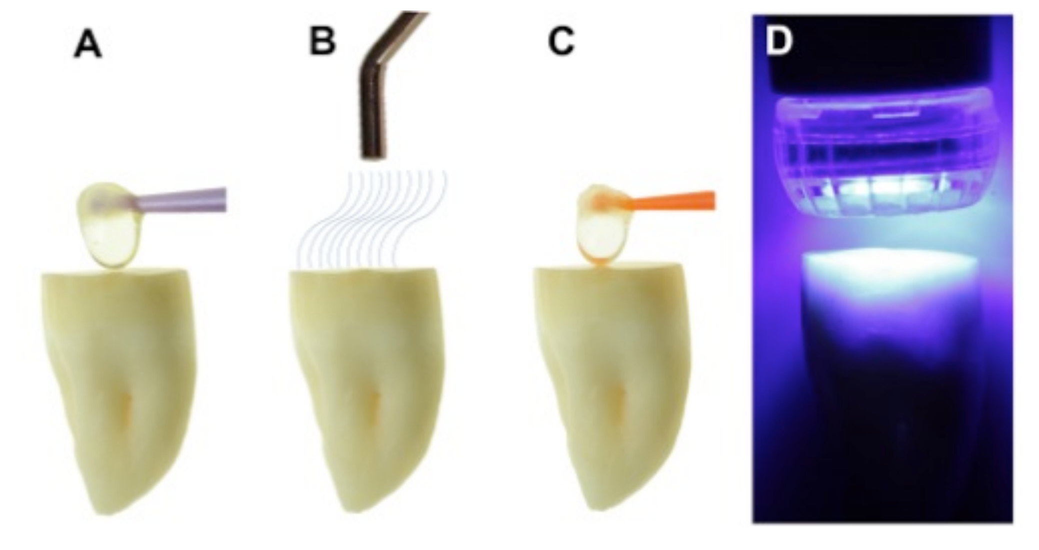

Figura 5 - Estratégia adesiva autocondicionante, a qual dispensa o condicionamento com ácido fosfórico, com aplicação de camada hidrofóbica; (A) aplicação do adesivo; (B) secagem para evaporação do solvente; (C) aplicação de camada de resina hidrofóbica; (D) fotoativação. Vide Quadro 3.

Quadro 2 - Sistemas adesivos e estratégias de aplicação de acordo com as instruções do fabricante para os Experimentos 1 e 2

Sistema adesivo (lote),	Estratégia Autocondicionate	Estratégia convencional
Adper Single Bond 2 (BPBR)	N.A	1. Aplicar condicionador por 15 s 2. Lavar por 10 s 3. Remover o excesso de água 4. Aplicar 2-3 camadas consecutivas do adesivo por 15 s com agitação suave 5. Aplicar suave jato de ar por 5 s 6. Fotoativar por 10 s*
Clearfil SE Bond (Primer: 00954A - Bond: 01416ª)	1. Aplicar o *primer* sobre a superfície do dente e deixar por 20 s 2.Secar com jato de ar para evaporar os componentes voláteis 3. Aplicar *bond* na superfície do dente, criando uma película uniforme usando um jato de ar suave 4. Fotoativar por 10 s*	N.A
Peak Universal Adhesive System (Peak SE Primer: 0N062 – Peak LC Bond: Y062)	1. Ativar os dois componentes separados na seringa 2. Aplicar "Peak SE" com microbrush por 20 s esfregando continuamente em dentina. 3. Aplicar 3 s de jato de ar/água ou sucção de alto volume diretamente sobre a preparação para afinar a camada 4. Aplicar uma generosa camada de Peak LC Bond, com agitação suave durante 10 s 5. Afinar e secar a camada usando 10 s com ¼ a ½ da pressão de ar 6. Fotoativar por 10 s*	1. Fazer condicionamento ácido por 20 s 2. Lavar por 5 s 3. Secar com jato de ar por 2 s 4. Aplicar uma generosa camada de Peak LC Bond, com agitação suave durante 10 s 5. Afinar e secar a camada usando 10 s com ¼ a ½ da pressão de ar 6. Fotoativar por 10 s*
Scotchbond Universal (D-82229)	1. Aplicar o adesivo na preparação com um microbrush e esfrega-lo por 20 s. Se necessário, molhe com adesivo a microbrush durante o tratamento 2. Dirigir um ligeiro jato de ar sobre o líquido durante 5 s aproximadamente até a camada não se mover e o solvente estiver completamente evaporado 3. Fotoativar por 10 s*	1. Fazer condicionamento ácido por 15 s 2. Lavar por 10 s 3. Secar com jato de ar por 2 s 4. Aplicar adesivo como indicado na estratégia autocondicionante
All-Bond Universal (1200006111)	1. Aplicar duas camadas de adesivo separadamente, esfregar sobre a preparação por 10-15 s por cada camada. Não fotoativar entre as camadas. 2. Evaporar cuidadosamente o excesso de solvente secando com jato de ar durante pelo menos 10 s, não devendo haver nenhum movimento visível do material. A superfície deve ter uma aparência brilhante e uniforme 3. Fotoativar por 10 s*	1. Fazer condicionamento ácido por 15 s 2. Lavar bem 3. Remover excessos de água com pellets absorventes ou sucção de alta potência por 1-2 s 4. Aplicar adesivo como indicado na estratégia autocondicionante

* aparelho LED de 1200 mW/cm^2 de intensidade (Radii-cal, SDI Limited, Bayswater, Victoria, Austrália)

Quadro 3 - Materiais adesivos e estratégias de aplicação dos sistemas adesivos utilizados no Experimento 3

Material adesivo (lote)	**Estratégia de aplicação**			
Heliobond - HE (N37749)	**Autocondicionante**		**Convencional**	
	Sem camada hidrofóbica	**Com camada hidrofóbica**	**Sem camada hidrofóbica**	**Com camada hidrofóbica**
Scotchbond Universal Adhesive - SBU (448716)	1. Aplicar o adesivo na preparação com um microbrush e esfregue-o por 20 s. Se necessário, molhe novamente com adesivo o microbrush durante o tratamento 2. Dirigir um ligeiro jato de ar sobre o líquido durante 5 s aproximadamente até que a camada não se mova e o solvente esteja completamente evaporado 3. Fotoativar por 10 s*	1. Repetir os passos da estratégia autocondicionante 2. Aplicar uma camada muito fina de Heliobond com microbrush sobre a superfície dentinária 3. Aplicar jato de ar para conseguir uma camada fina 4. Fotoativar por 10 s*	1. Condicionamento com ácido fosfórico por 15 s 2. Lavar por 10 s 3. Secar com ar durante 5 s 4. Aplicar adesivo como na estratégia autocondicionante	1. Repetir os passos da estratégia convencional 2. Aplicar uma camada muito fina de Heliobond com microbrush sobre a superfície dentinária 3. Aplicar jato de ar para conseguir uma camada fina 4. Fotoativar por 10 s*
All-Bond Universal - ABU (1200002722)	1. Aplicar duas camadas de adesivo separadamente, esfregar sobre a preparação por 10-15 s por cada camada. Não fotoativar entre as camadas. 2. Evaporar cuidadosamente o excesso de solvente secando com jato de ar durante pelo menos 10 s, não devendo haver nenhum movimento visível do material. A superfície deve ter uma aparência brilhante e uniforme 3. Fotoativar por 10 s*	1. Aplicar da mesma forma autocondicionante 2. Aplicar uma camada muito fina de Heliobond com microbrush sobre a superfície dentária 3. Jato de ar para conseguir uma camada fina 4. Fotoativar por 10 s*	1. Fazer condicionamento ácido por 15 s 2. Lavar bem 3. Remover excessos de água com pellets absorventes ou sucção de alta potência por 1-2 s 4. Aplique adesivo como indicado na estratégia autocondicionante	1. Repetir os passos da estratégia convencional 2. Aplicar uma camada muito fina de Heliobond com microbrush sobre a superfície dentinária 3. Aplicar jato de ar para conseguir uma camada fina 4. Fotoativar por 10 s*
G-Bond Plus - GBP (1102221)	1. Aplicar usando um microbrush 2. Deixar em repouso durante 10 s após o fim da aplicação 3. Secar cuidadosamente por 5 s com ar livre de óleo sob pressão de ar máxima, utilizando sucção a vácuo 4. Fotoativar por 10 s*	1. Repetir os passos da estratégia autocondicionante 2. Aplicar uma camada muito fina de Heliobond com microbrush sobre a superfície dentária 3. Jato de ar para se obter camada fina 4. Fotoativar por 10 s*	1. Aplicar ácido fosfórico gel 34% por 10 s (Scotchbond Universal Etchant) 2. Lavar por 5s e secar suavemente 3. Aplicar o adesivo como na estratégia de autocondicionante **NOTA:** O fabricante não indica condicionamento com ácido fosfórico em dentina	1. Aplicar da mesma forma como na estratégia convencional 2. Aplicar uma camada muito fina de Heliobond com microbrush sobre a superfície dentinaria 3. Jato de ar para conseguir uma camada fina 4. Fotoativar por 10 s*

* Aparelho LED de 1200 mW/cm^2 de intensidade (Radii-cal, SDI Limited, Bayswater, Victoria, Austrália)

Os sistemas adesivos foram aplicados em conformidade com as instruções dos respectivos fabricantes, a partir das quais foram desenhadas as variáveis adesivas de todos os experimentos. As únicas exceções ocorreram no Experimento 3, pois alguns grupos receberam a camada final de resina hidrofóbica, e, ainda no mesmo experimento, pois se realizou o condicionamento com ácido fosfórico para o material G-bond Plus. Todos os procedimentos adesivos e restauradores foram realizados por um único operador em ambiente controlado (24^{o}C).

Os sistemas adesivos foram fotoativados usando um aparelho LED de 1200 mW/cm^2 de intensidade (Radii-cal, SDI Limited, Bayswater, Victoria, Austrália). Após os procedimentos adesivos, todos os dentes foram restaurados com uma resina composta microhíbrida de restauração (Opallis, FGM Produtos Odontológicos, Joinville, Santa Catarina, Brasil) em dois incrementos de 2 mm sendo cada incremento fotoativado por 40 s com o mesmo aparelho LED **(Figura 6).**

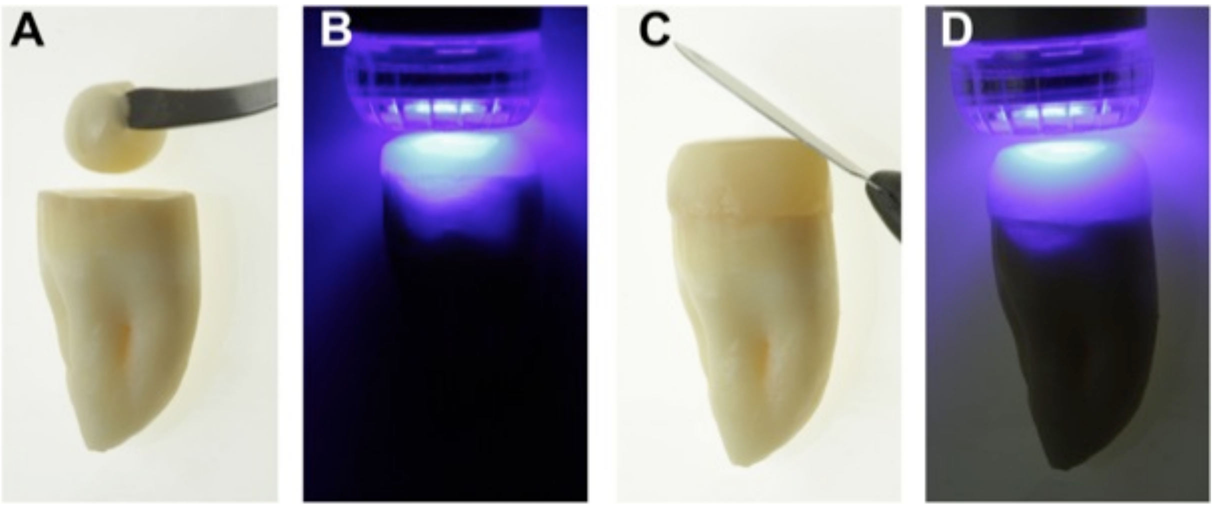

Figura 6 – Restauração dos dentes após aplicação das variáveis adesivas. (A) Colocação da resina composta; (B) fotoativação do primeiro incremento; (C) Colocação do incremento final; (D) fotoativação do segundo incremento.

Os dentes restaurados foram armazenados em água destilada a 37^{o} C por 24 h e levados a uma máquina de corte (Isomet 1000, Buehler Ltd., Lake Bluff, Illinois, EUA). Com ajuda de um disco diamantado (15 HC-4in, Buehler Ltd., Lake Bluff, Illinois, EUA) sob irrigação a 300 rpm os dentes foram seccionados em duas sequências de cortes perpendiculares uma no eixo *x* e outra no eixo *y,* obtendo-se 25-30 "palitos" **(Figura 7)** com uma área de secção transversal de 0,8 ± 0,1 mm^2, medida com um paquímetro digital (Digimatic Caliper, Mitutoyo, Kanagawa, Tóquio, Japão, **Figura 8B**).

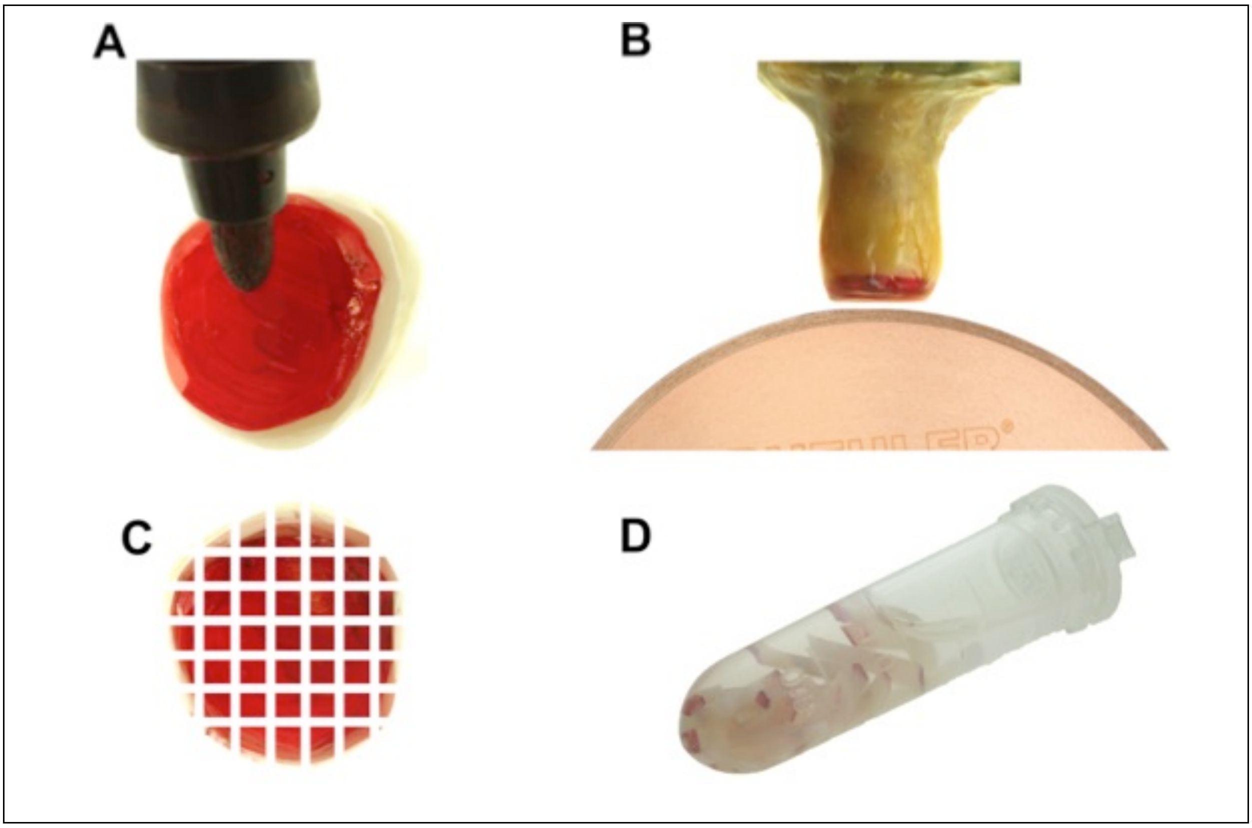

Figura 7 - Sequência de corte para a obtenção dos palitos. (A) superfície oclusal das coroas de resina compostas pintadas com caneta marcadora permanente de cores diferentes para identificação dos palitos de cada grupo. (B) dente fixado com cera pegajosa ao dispositivo da máquina de corte montada com um disco diamantado; (C) duas sequências perpendiculares de corte, uma no eixo x e outra no eixo y, permitiram a obtenção dos palitos para o teste de resistência de união; (D) em *eppendorfs*, os palitos de cada dente foram armazenados em água destilada por 24 h.

Nos Experimentos 1 e 3 a totalidade dos palitos foi utilizada imediatamente após as 24 h de armazenamento. No Experimento 2, metade dos palitos de cada dente foi selecionada aleatoriamente para serem testados imediatamente (24 h) e o restante após 6 meses de armazenamento em água destilada. Alguns palitos de cada tempo de avaliação foram aleatoriamente selecionados e distribuídos nos testes de grau de conversão (n = 3) e nanoinfiltração (n = 3 no tempo de 24 h e n = 3 aos 6 meses) como será descrito.

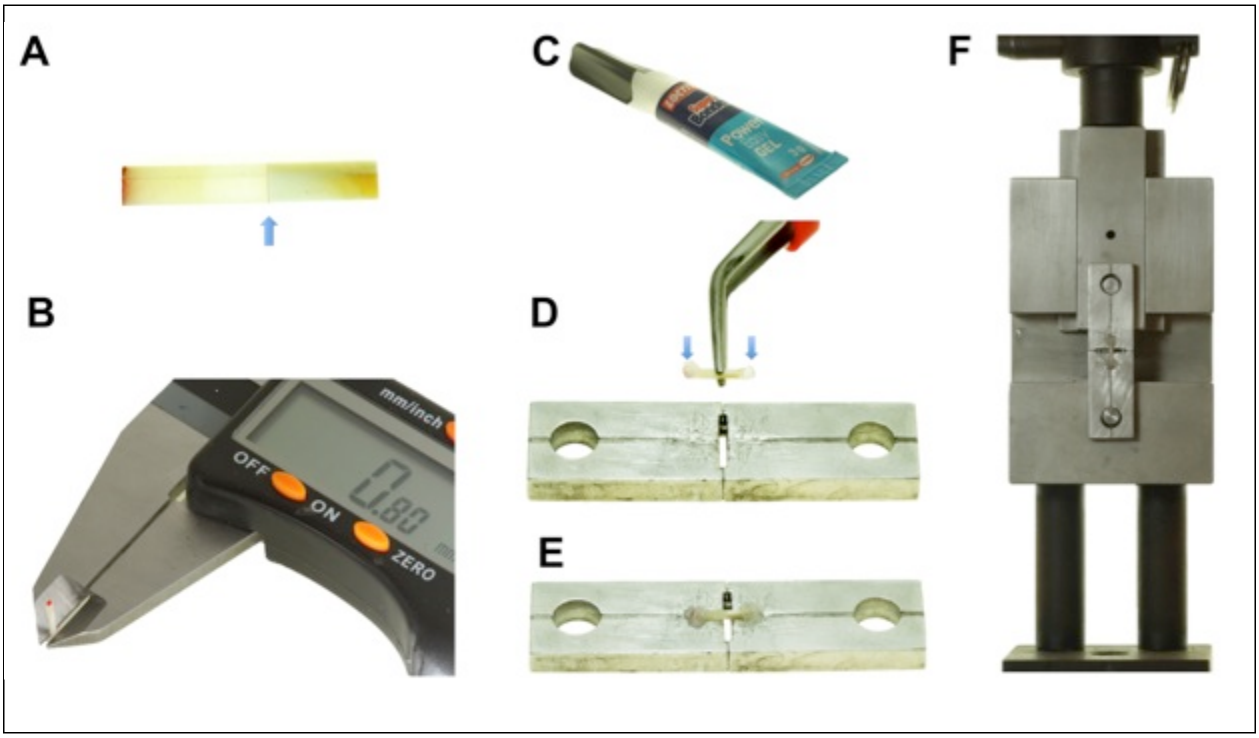

Figura 8 – Sequência para o teste de microtração. (A) a seta azul indica a interface adesiva entre dente e restauração, numa vista lateral do palito. (B) mensuração da largura e da espessura do mesmo palito, com paquímetro digital, para o cálculo da área da interface adesiva. (C) cola de cianocrilato em gel (que permite controle da aplicação). (D) aplicação da cola nas extremidades do palito, protegendo a interface com a pinça; (E) posicionamento do palito nas garras de microtração deixando a interface adesiva no espaço central da garra; (F) adaptação das garras na máquina de ensaios universais após a polimerização da cola.

3.1.3 Teste de resistência de união à microtração (RU)

Os palitos obtidos foram colados individualmente em garras de Geraldeli (Odeme Biotechnology, Joaçaba, Santa Catarina, Brasil) (Perdigao et al.[64] 2002) aplicando em cada um dos extremos do palito cola de cianocrilato gel (Loctite Superbonder Power Easy Gel, Henckel, São Paulo, São Paulo, Brasil) de forma a não contaminar a interface dente-restauração **(Figura 8).** Para acelerar o tempo de polimerização da cola, sobre sua superfície foi pincelado (Cavibrush, FGM Produtos Odontológicos, Joinville, Santa Catarina, Brasil) líquido de resina acrílica autopolimerizável para prótese removível (Jet, Artigos Odontológicos Clássico, São Paulo, SP, Brasil). Após o tempo de polimerização da cola, as garras com os palitos foram acopladas na máquina de ensaios universal (Kratos Dinamômetros, Cotia, São Paulo, Brasil, **Figura 8F**) e estes foram testados por tração a uma velocidade de 0,5 mm/min com célula de carga de 100 N até rompimento da interface. Os valores de

RU de cada palito em Megapascal (MPa) foram calculados pela divisão da tensão máxima a ruptura (N) e a área de secção transversal (mm^2) obtida previamente de cada palito.

A unidade de medida foi o dente, assim uma média do valor RU em MPa dos palitos provenientes do mesmo dente foi obtida, totalizando cinco valores médios por grupo experimental.

3.1.4 Tipos de fratura

Utilizando uma lupa esteoreoscópica a 100x de magnificação (Olympus SZ40, Shinjuku-ku, Tóquio, Japão) a falha de cada palito foi classificada segundo o tipo de fratura como **(Figura 9)**:

- Adesiva, falha na interface adesiva dente/restauração.
- Coesiva, falha exclusivamente em dentina ou resina composta.
- Adesiva/Mista, falha na interface adesiva dente/restauração, mas que incluiu falha coesiva dos substratos vizinhos.
- Falhas prematuras, palitos que quebraram antes dos testes.

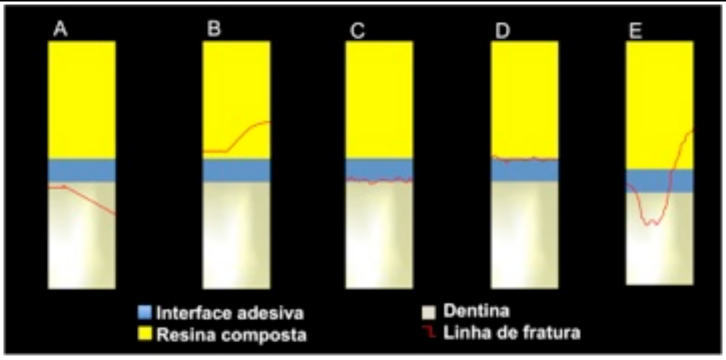

Figura 9 - Tipos de fratura: (A) coesiva de dentina; (B) coesiva de resina composta; (C) adesiva na camada híbrida; (D) adesiva na camada adesiva; (E) adesiva/mista.

3.1.5 Grau de conversão (GC)

Como descrito anteriormente, três palitos por cada dente foram selecionados aleatoriamente e submetidos ao teste de GC. Previamente ao teste, os palitos foram colados com parafina em *stubs* **(Figura 10)** e levados a uma politriz manual que permite a irrigação com água constante no momento do polimento, limpando continuamente as partículas geradas. A sequência de lixas de SiC foi #1500; 2000 e 2500 (Buehler Ltd., Lake Bluff, Illinois, EUA) foi aplicada por 30 s cada uma. A seguir os palitos foram lavados em cuba ultrassônica (Dabi Atlante – 3L, Ribeirão Preto, São Paulo, Brasil) por 20 min e armazenados em água destilada a 37° C por 24 h antes da leitura de GC.

A análise de GC foi realizada na camada híbrida com um espectrofotômetro de Raman (Senterra equipment, Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Alemanha). Antes das análises, o equipamento era calibrado para redefinir o zero utilizando padrões de silicone com valores de coeficientes conhecidos. As análises foram realizadas sob os seguintes parâmetros: laser de Neônio com 532 nm de comprimento de onda a 20 mW de potência; resolução espacial de ≈ 3 µm; resolução espectral de ≈ 5 cm^{-1}, com 30 s de tempo de acumulação e 6 co-adições, em magnificação de 100X para um laser de ≈ 1 µm de diâmetro. A análise iniciou obtendo-se os espectros com os picos de referência dos adesivos não polimerizados (*np*). Para isso, cada sistema adesivo teve todos os seus componentes dispensados e misturados em uma placa de vidro, a qual foi levada ao espectrofotômetro para leitura conforme os parâmetros já descritos. A leitura dos adesivos polimerizados (*p*) foi realizada diretamente na camada híbrida. Considerando as duplas ligações dos compostos alifáticos (1639 cm^{-1}, pico *reativo*, aqui são detectadas mudanças) e aromáticos (1609 cm^{-1}, pico *referência*, estável sem mudanças). Para ambas as análises (*np* e *p*) foram determinados os valores da integral (I) dos picos registrados utilizando o software do equipamento Opus Spectroscopy 6.5 (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Alemanha). Assim, o GC de duplas ligações do monômero em polímero foi calculado com a seguinte fórmula:

$$GC\ \% = \left[1 - \frac{\mathrm{I}\ Reativo\ (p)_{1639} / \ \mathrm{I}\ Referência(p)_{1609}}{\mathrm{I}\ Reativo(np)_{1639} / \mathrm{I}\ Referência(np)_{1609}}\right] \times 100$$

As análises espectrais foram obtidas de três sítios aleatoriamente selecionados na camada híbrida de cada palito. A partir disso foi possível a obtenção das médias de cada dente, as quais foram utilizadas na análise estatística (n = 3). As leituras iniciais (*np*) também foram realizadas em triplicata.

Figura 10 - *Stub* com palitos lixados previamente à leitura no micro-Raman. Isto facilita a localização da camada híbrida.

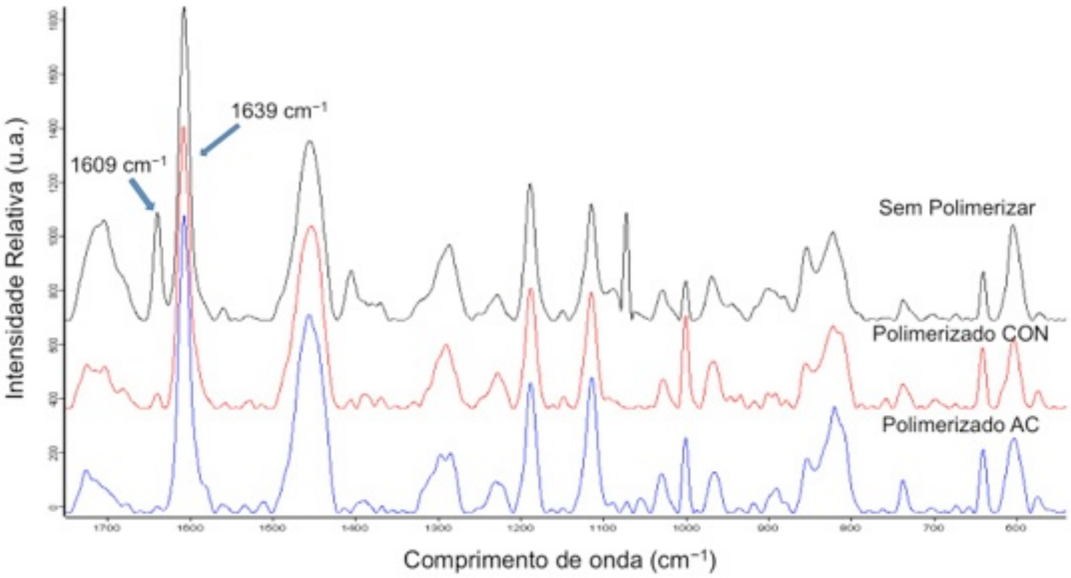

Figura 11 – Espectros representativos obtidos do sistema adesivo Peak Bond Universal não polimerizado, e após sua fotoativação na camada hibrida *in situ* em sua versão ER e SE. As setas indicam os picos de referencia (1609 cm^{-1}) e reativo (1639 cm^{-1}).

3.1.6 Avaliação da Nanoinfiltração (NI) em Microscopia Eletrônica de Varredura (MEV)

Três palitos provenientes de cada dente para os tempos imediato e 6 meses, foram reservados para análise de NI **(Figura 11)**. Utilizando eppendorfs (OA12, Odeme Biotechnology, Joaçaba, SC, Brasil), os palitos foram imersos em uma solução aquosa (pH=7,0) de nitrato de prata amoniacal 50% em peso (Tay et al.[65] 2002) e armazenados por 24 h em ausência de luz. Após o tempo de infiltração, os palitos foram lavados profusamente com água destilada e imersos em solução reveladora (Kodak, Rochester, Nova York, EUA) por 8 h sob luz fluorescente para a redução dos íons de prata ao longo das nanoporosidades da interface de união. Assim, os palitos foram novamente lavados em água corrente e fixados com parafina em *stubs* de alumínio para facilitar o polimento uniforme das amostras, realizado em uma politriz de bancada (ALM 4, Arotec, Cotia, São Paulo, Brasil) sob escoamento contínuo da água com lixas de granulações 1000, 1200, 1500, 2000, 2500 e pastas diamantadas com granulações decrescentes de 1 µm e 0,25 µm (Buehler Ltd, Lake Bluff, IL, USA). Entre cada lixa e pasta de polimento as amostras foram lavadas em cuba ultrassônica por 8 min seguidos de escoamento de água sobre a superfície por 2 min. As amostras foram deixadas, então, por 30 min em cuba ultrassônica. Para o processo de desidratação foram deixadas em um dessecador contendo sílica coloidal por 24 h para, então, serem metalizadas em carbono/ouro (MED 010, Balzers Union, Balzers, Liechtenstein).

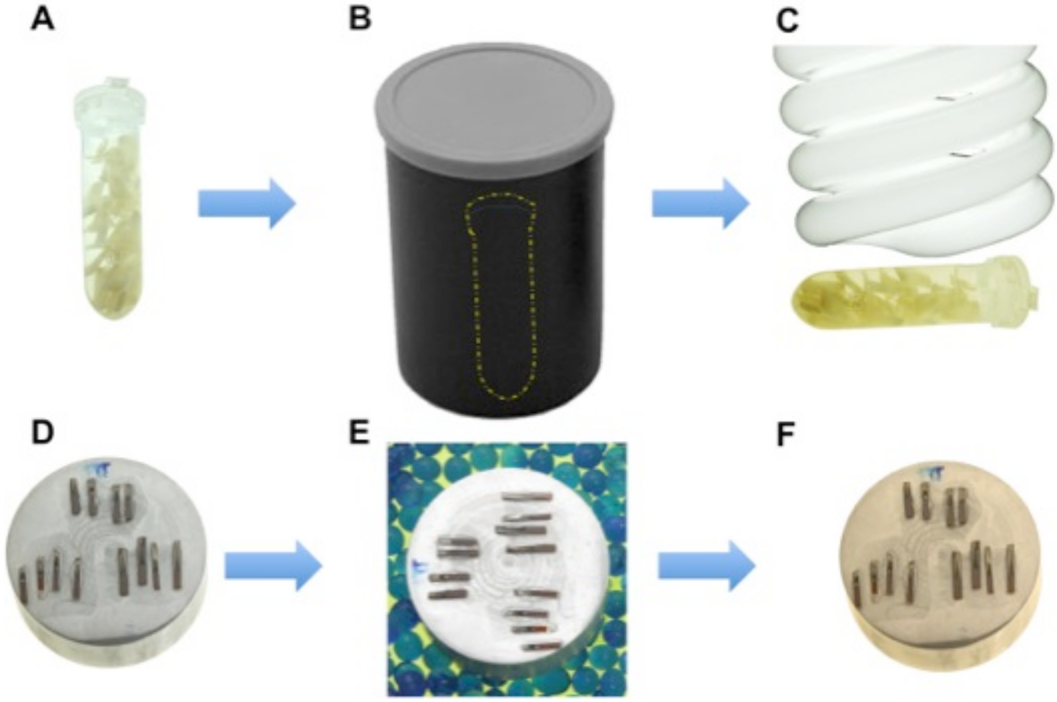

Figura 12 - Preparação de amostras para NI: (A) palitos imersos em uma solução aquosa de nitrato de prata (pH=7,0); (B) armazenados por 24 h sob total escuridão; (C) após lavagem, imersos em solução reveladora por 8 h sob luz fluorescente; (D) palitos revelados, montados em *stubs* de alumínio para facilitar o polimento uniforme; (E) palitos dessecados em sílica por 24 h; (F) palitos metalizados para a análise de MEV.

As interfaces resina-dentina foram analisadas num microscópio eletrônico de varredura de emissão de campo (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, United Kingdom) em modo de elétrons retroespalhados. Um operador que desconhecia a origem dos palitos a serem analisados realizou três fotomicrografias com aumento 1200X da interface dente-restauração de cada um dos palitos, sendo uma no centro e outras duas 3 µm à esquerda e à direita do centro. A porcentagem relativa de nitrato de prata nas áreas ocupadas pela camada híbrida e camada de adesivo foram consideradas na mensuração automatizada da NI utilizando um software UTHSCSA ImageTool 3.0 software (Department of Dental Diagnostic Science, University of Texas, San Antonio, Texas, EUA) operado por apenas um avaliador, o qual desconhecia a que grupo experimental pertencia a imagem. Assim, foram obtidas as médias dos valores percentuais de NI dos palitos provenientes do mesmo dente para fins estatísticos.

3.1.7 Medição do pH

O pH dos sistemas adesivos foi mensurado com um pH-metro E520 (Metrohm, Appenzell Exterior, Herisau, Suíça). O equipamento foi calibrado em soluções tampão de pH 4,0 e 7,0. Para a extração dos íons de hidrogênio dos sistemas adesivos foram realizadas três misturas por cada adesivo com água destilada na proporção 1:9 em peso, as quais foram homogeneizadas com agitação manual e armazenadas por 12 h em escuridão. A seguir foram realizadas três leituras por cada mistura. Entre cada medição, o eletrodo do equipamento foi lavado por escoamento com álcool 90° por 30 s, seguido de água destilada por 30 s, finalizando com uma medição de ambas as soluções tampão (4,0 e 7,0) para conferir sua estabilidade. A média de valores por adesivo foi utilizada para fins estatísticos.

Figura 13 - Análise de pH. Vista do eletrodo totalmente submerso na solução para a leitura do pH do adesivo após 12 h de armazenamento em escuridão.

4. ARTIGOS

4.1 Capítulo 1 (do experimento 1)

IMMEDIATE BONDING PROPERTIES OF UNIVERSAL ADHESIVES TO DENTINE

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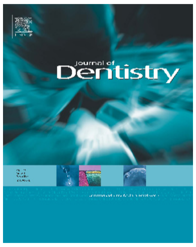

Immediate bonding properties of universal adhesives to dentine

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ABSTRACT

Objectives: To evaluate the dentine microtensile bond strength (μTBS), nanoleakage (NL), degree of conversion (DC) within the hybrid layer for etch-and-rinse and self-etch strategies of universal simplified adhesive systems.

Methods: forty caries free extracted third molars were divided into 8 groups for μTBS ($n = 5$), according to the adhesive and etching strategy: Clearfil SE Bond [CSE] and Adper Single Bond 2 [SB], as controls; Peak Universal Adhesive System, self-etch [PkSe] and etch-and-rinse [PkEr]; Scotchbond Universal Adhesive, self-etch [ScSe] and etch-and-rinse [ScEr]; All Bond Universal, self-etch [AlSe] and etch-and-rinse [AlEr]. After restorations were constructed, specimens were stored in water (37 °C/24 h) and then resin–dentine sticks were prepared (0.8 mm^2). The sticks were tested under tension at 0.5 mm/min. Some sticks from each tooth group were used for DC determination by micro-Raman spectroscopy or nanoleakage evaluation (NL). The pH for each solution was evaluated using a pH metre. Data were analyzed with one-way ANOVA and Tukey's test ($\alpha = 0.05$).

Results: For μTBS, only PkSe and PkEr were similar to the respective control groups ($p > 0.05$). AlSe showed the lowest μTBS mean ($p < 0.05$). For NL, ScEr, ScSe, AlSe, and AlEr showed the lowest NL similar to control groups ($p < 0.05$). For DC, only ScSe showed lower DC than the other materials ($p < 0.05$).

Conclusions: Performance of universal adhesives was shown to be material-dependent. The results indicate that this new category of universal adhesives used on dentine as either etch-and-rinse or self-etch strategies were inferior as regards at least one of the properties evaluated (μTBS, NL and DC) in comparison with the control adhesives (CSE for self-etch and SB for etch-and-rinse).

1. Introduction

The bonding mechanism of adhesive systems basically involves the replacement of minerals removed from the hard dental tissue by resin monomers, in such a way that a polymer becomes micro-mechanically interlocked to the dental substrate.[1] However, the adhesive systems available on the market can be classified into two categories: etch-and-rinse (Er) and those applied using self-etch strategies

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Abbreviations: Co, composite; De, dentine; HL, hybrid layer; AL, adhesive layer.

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(Se), in versions of three (only Er), two or one application step.[2,3]

When using the Er strategy, the first step involves the application of a phosphoric acid gel to both dental substrates, which allows removal of the smear layer, exposure of the collagen fibrils in dentine, and increase in surface area and surface energy in the enamel substrate. The primer is then applied (second step) followed by the bond (third step) resin separately or in a single solution.[2–4] Irrespective of the number of steps, the main disadvantage of the Er system, mainly two-step versions, is that there is risk of collagen fibre collapse during the process of demineralized dentine drying, which leads to a decrease in bond strength.[5,6] The collagen collapse is prevented by keeping demineralized dentine moist, which is a difficult task to perform clinically. In fact, adequate moisture depends on both the solvent used in the material[7] and on the clinician's interpretation of the manufacturer's directions.

The incomplete impregnation of collagen fibers[8] and the need to protect them against the degrading mechanisms present in the oral cavity environment,[9,10] led to the development of the second category, an adhesive using the self-etch strategy.

In the Se strategy (one-step or two-step), there is no need to apply a preliminary phosphoric acid gel on dental substrates as dentine demineralization and priming occur simultaneously.[3,11] The dissolved hydroxyapatite crystals and residual smear layer are incorporated in the hybridized complex.[3,12] Except for very acidic Se systems,[13,14] the whole extension of the demineralized dentine depth is impregnated by resin monomers, which may be the reason why Se systems are not associated with the technique sensitivity characteristic of bonding to moist etched dentine.[7,15,16] This advantage makes Se materials suitable for areas where adequate control of moisture is rather difficult, such as in posterior restorations.

A clear disadvantage of the Se protocol is the reduction in enamel bonding effectiveness.[17,18] The increase in surface area in intact and ground enamel obtained with Se adhesives is lower than that achieved with phosphoric acid, and it depends on the pH of the Se adhesive.[18] The performance of Se adhesives has improved when these systems were applied to phosphoric acid-treated enamel.[12,19,20] However, this procedure has been shown to be unsuitable for use on the dentine substrate,[21–23] because accidental dentine etching may occur during the enamel-etching process, particularly when a low-viscosity etchant is used. The effect of intentionally etching dentine with phosphoric acid prior to the application of self-etch adhesives has been studied.[21,23–26] The results are controversial and material-dependent.

Considering the differences in professional judgement regarding the selection of the adhesive strategy and number of steps, some manufacturers have released more versatile adhesive systems that include etch-and-rinse (two step) and self-etch (one or two step) options. These new materials are called ‘‘Universal’’, ‘‘Multi-purpose’’ or ‘‘Multi-mode’’ adhesives.[23,27] There is little information in the literature about the performance of this new class of universal adhesives.[23,27] Thus, this study compared the immediate microtensile bond strengths (μTBS), nanoleakage (NL), *in situ* degree of conversion (DC) of three universal adhesives applied to dentine according to the etch-and-rinse and the self-etch strategies. The two-step etch-and-rinse, Adper Single Bond 2 (SB, 3M ESPE, St. Paul, MN, USA), and two-step self-etch, Clearfil SE Bond (CSE, Kuraray, Okayama, Japan) were also evaluated as control groups. The following null hypotheses were tested in this study: (1) universal adhesives applied to dentine according to the Er and the Se strategies when compared to their respective control groups do not affect the immediate resin–dentine bond strength; (2) universal adhesives applied to dentine according to the Er and the Se strategies when compared to their respective control groups do not affect the immediate silver nitrate deposition and (3) universal adhesives applied to dentine according to the Er and the Se strategies when compared to their respective control groups do not affect the degree of conversion of the adhesives.

2. Materials and methods

2.1. *Tooth selection and preparation*

Forty extracted, caries-free human third molars were used. The teeth were collected after obtaining the respective patients' informed consent under a protocol approved by the local Ethics Committee Review Board. The teeth were disinfected in 0.5% chloramine, stored in distilled water and used within six months after extraction. A flat dentine surface was exposed after wet grinding the occlusal enamel on a #180 grit SiC paper. The exposed dentine surfaces were further polished on wet #600-grit silicon-carbide paper for 60 s to standardize the smear layer.

2.2. *Experimental design*

The teeth were randomly assigned into eight groups ($n = 5$) according to the different bonding strategies of the selected adhesive system. As control materials, the 2-step etch-and-rinse (Er), Adper Single Bond 2 (SB, 3M ESPE, St. Paul, MN, USA); and the 2-step self-etch (Se), Clearfil SE Bond (CSE, Kuraray, Okayama, Japan) were used. The following three universal adhesive systems were tested: Peak Universal Adhesive System (Peak LC Bond and Peak SE Primer, Ultradent Products Inc., South Jordan, UT, USA), applied as a 2-step Er (PkEr) and 2-step Se (PkSe); Scotchbond Universal Adhesive (3M ESPE, St. Paul, MN, USA), applied as a 2-step Er (ScEr) and 1-step Se (ScSe); and All Bond Universal (Bisco Inc., Shaumburg, IL, USA) applied as a 2-step Er (AlEr) and 1-step Se (AlSe).

2.3. *Restorative procedure and specimen preparation*

The adhesive systems were applied strictly in accordance with the respective manufacturer's instructions, described in Table 1. After the bonding procedures, all teeth received a microhybrid composite restoration (Opallis, FGM Produtos Odontológicos, Joinville, SC, Brazil) in two increments of 2 mm. Each increment was light polymerized for 40 s using a LED light curing unit set at 1200 mW/cm^2 (Radii-cal, SDI Limited, Bayswater, Victoria, Australia).

After the restored teeth had been stored in distilled water at 37 °C for 24 h, the specimens were sectioned longitudinally in the mesio-distal and buccal-lingual directions across the

Table 1 – Adhesive system (batch number), composition and application mode* of the adhesive systems used (*) according to the manufacturer's instructions.

Adhesive system (batch number)	Composition	Self-etch strategy	Etch-and-rinse strategy
Adper Single Bond 2 (BPBR)	1. Etchant: 35% phosphoric acid (Scotchbond Etchant) 2. Adhesive: bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly(itaconic) acids,10% by weight of 5 nm-diameter spherical silica particles	N.A	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Blot excess water 4. Apply 2–3 consecutive coats of adhesive for 15 s with gentle agitation 5. Gently air dry for 5 s 6. Light polymerize for 10 s at 1200 mW/cm^2
Clearfil SE Bond (Primer: 00954 A – Bond: 01416A)	1. Primer: water, MDP, HEMA, camphorquinone, hydrophilic dimethacrylate 2. Bonding: MDP, bis-GMA, HEMA, camphorquinone, hydrophobic dimethacrylate, *N*,*N*-diethanol *p*-toluidine bond, colloidal silica	1. Apply primer to tooth surface and leave in place for 20 s 2. Dry with air stream to evaporate the volatile ingredients 3. Apply bond to the tooth surface and then create a uniform film using a gentle air stream 4.Light polymerize for 10 s at 1200 mW/cm^2	N.A
Peak Universal Adhesive System (Peak SE Primer: 0N062 – Peak LC Bond: Y062)	1. Etchant: 35% phosphoric acid (Ultraetch) 2. Peak SE Primer: ethyl alcohol, methacrylic acid, 2-hydroxyethyl methacrylate (Peak SE Primer) 3. Peak LC Bond resin: ethyl alcohol, 2-hydroxyethyl methacrylate (Peak LC Bond)	1. Initial use of Peak SE requires activation of the two components separated in the syringe 2. Application of the Peak SE with microbrush for 20 s using continuous scrubbing on dentine. Do not scrub enamel 3. Thin/dry for 3 s using air/water syringe or high volume suction directly over preparation 4. Apply a puddle coat of Peak LC Bond and gently agitate for 10 s 5. Thin/dry 10 s using ¼ to ½ air pressure 6. Light polymerize for 10 s at 1200 mW/cm^2	1. Apply etchant for 20 s 2. Rinse for 5 s 3. Air dry 2 s 4. Apply a puddle coat of Peak LC Bond with gently agitate for 10 s 5. Dry 10 s using ¼ to ½ air pressure 6. Light polymerize for 10 s at 1200 mW/cm^2
Scotchbond Universal Adhesive (D-82229)	1. Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. (Scotchbond Universal Etchant) 2. Adhesive: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. If necessary, rewet the disposable applicator during treatment 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely 3. Light polymerize for 10 s	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Air dry 2 s 4. Apply adhesive as for the self-etch mode
All-Bond Universal (1200006111)	1. Etchant Uni-Etch: 32% phosphoric acid, benzalkonium Chloride 2. Adhesive: MDP, bis-GMA, HEMA, ethanol, water, initiators	1. Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10–15 s per coat. Do not light polymerize between coats 2. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 s, there should be no visible movement of the material. The surface should have a uniform glossy appearance 3. Light polymerize for 10 s at 1200 mW/cm^2	1. Apply etchant for 15 s 2. Rinse thoroughly 3. Remove excess water with absorbent pellet or high volume suction for 1–2 s 4. Apply adhesive as for the self-etch mode

bonded interface, using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) to obtain 15–20 resin–dentine sticks with a cross sectional area of approximately 0.8 mm^2 measured with a digital calliper (Digimatic Calliper, Mitutoyo, Tokyo, Japan).

All the specimens from each tooth were used for the microtensile bond strength evaluation (μTBS), except for six that were randomly selected. These six resin–dentine bonded specimens were divided for measurement of the *in situ* degree of conversion (DC) and nanoleakage (NL).

2.4. *Microtensile bond strength test (μTBS)*

Resin–dentine bonded sticks were attached to a Geraldeli's jig[28] with cyanoacrylate adhesive and tested under tension (Kratos Dinamometros, Cotia, SP, Brazil) at 0.5 mm/min until failure. The μTBS values were calculated by dividing the load at failure by the cross-sectional bonding area.

The failure mode of the specimens was classified as cohesive ([C] failure exclusive within dentine or resin composite), adhesive ([A] failure at resin/dentine interface), or mixed ([M] failure at resin/dentine interface, which included cohesive failure of the neighbouring substrates). The classification was performed under a stereomicroscope at 100× magnification (Olympus SZ40, Tokyo, Japan). Specimens with premature failures (PF) were included in the tooth mean.

2.5. *Degree of conversion in situ (DC)*

Three resin–dentine bonded sticks from each tooth were wet polished with #1500, 2000 and 2500-grit SiC paper. Then they were ultrasonically cleaned for 20 min and stored in water at 37 °C for 24 h, before taking the DC readings. The micro-Raman spectroscopy analysis was performed using the Senterra equipment (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany). The micro-Raman spectrometer was first calibrated by resetting to zero, and then for coefficient values using a silicon specimen. Specimens were analyzed using the following micro-Raman parameters: 20 mW Neon laser with 532 nm wavelength, spatial resolution of ≈3 μm, spectral resolution ≈5 cm^{-1}, accumulation time of 30 s with 6 co-additions, and 110× magnification (Olympus UK, London, UK) to a ≈1 μm beam diameter. Spectra were taken at the dentine–adhesive interface at three different sites within intertubular dentine for each resin–dentine bonded stick. The average value of the measurements taken from the same tooth was used for statistical purposes. Spectra of unpolymerized adhesives were taken as reference. Post-processing of spectra was performed using the dedicated Opus Spectroscopy Software version 6.5. The ratio of double-bond content of monomer to polymer in the adhesive was calculated according to the following formula:

$$\mathrm{DC}\,(\%) = \left(1 - \frac{R_{(\mathrm{cured})}}{R_{(\mathrm{uncured})}}\right) \times 100$$

where "R" is the ratio of aliphatic and aromatic peak areas at 1639 cm^{-1} and 1609 cm^{-1} in polymerized and unpolymerized adhesives. The *in situ* DC of all resin–dentine bonded sticks from the same tooth was averaged for statistical purposes.

2.6. *Nanoleakage (NL) evaluation*

Three resin-bonded sticks, from each tooth, were used for NL evaluation. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al.[29] The sticks were placed in the ammoniacal silver nitrate solution in darkness for 24 h, rinsed thoroughly in distilled water, and immersed in photo developing solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface. Specimens were polished with a wet #600, 1000, 1200, 1500, 2000 and 2500-grit SiC paper and 1 and 0.25 μm diamond paste (Buehler Ltd., Lake Bluff, IL, USA) using a polishing cloth. They were ultrasonically cleaned, air dried, mounted on stubs, and coated with carbon-gold (MED 010, Balzers Union, Balzers, Liechtenstein). Resin–dentine interfaces were analyzed in a field-emission scanning electron microscope operated in the backscattered mode (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, UK).

Three images were captured of each resin–dentine bonded stick. The relative percentage of NL within the adhesive and hybrid layers in each specimen was measured in all images using the UTHSCSA ImageTool 3.0 software (Department of Dental Diagnostic Science at The University of Texas Health Science Centre, San Antonio, Texas) by a blinded researcher. The values originating from the same specimen were averaged for statistical purposes. The mean NL of all sticks from the same tooth was averaged for statistical purposes.

2.7. *pH measurement*

The pH of the adhesive systems was measured with a pH metre E520 (Metrohm, Herisau, Switzerland). Three blends were prepared by mixing each adhesive solution and deionized water in a proportion of 1:9 (by weight). This blend was kept stored in darkness for 12 h to allow uptake of H^+. For each blend, three readings were performed at room temperature, in darkness and a mean was obtained.

2.8. *Statistical analysis*

Data from μTBS, NL, DC and pH were analyzed by one-way ANOVA and Tukey's *post hoc* test at $\alpha = 0.05$.

3. Results

3.1. *Microtensile bond strength test*

The overall values of all tested properties are shown in Table 2. Significant differences were detected in the mean μTBS among the adhesives tested ($p < 0.05$). Only the universal adhesive PK, used in both the Er and Se approach, showed mean μTBS statistically similar to those of the controls SB and CSE respectively ($p > 0.05$). The other universal materials resulted in lower mean μTBS than those of the Er and Se controls ($p < 0.05$). The lowest mean μTBS was observed for AISe ($p < 0.05$).

The majority of the specimens (83.1%) showed adhesive/mixed failures. Cohesive failures were observed in 8.3% of the

Table 2 – Microtensile bond strength (μTBS), nanoleakage (NL) and degree of conversion (DC) values (means ± standard deviations) of the different experimental groups.*

Adhesive system	Application mode	Test		
		μTBS (MPa)	NL (%)	DC (%)
Adper Single Bond 2	Etch-and-rinse	49.3 ± 4.6A	12.4 ± 2.5b	85.4 ± 4.7^{a}
Clearfil SE Bond	Self-etch	43.0 ± 4.5A,B	7.6 ± 2.a	87.7 ± 2.8^{a}
Peak Universal Adhesive System	Etch-and-rinse	43.6 ± 4.6A,B	23.4 ± 5.9c	89.2 ± 6.3^{a}
	Self-etch	39.9 ± 4.5B,C	34.4 ± 11c	78.9 ± 9.5^{a}
Scotchbond Universal Adhesive	Etch-and-rinse	35.1 ± 6.6B,C	5.1 ± 2.5a	88.3 ± 5.6^{a}
	Self-etch	32.4 ± 4.5C	5.5 ± 3.2a	69.1 ± 9.8^{b}
All Bond Universal	Etch-and-rinse	39.3 ± 3.7B	9.3 ± 2.9b	77.9 ± 0.1^{a}
	Self-etch	13.4 ± 1.9D	6.2 ± 4.1a	77.8 ± 0.1^{a}

*Similar capital (μTBS), lower (NL) and superscript letter (DC) are not statistically significant ($p < 0.05$)

Table 3 – Number and percentage of specimens (%) according to the fracture mode and premature failures of all experimental group.

Adhesive system	Application mode	Fracture pattern			
		A	*C*	*A/M*	*PF*
Adper Single Bond 2	Etch-and-rinse	50 (56.8)	18 (20.5)	8 (9.1)	12 (13.6)
Clearfil SE Bond	Self-etch	66 (76.7)	4 (4.7)	8 (9.3)	8 (9.3)
Peak Universal Adhesive System	Etch-and-rinse	60 (68.2)	6 (6.8)	12 (13.6)	10 (11.4)
	Self-etch	56 (66.7)	8 (9.5)	12 (14.3)	8 (9.5)
Scotchbond Universal Adhesive	Etch-and-rinse	64 (82.1)	0 (0)	12 (15.4)	2 (2.5)
	Self-etch	58 (76.3)	4 (5.3)	12 (15.8)	2 (2.6)
All Bond Universal	Etch-and-rinse	46 (60.5)	10 (13.2)	16 (21.1)	4 (5.2)
	Self-etch	52 (66.7)	0 (0)	20 (25.6)	6 (7.7)

specimens. A small number of premature failures (8.6%) were observed in the present study (Table 3).

3.2. *Nanoleakage evaluation*

Significant differences were observed among the adhesives tested ($p = 0.0001$; Table 2 and Fig. 1). PKEr and PKSe adhesives showed the highest percentage of NL ($p < 0.05$). AlEr and AlSe adhesives were statistically similar to their respective control groups (SB and CSE; $p > 0.05$). Scotchbond Universal (ScEr and ScSe) showed the lowest nanoleakage ($p < 0.05$); in the Er approach, the NL was lower than that of SB; and in the Se approach NL was similar to that of CSE.

3.3. *Degree of conversion*

All materials showed similar DC ($p > 0.05$, Table 2), except for ScSe, which showed a statistically lower DC compared with the other materials ($p = 0.01$).

3.4. *pH measurement*

The pH of the adhesives is shown in Table 4. SB showed the highest pH (4.1) and the Peak LC Bond showed the lowest (1.2).

4. Discussion

Although all the new universal adhesive systems tested share the same versatility of being used in the Er and Se approaches, there are differences in their compositions and number of steps, which seems to be the key reason for the different performance of these materials when compared with the controls.

The 2-step Se CSE and, the 1-step Se ScSe and AlSe have a pH within a similar range (around 2). Se adhesives within this pH range partially demineralize dentine, leaving a substantial amount of hydroxyapatite crystals around the collagen fibrils.[30] As these three materials have 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP) in their composition, they bond chemically to dentine.[31–33] Yoshida et al.[34] showed that an effective chemical interaction occurs between MDP and hydroxyapatite forming a stable nano-layer that could form a stronger phase at the adhesive interface, which increases the mechanical strength of the adhesive interface. In addition, stable MDP-Ca salt deposition along with nano-layering may explain the high bond stability,[34,35] which has previously been proven both in laboratory and clinical research.[33,36,37]

Based on these similarities one would expect that these two universal adhesives (ScSe and AlSe) would be equivalent to the control 2-step Se CSE; however, this was not observed in the present investigation. The slight difference in terms of μTBS between CSE and ScSe may be due to the presence of polyalkenoic acid copolymer in the composition of ScSe and this leads to partially reject the 1st. null hypothesis. This copolymer may have competed with the MDP by binding to the calcium of the hydroxyapatite.[34] In addition to impairing the bond of MDP to dentine, the polyalkenoic acid copolymer could have prevented

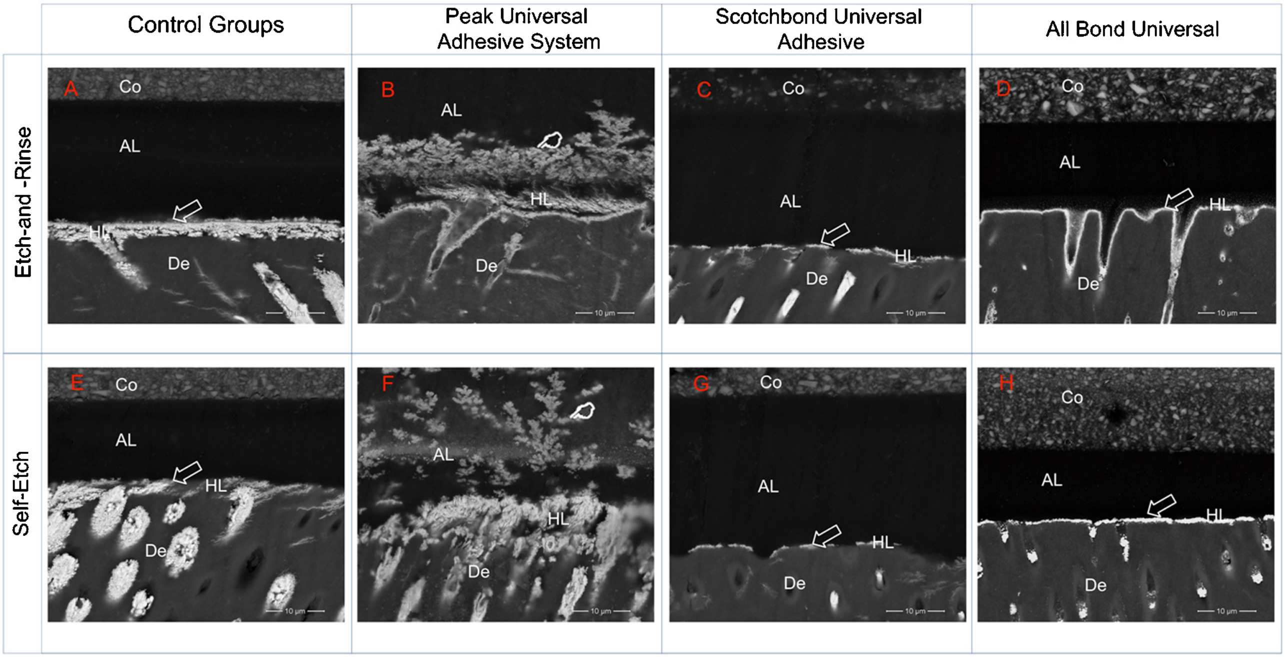

Fig. 1 – Representative backscattered SEM images of the resin–dentine adhesive interfaces of each experimental group (Control etch-and-rinse group = Adper Single Bond 2 and control self-etch group = Clearfil SE Bond). The amount of nanoleakage was lower and practically occurred within the hybrid layer for Scotchbond Universal (C, G); All Bond Universal (D, H) and controls (*white arrows*) (A, E). For Peak Universal Adhesive System, the amount of nanoleakage was higher than the other materials; with most silver nitrate uptake occurring throughout the entire thickness of the HL (*white arrows*) and in the AL, forming the so-called "water trees (*white hands*) (B, F).

monomer approximation during polymerization, due to its high molecular weight. Consequently a lower DC was observed for this material in comparison with the others leads to partially reject the 3rd. null hypothesis. Furthermore, ScSe and AlSe are 1-step self-etch adhesives and this probably makes the MDP concentration lower than that of CSE, which has this monomer incorporated into both the primer and bond components.[34]

Among all Se adhesives, AlSe showed the poorest performance in terms of μTBS, which might be attributed to its application mode. AlSe was the only material that was not applied actively on dentine, as the materials were applied in accordance with the respective manufacturer's directions. Previous literature findings clearly pointed out that active application improves the bonding performance of Se adhesive systems to dentine.[38,39] Future studies need to be conducted to test this hypothesis.

Among adhesives that are used with the Se strategy, PKSe provided μTBS values similar to those of the control CSE. The placement of an additional layer has been shown to increase the performance of 1-step Se materials both *in vitro*[37,40–42] and clinically.[43,44] This is also a disadvantage of ScSe and AlSe. In general, 1-step SE adhesives present inferior performance to 2-step Se adhesives.[45,46] Usually, 1-step Se adhesives result in thinner adhesive layers, which are likely to be prone to polymerization inhibition by oxygen.[47]With regard to the Er approach, the universal adhesives showed improved bond strength in comparison with the Se strategy, in spite of ScPK being statistically similar to the ScSe. It is known that the smear layer constitutes a true physical barrier and makes it extremely difficult for the bonding and hybrid layer formation to be fully integrated with the dentine.[48] After preliminary etching with phosphoric acid, the smear layer is removed and superficial dentine is demineralized. This increases impregnation by the adhesive, allowing the creation of a well impregnated hybrid layer, without modifying the potential for interfacial nanoleakage, as has been shown by several authors when a 1-step self-etch adhesive was applied on phosphoric acid etched dentine.[23,25–27]

NL represents the location of defects at the resin–dentine interface that might serve as the pathway for degradation of resin/dentine bonds over time.[49] Silver

Table 4 – The pH values (means ± standard deviations) of each adhesive system.

Adhesive system		pH
Adper Single Bond 2		4.1 ± 0.02
Clearfil SE Bond	Primer	2.1 ± 0.01
	Bond	2.6 ± 0.08
Peak Universal Adhesive System	Peak SE Primer	1.2 ± 0.01
	Peak LC Bond resin	2.0 ± 0.04
Scotchbond Universal Adhesive		3.0 ± 0.05
All Bond Universal		2.4 ± 0.05

nitrate is capable of occupying the nanometer-sized spaces around naked collagen fibrils, where resin failed to infiltrate, or where residual water has not been displaced by adhesive or even in areas of incomplete monomer conversion.[49]

Among all the adhesives, PKEr and PKSe showed the highest NL at the adhesive interfaces, and this leads to partially reject the 2nd. null hypothesis. In the case of PkSe, Peak SE primer has a low pH value (pH = 1.2), which is within the range of the most aggressive Se adhesives.[30,45] It has previously been reported that unpolymerized, acidic and aggressive monomers from Se adhesives are able to continue to demineralize the dentine even after polymerization.[13,14] This is probably the result of hydrolysis of the ester bond from the acid monomer that results in a strong phosphoric acid[14] and might explain the intense silver nitrate deposition within the hybrid layer.

Although one could expect reduced NL within the adhesive layer due to the fact that PKSe is a 2-step Se, the second adhesive layer seems to be as hydrophilic as the primer, since no hydrophobic monomer was listed in the composition of the Peak LC Bond (Table 1). Thus, although increased impregnation of resin monomers might have occurred during the application of an additional adhesive coat, the nature of this impregnation is hydrophilic, which resulted in extensive NL in the hybrid layer. In fact, Peak LC bond (pH = 2.0) may cause an additional etching and an increased conditioning pattern of the dentine substrate. This is likely to result in increased demineralization and production of a hydroxyapatite-denuded, collagen-rich, network,[45,50,51] increasing the risk of NL [10,52–54] for PkEr.

Taking all the properties together, none of the materials showed similar behaviour to the controls (a gold standard two-step self-etch Clearfil SE Bond and a gold standard two-step etch-and-rinse Adper Single Bond 2) leads to reject all null hypothesis tested. Future studies need to be conducted to evaluate the long-term performance of this new category of adhesives.

5. Conclusions

Within the limitations of the present study, the results indicate that when the universal adhesives were tested using the self-etch or etch-and-rinse strategy on dentine, they were inferior to the respective controls (Clearfill SE Bond, a two-step etch-and-rinse or Adper Single Bond 2, a two-step etch-and-rinse) with respect to at least one of the properties tested (microtensile bond strength, nanoleakage and *in situ* degree of conversion.

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4.2 Capítulo 2 (do experimento 2)

LONGEVITY OF BONDING PROPERTIES OF UNIVERSAL ADHESIVES TO DENTIN

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Abstract	Objectives: To evaluate the dentine microtensile bond strength (μTBS) and nanoleakage (NL) for etch-and-rinse and self-etch strategies of universal adhesive systems in the immediate time (IM) and after 6 months of storage (6M). Methods: forty caries-free extracted third molars were divided into 8 groups for μTBS (n = 5), according to the adhesive and etching strategy: Clearfil SE Bond [CSE] and Adper Single Bond 2 [SB], as controls; Peak Universal, self-etch [PkSe] and etch-and rinse [PkEr]; Scotchbond Universal Adhesive, self-etch [ScSe] and etch-and-rinse [ScEr]; All Bond Universal, self-etch [AlSe] and etch-and-rinse [AlEr]. After composite restorations, specimens were stored in water (37oC/24 h) and longitudinally sectioned to obtain resin-dentine bonded sticks (0.8 mm2). Half of the sticks were tested under tension at 0.5 mm/min in the IM and the other half in 6M. Some sticks from each tooth group and time evaluations were used for nanoleakage evaluation (NL). Data were analyzed with two-way repeated measure ANOVA and Tukey's test (a = 0.05). Results: Despite higher μTBS in the immediate time, PkSe and PkEr, similar to the respective control groups (IM; p>0.05), these strategies showed higher NL pattern and lower μTBS after 6M (p<0.05). ScSe and ScEr showed intermediary μTBS values, but it was maintained after 6 months (p>0.05). For Al, AlSe showed the lowest μTBS mean (p<0.05), but μTBS and NL values did not degrade (p>0.05). However, AlEr, despite a higher immediate μTBS mean, showed signs of degradation after 6 months (p<0.05). Conclusions: Universal adhesives that contain MDP showed better results after 6 months of water storage.
Associate Editor	Assigned
Key Words	Microtensile bond strength, nanoleakage, degree of conversion, etch-and-rinse, self-etch,

Longevity of Bonding Properties of Universal Adhesives to Dentin

Abstract

Objectives: To evaluate the dentine microtensile bond strength (μTBS) and nanoleakage (NL) for etch-and-rinse and self-etch strategies of universal adhesive systems in the immediate time (IM) and after 6 months of storage (6M). Methods: forty caries-free extracted third molars were divided into 8 groups for μTBS (n = 5), according to the adhesive and etching strategy: Clearfil SE Bond [CSE] and Adper Single Bond 2 [SB], as controls; Peak Universal, self-etch [PkSe] and etch-and rinse [PkEr]; Scotchbond Universal Adhesive, self-etch [ScSe] and etch-and-rinse [ScEr]; All Bond Universal, self-etch [AlSe] and etch-and-rinse [AlEr]. After composite restorations, specimens were stored in water (37°C/24 h) and longitudinally sectioned to obtain resin–dentine bonded sticks (0.8 mm^2). Half of the sticks were tested under tension at 0.5 mm/min in the IM and the other half in 6M. Some sticks from each tooth group and time evaluations were used for nanoleakage evaluation (NL). Data were analyzed with two-way repeated measure ANOVA and Tukey's test (a = 0.05). Results: Despite higher μTBS in the immediate time, PkSe and PkEr, similar to the respective control groups (IM; $p>0.05$), these strategies showed higher NL pattern and lower μTBS after 6M ($p<0.05$). ScSe and ScEr showed intermediary μTBS values, but it was maintained after 6 months ($p>0.05$). For Al, AlSe showed the lowest μTBS mean ($p<0.05$), but μTBS and NL values did not degrade ($p>0.05$). However, AlEr, despite a higher immediate μTBS mean, showed signs of degradation after 6 months ($p<0.05$). Conclusions: Universal adhesives that contain MDP showed better results after 6 months of water storage.

Keywords: Microtensile bond strength; nanoleakage; degree of conversion; etch-and-rinse; self-etch; universal adhesive systems

Introduction

Current adhesive materials simplify the bonding procedures by reducing the number of application steps/time required for application. This simpler protocol makes them less technique-sensitive and allows for better application standardization.[1] This is the possibility some of the factors responsible for the large increase in the use of self-etch adhesives among clinicians.[2]

Self-etch materials (also known as non-rinsing adhesives or etch-and-dry adhesives) do not require a separate acid step as demineralization and priming occurs simultaneously.[3] The preliminary use of phosphoric acid increases the probability of clinical errors due to the need of rinsing and adequate management of dentin moisture.[4] Contrary to the etch-and-rinse approach, self-etch adhesives do not remove but incorporate the smear layer in the hybridized complex. Although a complete resin infiltration through is not observed for some acidic self-etch systems, [5, 6] some studies report lower incidence of post-operative sensitivity after placement of direct composite posterior restorations.[7]

On the other hand, some drawbacks may be listed for these self-etch materials. They do not produce an enamel conditioning pattern as retentive as that produced by phosphoric acid, [8, 9] which is likely responsible for the higher rates of marginal discoloration in the enamel margins of cervical restorations.[10] Selective enamel etching on the enamel margins with phosphoric acid is the most current accepted technique to solve this problem, showing good results either in vitro[11-13] and in vivo studies.[14]

One clinical problem of this technique is that accidental dentin etching may occur, particularly in small cavities[15] or when a low-viscosity acid is used.[16] The preliminary etching of dentin with phosphoric acid before application of self-etch application shows controversial results and seems to be material-dependent.[15-18] For one-step self-etch materials, the previous phosphoric acid etching can increase the bond strength of these adhesives to dentin.[15, 17, 18]

Based on that, one may conclude that clinical application of the adhesive systems is limited not only by the bonding strategy, but also by their number of clinical steps. Although simplified adhesives (2-step ER and 1-step SE) are suitable for direct clinical procedures due to their fast and easy application, they may not be adequate in luting procedures when chemically-cured cements are employed, in the face of the possibility of chemical and physical incompatibilities between the adhesive system and the luting materials.[19, 20]

Keeping this concept in mind, a novel family of bonding systems known as ‘‘universal’’ or ‘‘multi-mode’’ adhesives[21, 22] was recently launched in the market. They are one-step self-etch adhesives that can be associated or not with phosphoric acid etching, which gives the dentist a more versatile adhesive system.[23] The main difference between the oldest self-etch systems and these newest ones is the presence of monomers capable to produce chemical adhesion to the dental substrates.[21, 22] This is expected to increase the durability of the bonds produced with simplified self-etch adhesive, which has been considered less durable in in vitro and in vivo studies.[10, 24] To the extent of our knowledge, the longevity of bonds produced by universal adhesives is still scarce in the literature,[25] as well as their immediate bonding properties.[16, 21-23]

Thus, the aim of this study was to evaluate the immediate and 6-month microtensile bond strength (μTBS) and nanoleakage (NL) of universal adhesive systems used in the etch-and-rinse and self-etch approaches. The following null hypotheses were tested in this study: (1) the immediate and 6-month μTBS and nanoleakage of universal adhesives used as etch-and-rinse or self-etch or will be similar.

Materials and methods

Tooth selection and preparation

Forty extracted caries-free human third molars were used. The teeth were collected after obtaining the respective patients' informed consent under a protocol approved by the local Ethics Committee Review Board. The teeth were disinfected in 0.5% chloramine, stored in distilled water, and used within six months after extraction. A flat dentine surface was exposed after wet-grinding the occlusal enamel on a #180-grit SiC paper. The exposed dentine surfaces were further polished on wet #600-grit silicon-carbide paper for 60 s to standardize the smear layer.

Experimental design

The teeth were randomly assigned into eight groups (n = 5) according to the different bonding strategies of the selected adhesive system. As control materials, the 2-step etch-and-rinse (Er), Adper Single Bond 2 (SB, 3M ESPE, St. Paul, MN, USA); and the 2-step self-etch (Se), Clearfil SE Bond (CSE, Kuraray, Okayama, Japan) were used. The following three universal adhesive systems were tested: Peak Universal Adhesive System (Peak LC Bond and Peak SE Primer Ultradent Products Inc., South Jordan, UT, USA), applied as a 2-step Er (PkEr) and 2-step Se (PkSe); Scotchbond Universal Adhesive (3M ESPE, St. Paul, MN, USA), applied as a 2-step

Er (ScEr) and 1-step Se (ScSe); and All Bond Universal (Bisco Inc., Shaumburg, IL, USA) applied as a 2-step Er (AlEr) and 1-step Se (AlSe).

Restorative procedure and specimen preparation

The adhesive systems were applied in accordance with the respective manufacturer's instructions; however, with vigorous rubbing action,[26] described in Table 1. After the bonding procedures, all teeth received a microhybrid composite restoration (Opallis, FGM Produtos Odontológicos, Joinville, SC, Brazil) in two increments of 2 mm. Each increment was light polymerized for 40 s using an LED light curing unit set at 1200 mW/cm^2 (Radii-cal, SDI Limited, Bayswater, Victoria, Australia).

After the restored teeth had been stored in distilled water at 37 °C for 24 h, the specimens were sectioned longitudinally in the mesio-distal and buccal-lingual directions across the bonded interface using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) to obtain 25–30 resin–dentine sticks with a cross sectional area of approximately 0.8 mm^2 measured with a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan).

The halves of specimens from each tooth were used for the immediate microtensile bond strength evaluation (μTBS) and another one after 6 months of water storage (37 °C). The same amount of distilled water was used throughout the study period.

Microtensile bond strength test (μTBS)

Resin–dentine bonded sticks were attached to a Geraldeli's jig[27] with cyanoacrylate adhesive and tested under tension (Kratos Dinamometros, Cotia, SP, Brazil) at 0.5 mm/min until failure. The µTBS values were calculated by dividing the load at failure by the cross-sectional bonding area.

The failure mode of the specimens was classified as cohesive ([C] failure exclusive within dentine or resin composite), adhesive ([A] failure at resin/dentine interface), or mixed ([M] failure at resin/dentine interface, which included cohesive failure of the neighboring substrates). The classification was performed under a stereomicroscope at 100× magnification (Olympus SZ40, Tokyo, Japan). Specimens with premature failures (PF) were included in the tooth mean.

Nanoleakage (NL) evaluation

Three resin-bonded sticks from each tooth in each evaluation time were used for NL. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al.[28] The sticks were placed in the ammoniacal silver nitrate solution in darkness for 24 h, rinsed thoroughly in distilled water, and immersed in photo developing solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface. Specimens were polished with a wet #600, 1000, 1200, 1500, 2000 and 2500-grit SiC paper and 1 and 0.25 µm diamond paste (Buehler Ltd., Lake Bluff, IL, USA) using a polishing cloth. They were ultrasonically cleaned, air dried, mounted on stubs, and coated with carbon-gold (Shimadzu IC 50, Tóquio, Japão). Resin–dentine interfaces were analyzed in a field-emission scanning electron microscope operated in the backscattered mode (SSX-550, Shimadzu, Tóquio, Japão).

Three images were captured of each resin–dentine bonded stick. The relative percentage of NL within the adhesive and hybrid layers in each specimen was measured in all images using the UTHSCSA ImageTool 3.0 software (Department of

Dental Diagnostic Science at The University of Texas Health Science Centre, San Antonio, Texas) by a blinded researcher. Values originating from the same specimen were averaged for statistical purposes. The mean NL of all sticks from the same tooth was averaged for statistical purposes.

Statistical analysis

The experimental unit in the current study was the hemi-tooth, since half of the sample was tested immediately and the other half tested only after 6 months. The μTBS and NL of all sticks from the same hemi-tooth were averaged for statistical purposes. The μTBS (MPa) and NL (%) means for every testing group was expressed as the average of five hemi-teeth used per group. The premature failures from MTBS were not included in the hemi-tooth mean. The data of μTBS (MPa) and NL (%) were subjected to a two-way repeated measures ANOVA (Adhesive vs. Storage time) and a post hoc test (Tukey's post hoc test at $\alpha = 0.05$) for pair-wise comparisons.

Results

The percentage of specimens with premature failure and the frequency of each fracture pattern mode are shown in Table 2. For all experimental conditions, a lower percentage of premature (5.7 % on average) and cohesive failures (4.6% on average) of the specimens were observed. The majority of the specimens showed adhesive or adhesive/mixed failures.

The overall μTBS values of all adhesives are shown in Table 3. The cross-product Adhesive vs. Storage time was statistically significant ($p = 0.001$). Despite PkSe and PkEr showing higher immediate μTBS similar to controls (CSE and SB; $p > 0.05$), a significant decrease of μTBS values occurred after 6 months of water storage ($p < 0.05$). Adhesives ScSe, ScEr, AlSe and AlEr showed inferior immediate μTBS in comparison with controls (CSE and SB; $p < 0.05$), but only when applied in the Se mode (ScSe and AlSe) there is not significant decrease of μTBS values after 6 months of water storage ($p > 0.05$). In the Er mode, only Al showed significant and lower μTBS values after 6 months of water storage ($p < 0.05$).

The overall NL values of all adhesives are shown in Table 4. The cross-product Adhesive vc. Storage time was statistically significant ($p = 0.001$). Only PkSe and PkEr in both modes of application showed higher and significant amounts of NL in immediate time ($p < 0.05$), and this NL pattern significantly increased after 6 months of water storage ($p < 0.05$). Sb and Al when applied in Se and Er mode showed a lower NL pattern in both periods of time and similar to controls (CSE and SB; $p > 0.05$).

Discussion

The results of the present study demonstrated that the universal adhesive tested had a heterogeneous behavior, since some diminished the bond over the course of time and some did not.

The adhesive Peak, in spite of presenting excellent bond strength results, formed an adhesive and hybrid layer with a large quantity of NL in the immediate time interval. NL represents the location of defects at the resin-dentin interface that may serve as pathways for degradation of the resin/dentin bond over time.[29] Silver nitrate is capable of occupying nanometric-sized spaces present around the exposed collagen fibrils where the monomers were unable to infiltrate or where residual water was not displaced by the adhesive, or even in areas with incomplete monomer conversion[29], factors preponderant for the degradation of the bond interface.

This large quantity of silver nitrate, in the case of PkSe, was owing to the fact that the primer of the system has a very low pH (Peak SE Primer - pH = 1.2) when compared with those of the other adhesives (Scotchbond Universal=3; Allbond Universal=2.4)[16] characterizing this material within the category of the more aggressive Se.[30, 31] It has been related that acid and unpolymerized monomers present in acidic adhesive do not completely infiltrate into the region of dentin that was demineralize, [5, 6] probably the result of hydrolysis of the ester bond of the acidic monomer that results in a strong phosphoric acid[5] which may also explain the intense deposition of silver nitrate within the hybrid layer of this adhesive.

Furthermore, in the case of PkSe, only in this material does the manufacturer indicate the application of Peak LC Bond, compared to the other universal adhesives. Various in vitro[13, 32-34] and clinical studies[35, 36] have shown that the application of an additional layer increases the performance of 1-step Se adhesives, provided that this is a layer with a hydrophobic nature. An additional layer of a traditional Bond incorporates non-solvated hydrophobic monomers at the bond interface, which diminishes the relative concentration of solvents retained and non-reacted monomers in the adhesive layer [37], making it less permeable[38, 39] and have less propensity to the effects of degradation over the course of time.[40, 41]

However, Peak LC Bond appears to be as hydrophilic as Peak SE Primer, since no hydrophobic monomers were listed in the composition of Peak LC Bond (Table 1). This high level of hydrophilicity must be responsible for the increase in the patterns of degradation observed for this adhesive in the present study. Indeed, it is important for Peak LC Bond to be hydrophilic to be applied in the Er technique. Nevertheless, in the case of PkEr, in which the primer is not applied, Peak LC Bond (pH=2.0) may have caused an additional etching and an increase in the pattern of conditioning of the dentinal substrate. This probably resulted in an increase in demineralization and collagen exposure,[31, 42] thereby increasing the NL[43] for PkEr, as may be seen in Figure 1.

On the other hand, in spite of Sc and Al being one-step Se adhesives and therefore highly hydrophilic, for 3 of the 4 groups tested, no degradation occurred at the bond interface after 6 months of storage in water. This must be attributed, as described in the introduction, to the presence of monomers capable of producing a chemical bond to the hard structures of teeth,[21, 22] as opposed to the lack of this compound in Peak.

In this case, Sc and Al contain 10-MDP in their composition, as does CSE, which was the first SE adhesive to contain this component. Studies with CSE have demonstrated that 10-MDP allows for a stable chemical bond to dentin over the course of time, both in vitro[32, 44-46] and in clinical[14, 47, 48] studies of long-term. This compound forms a stable nanolayer together with a deposition of stable MDP-Ca salts at the adhesive interface,[49] which increases its mechanical strength[49, 50]. However, in spite of the presence of MDP, the adhesives had different behaviors.

Please note that in spite of its stability, the μTBS values of ScSe and AlSe in the immediate time interval were not equivalent to those of the control CSE. ScSe and AlSe are one-step adhesives and this probably leads to the concentration of MDP being lower than it is in CSE, which has MDP incorporated into both the *primer* and the bond.[49] Moreover, it has been demonstrated that the presence of HEMA, a component of Sc and Al may compete with MDP by the bond to the calcium of hydroxyapatite, thereby harming the chemical bond of MDP to dentin.[49] Nevertheless, both ScSe and AlSe demonstrated excellent results with regard to bond stability.

The difference between ScSe and AlSe may be explained by two factors: presence of the copolymer of polyalkenoic acid in Sc; and the high quantity of solvent of Al. Sc contains a specific polyalkenoic acid copolymer (PAC) used in resin-modified glass ionomer Vitrebond (3M ESPE). PAC bonds chemically and spontaneously to hydroxyapatite in glass ionomer materials,[51] and a recent study demonstrated that the presence of PAC showed more enhanced bond strength than a PAC-free adhesive with the same composition.[47, 51] Yoshida[49] hypothesized that PAC may compete with the MDP present in Sc; however, if we compare the longevity results of Sc (MDP + PAC) in comparison with SB (PAC), two materials with similar compositions, the only difference being the presence of MDP in the former, we may observe that the association MDP-PAC enhanced the results observed, since both ScSe and ScEr demonstrated stable results after 6 months of storage in water.

Whereas Al contains more solvent when compared with Sc (30-60 wt% and 10-15 wt%0 respectively.[52, 53] This will lead to a larger quantity of residual solvent retained in the hybrid layer and adhesive layer,[54] preventing the formation of a polymer with high reticulation,[55-57] thus diminishing the degree of conversion,[38] reducing the resin-dentin bond strength[58-61] and increasing the permeability of the adhesive layer after polymerization.[62, 63] Consequently, the polymers will be more susceptible to degradation over time.[3, 64]

This may explain the lower results of AlSe in the immediate time interval in comparison with the other adhesives, and this is in agreement with a recent study published by Munoz et al.,[16] even when applied actively; whereas in the mentioned study, AI was applied passively.[16] Active/vigorous application improves the immediate and long-term results of the bond to dentin of 1-step SE adhesive systems[65-69] because it increases the penetration of monomers into dentin and solvent evaporation. Agitation will also improve the efficacy of polymerization by improving the chemical interaction of the adhesive with the dental substrate, particularly for the acid SE adhesives.[43, 70] In addition, unreacted acid monomers present in the superficial layer of the adhesive may be taken to a basal area of dentin, increasing demineralization of the substrate, diffusion of monomers, and improving the interaction with the smear layer and subjacent dentin.[65-67]

As only AIEr demonstrated degradation of the bond strength values over the course of time after 6 months of evaluation, we could hypothesize that the presence of PAC is more important for Er adhesives than for Se. Some authors have indicated that the function of PAC is to improve the stability to humidity,[71, 72] a crucial factor for Er adhesives, which due to dentin demineralization has a more sensitive technique when compared with that of the Se adhesives.[2]

Although there are various universal adhesive systems available on the market, among the materials evaluated, only those that contain MDP presented better bond longevity characterized by the maintenance of bond strength and nanoleakage pattern, as observed for the 2-step Se adhesive (CSE) tested.

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Legend of Figure

Figure 1. Representative backscatter SEM images of the resin-dentin adhesive interfaces of each experimental group for immediate and 6-month times. (Control etch-and-rinse group= Adper Single Bond 2 and control self-etch group=Clearfil SE Bond). For the Peak Universal Adhesive System, the amount of nanoleakage was higher than the other materials and increased after 6 months for strategies ER (B, F) and SE (J, N). The amount of nanoleakage was lower and stable after 6M within the hybrid layer for Scotchbond Universal (C, G, K, O); AllBond Universal (D, H, L, P) and controls (white arrows) (A, E. I, M). Abbreviations: Co, composite; De, dentine; HL, hybrid layer; AL, adhesive layer; SE, self-etch; ER, etch-and-rinse

Table 1. Adhesive system (batch number), composition and application mode* of the adhesive systems used (*) according to the manufacturer's instructions.

Adhesive (batch number),	Composition	Self-etch strategy	Etch-and-rinse strategy
Adper Single Bond 2 (BPBR)	1. Etchant: 35% phosphoric acid (Scotchbond Etchant) 2. Adhesive: Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and polyitaconic acids,10% by weight of 5 nm-diameter spherical silica particles	N.A	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Blot excess water 4. Apply 2-3 consecutive coats of adhesive for 15 s with gentle agitation 5. Gently air thin for 5 s 6. Light-cure for 10 s at 1200 mW/cm2
Clearfil SE Bond (Primer: 00954A - Bond: 01416ª)	1. Primer: water, MDP, HEMA, camphorquinone, hydrophilic dimethacrylate 2. Bonding: MDP, Bis-GMA, HEMA, camphorquinone, hydrophobic dimethacrylate, N,N-diethanol p-toluidine bond, colloidal silica	1. Apply primer to tooth surface and leave in place for 20 s 2.Dry with air stream to evaporate the volatile ingredients 3. Apply bond to the tooth surface and then create a uniform film using a gentle air stream 4.Light-cure for 10 s at 1200 mW/cm2	N.A
Peak Universal Adhesive System (Peak SE Primer: 0N062 – Peak LC Bond: Y062)	1. Etchant: 35% phosphoric acid (Ultraetch) 2.Primer: ethyl alcohol, methacrylic acid, 2-hydroxyethyl methacrylate (Peak SE Primer) 3. Adhesive: Ethyl alcohol, 2-hydroxyethyl methacrylate (Peak LC Bond)	1. Initial use of Peak SE requires activation of the two components separated in the syringe 2. Application of the Peak SE with microbrush for 20 s using continuous scrubbing on dentin. Do not scrub enamel 3. Thin/dry for 3 s using air/water syringe or high volume suction directly over preparation 4. Apply a puddle coat of Peak LC Bond with gently agitate for 10 s 5. Thin/dry 10 s using ¼ to ½ air pressure 6. Light polymerize for 10 s at 1200mW/cm2	1. Apply etchant for 20 s 2. Rinse for 5 s 3. Air dry 2 s 4. Apply a puddle coat of Peak LC Bond with gently agitate for 10 s 5. Thin/dry 10 s using ¼ to ½ air pressure 6. Light-cure for 10 s at 1200mW/cm2
Scotchbond Universal Adhesive (D-82229)	1. Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide. (Scotchbond Universal Etchant) 2. Adhesive: MDP Phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. If necessary, rewet the disposable applicator during treatment 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely 3. Light-cure for 10 s	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Air dry 2 s 4. Apply adhesive as for the self-etch mode
All-Bond Universal (1200006111)	1. Etchant Uni-Etch: 32%phosphoric acid, benzalkonium chloride. 2. Adhesive: MDP, Bis-GMA, HEMA, ethanol, water, initiators	1. Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10-15 s per coat. Do not light cure between coat. Do not light polymerize between coats. 2. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 s, there should be no visible movement of the material. The surface should have a uniform glossy appearance 3. Light cure for 10 s at 1200 mW/cm2	1. Apply etchant for 15 s 2. Rinse thoroughly 3. Remove excess water with absorbent pellet or high volume suction for 1-2 s 4. Apply adhesive as for the self-etch mode

Table 2. Number of specimens (%) according to fracture mode and the premature failure of all experimental groups.

Adhesive system	Application mode	Time	Fracture pattern			
			A	C	A/M	PF
Adper Single Bond 2	ER control	Immediate	51(73.9)	10(14.5)	6(8.7)	2(2.9)
		6 month	49(73.1)	4(6.0)	9(13.4)	5(7.5)
Clearfil SE Bond	SE control	Immediate	50 (74.6)	3(4.5)	10(14.9)	4(6.0)
		6 month	53(80.3)	2(3.0)	8(12.1)	3(4.6)
Peak Universal	ER	Immediate	56(80)	0(0)	10(14.3)	4(5.7)
		6 month	52(75.4)	2(2.9)	12(17.4)	3(4.3)
	SE	Immediate	58(82.8)	3(4.3)	7(10)	2(2.9)
		6 month	48(69.6)	1(1.5)	13(18.8)	7(10.1)
Scotchbond Universal	ER	Immediate	53(79.1)	2(3.0)	10(14.9)	2(3.0)
		6 month	50(78.1)	0(0)	13(20.3)	1(1.6)
	SE	Immediate	47 (71.2)	3(4.6)	11(16.7)	5(7.5)
		6 month	51(73.9)	1(1.4)	14(20.3)	3(4.4)
Allbond Universal	ER	Immediate	49(71)	8(11.6)	7(10.1)	5(7.3)
		6 month	49(73.1)	2(3.0)	12(17.9)	4(6.0)
	SE	Immediate	51(72.85)	4(5.7)	9(12.9)	6(8.6)
		6 month	47(69.1)	5(7.4)	10(14.7)	6(8.8)

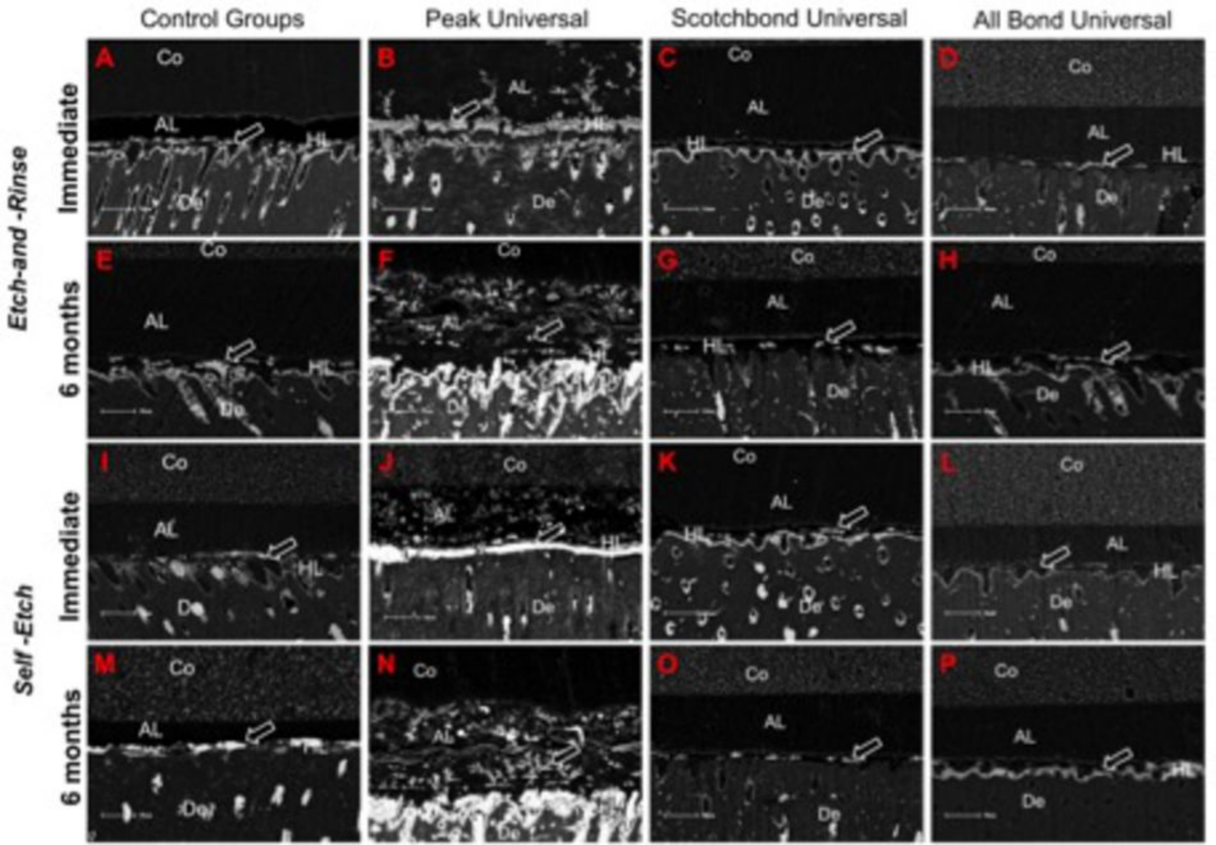
A – adhesive fracture mode; C – cohesive fracture mode; A/M – adhesive/mixed fracture mode; PF – premature failure

Table 3 – Microtensile bond strength (μTBS) values (means ± standard deviations) of the different experimental groups

	Adhesive System							
Time	**Adper Single Bond 2**	**Clearfil SE Bond**	**Peak Universal SE**	**Peak Universal ER**	**Scotchbond Universal SE**	**Scotchbond Universal ER**	**Allbond Universal SE**	**Allbond Universal ER**
Immediate	47.6±5.5 a	42.9±4.4 a, b	39.5±5.1 b	44.3±1.6 a, b	33.3±3.2 c	34.7±4.6 b, c	20.9±4.1 e	38.5±4 b
6 month	38.8±5.7 b	36.2±2.7 b, c	27.9±4.9 d	34.2±4.2 c	33.6±5.8 c	34.6±6.2 c	20.4±4.8 e	28.1±4.3 c

Table 4 – Nanoleakage (NL) values (means ± standard deviations) of the different experimental groups

	Adhesive System							
Time	**Adper Single Bond 2**	**Clearfil SE Bond**	**Peak Universal Bond SE**	**Peak Universal Bond ER**	**ScotchBond Universal SE**	**ScotchBond Universal ER**	**Allbond Universal SE**	**Allbond Universal ER**
Immediate	13.1±2.0 b	7.5±2.9 a, b	31.6±3.1 c	23.9±4.2 c	5.1±2.1 a	5.3±1.1 a	6.0±3.9 a	9.4±1.8 b
6 month	14.7±4.1 b	8.6±4.1 a, b	42.2±2.6 d	34.4±4.1 d	4.7±2.8 a	5.4±2.0 a	5.9±1.4 a	8.9±3.1 a, b


Control Groups
Peak Universal
Scotchbond Universal
All Bond Universal
Etch-and -Rinse
Immediate
6 months
Self -Etch
Immediate
6 months
A
Co
AL
HL
De
B
AL
HL
De
C
Co
AL
HL
De
D
Co
AL
HL
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4.3 Capítulo 3 (do experimento 3)

INFLUENCE OF A HYDROPHOBIC RESIN COAT ON THE BONDING EFFICACY OF THREE UNIVERSAL ADHESIVES

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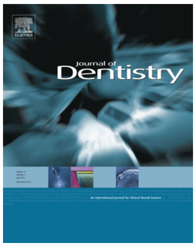

Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives

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ABSTRACT

Objectives: To evaluate the effect of an additional hydrophobic resin coating (HE) on the resin–dentine microtensile bond strengths (μTBS), nanoleakage (NL), and *in situ* degree of conversion (DC) of three universal adhesives used in the etch-and-rinse (ER) and the self-etch (SE) modes.

Methods: Sixty caries-free extracted third molars were divided into 12 groups according to the combination of the factors adhesive (All-Bond Universal [ABU]; G-Bond Plus [GBP] and Scotchbond Universal [SBU]), adhesive strategy (ER and SE), and the use of HE (Heliobond; yes or no). After restorations were constructed, specimens were stored in water (37 °C/24 h) and sectioned into resin–dentine beams (0.8 mm^2) to be tested under tension (0.5 mm/min). Selected beams from each tooth were used for DC quantification and for NL evaluation. Data from each adhesive were analyzed with two-way ANOVA and Tukey's test ($\alpha = 0.05$).

Results: ABU and GBP resulted in higher μTBS in the ER mode. The use of HE increased the μTBS of ABU and GBP only in the SE mode. Lower NL was observed for SBU and ABU in the ER mode + HE, and for GBP in the SE mode + HE. SBU and GBP showed higher DC when used in the ER mode, which was increased with HE application. The DC of ABU was similar in all conditions.

Conclusions: The conversion of 1-step SE to 2-step SE may increase the μTBS and DC of current universal adhesives. The reduction in the NL is more dependent on the adhesive composition than on the bonding strategy.

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1. Introduction

For each of the two current adhesive strategies dental manufacturers have introduced simplified adhesive systems, known as 2-step etch-and-rinse [ER] and 1-step self-etch [SE] adhesives,[1,2] making them more attractive to the clinician and reducing the sensitivity of the application technique. This task of simplification was possible through the inclusion of hydrophilic monomers and the increase in the amount of solvents, to make adhesives compatible with the inherent wet dentine substrate.[3,4] Recently, newly universal or multi-mode adhesive systems were introduced with manufacturers' claims that one monomer solution can be used for either adhesive strategy[5,6] without compromising the bonding effectiveness,[7] therefore being able to replace existing simplified adhesives.

The increased amount of solvents and hydrophilic monomers in the adhesive formulations lead to greater amount of residual solvents entrapped in the adhesive layer.[4] The accumulation of hydrophilic monomers and especially residual solvents may hinder the formation of a high cross-linking polymer,[8–10] decreasing the degree conversion (DC)[11] which may reduce resin–dentine bond strengths,[12–15] and increase the permeability of the adhesive layer after polymerization .[16,17] Consequently, the resulting polymers will be more susceptible to degradation over time.[2,18]

One of the methods used to circumvent these drawbacks includes the application of an additional layer of a hydrophobic resin coating over the polymerized simplified adhesive.[19] This extra resin coat aims at increasing the thickness and uniformity of the adhesive layer, as well as to reduce the fluid flow across the adhesive interface.[19,20] Excellent *in vitro* and clinical results have been reported after placement of an hydrophobic resin coating over 1-step SE adhesives[21,22]; however this technique has not been tested with new universal or multi-mode adhesives. Thus, the aim of this study was to compare the immediate resin–dentine microtensile bond strengths, nanoleakage and *in situ* degree of conversion of three universal adhesives with or without an additional hydrophobic resin coating. We tested the null hypothesis that the application of a hydrophobic resin coat on the cured adhesives will not influence the selected properties of the universal adhesives systems used in both adhesive strategies (see Fig. 1).

2. Materials and methods

Sixty caries-free extracted human third molars were disinfected in 0.5% chloramine, stored in distilled water and used within six months after extraction. The teeth were collected after obtaining the patients' informed consent under a protocol approved by the local Ethics Committee Review Board under the protocol number 17878/11.

A flat occlusal dentine surface was exposed in all teeth after wet-grinding the occlusal enamel with # 180 grit SiC paper. The exposed dentine surfaces were further polished with wet # 600-grit silicon-carbide paper for 60 s to standardize the smear layer.[23]

2.1. *Experimental design and specimen preparation*

The specimens were randomly assigned to twelve experimental conditions ($n = 5$) resulting from the combination of the factors "adhesive system" (Scotchbond Universal Adhesive [SBU, 3M ESPE, St. Paul, MN, USA, also known as Single Bond Universal in some countries], All-Bond Universal [ABU, Bisco Inc., Schaumburg, IL, USA] and G-Bond Plus [GBP, GC Corporation Tokyo, Japan – also known as G-ænial Bond]) (Table 1); "adhesive strategy" (etch-and-rinse [ER] or self-etch [SE]); and hydrophobic resin coating (yes or no; HE, Heliobond, Ivoclar Vivadent, Schaan, Liechtenstein).

The adhesive systems were applied according to the respective manufacturers' instructions (Table 1), except for G-Bond Plus, for which the manufacturer does not recommend dentine etching with phosphoric acid (Table 1). Composite resin crowns were built with a nanofilled composite (Filtek Z350 XT, 3M ESPE, St. Paul, MN, USA; also named Filtek Supreme XTE or Filtek Supreme Plus in some countries) in two increments of 2 mm each. Each increment was light-cured for 40 s using a LED light-curing unit set at 1200 mW/cm^2 (Radii-cal, SDI Limited, Bayswater, Victoria, Australia).

After storage in distilled water for 24 h at 37 °C, the specimens were longitudinally sectioned in mesio-distal and buccal–lingual directions across the bonded interface with a slow-speed diamond saw (Isomet Ltd., Buehler Ltd., Lake Bluff, IL, USA) to obtain resin–dentine beams with a cross sectional area of approximately 0.8 mm^2 measured with a digital calliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). All specimens from each tooth were used for the μTBS test, except four that were randomly selected for measurement of nanoleakage (NL) and *in situ* DC.

2.2. *Microtensile bond strength (μTBS)*

The resin–dentine bonded beams were attached to a Geraldeli jig[24] (Odeme Biotechnology, Joaçaba, SC, Brazil) with cyanoacrylate adhesive and tested under tension (Model 5565, Instron Co., Canton, MA, USA) at 0.5 mm/min until failure. The μTBS values were calculated by dividing the load at failure by the cross-sectional bonding area.

The failure mode was classified as cohesive [C] failure (exclusively within dentine or resin composite), adhesive [A] failure (at the resin/dentine interface), or mixed [M] failure (at the resin/dentine interface that included cohesive failure of the neighbouring substrates). The failure mode analysis was performed under a stereomicroscope at 100× magnification (Olympus SZ40, Tokyo, Japan). Specimens with premature failures (PF) were included in the tooth mean as zero MPa and those with cohesive failures were excluded.

2.3. *Nanoleakage (NL)*

Two resin-bonded beams from each tooth were used for NL evaluation. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al.[25] The beams were placed in the ammoniacal silver nitrate solution in darkness for 24 h, rinsed thoroughly in distilled water, and immersed in photo developing solution for 8 h under a fluorescent light to reduce silver ions into metallic silver

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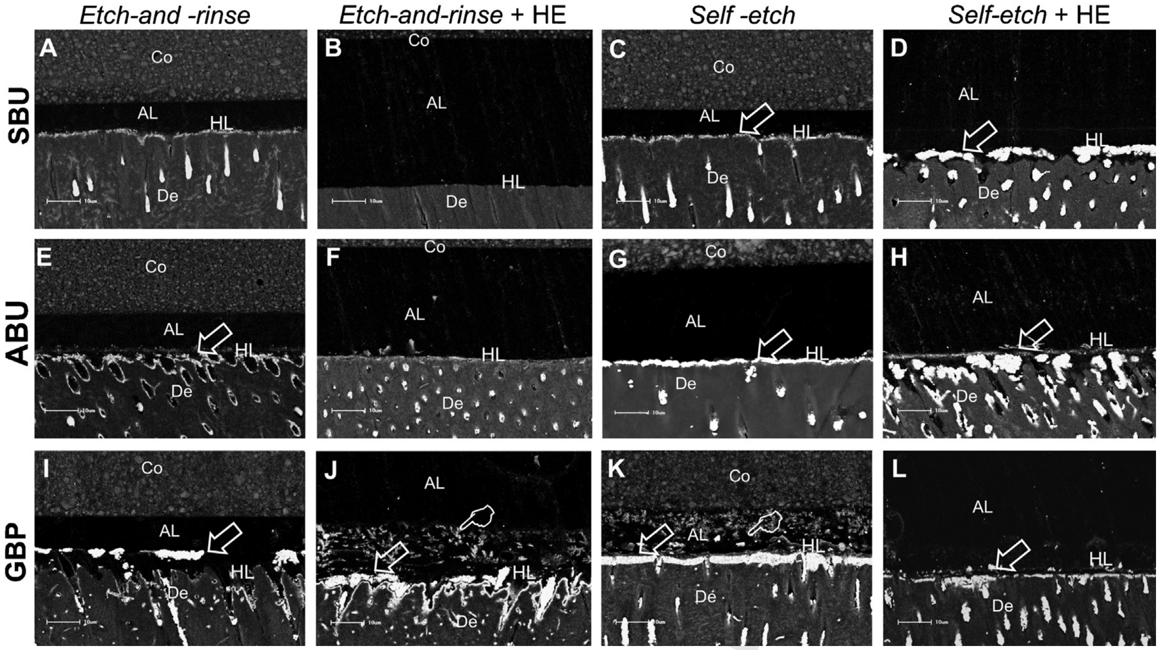

Fig. 1 – Representative backscattered SEM micrographs of the resin–dentine adhesive interfaces of each universal adhesive system (Scotchbond Universal Adhesive [SBU]; All-Bond Universal [ABU] and G-Bond Plus [GBP]), using two bonding strategies (etch-and-rinse [ER] and self-etch [SE]) with or without an additional hydrophobic resin coating [HE]. For SBU and ABU, lower NL was observed in the ER strategy when HE was used (B and F) compared with their controls (A and E). However, for the SE strategy, nanoleakage occurred similarly within the hybrid layer (HL) for both ABU and SBU using or not HE (white arrows) (C, D, G and H). GBP showed higher amount of nanoleakage under the ER strategy in the hybrid layer (white arrows) (I), when compared with SBU and ABU; most silver nitrate uptake occurred throughout the entire thickness of the HL (white arrows) and in the adhesive layer (AL), forming the so-called "water trees" (white pointers) (J and K). Only in the SE strategy with HE, the nanoleakage was lower and similar to SBU and ABU (D, H and L).

grains. Specimens were polished with wet 600-, 1000-, 1200-, 1500-, 2000- and 2500-grit SiC paper and 0.25 μm diamond paste (Buehler Ltd., Lake Bluff, IL, USA) using a polishing cloth. Specimens were then ultrasonically cleaned, air dried, mounted on Al stubs, and coated with carbon-gold (MED 010, Balzers Union, Balzers, Liechtenstein). Resin–dentine interfaces were analyzed in a field-emission scanning electron microscope operated in the backscattered mode (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, UK).

Three micrographs of each resin–dentine bonded beam were obtained. A blinded operator measured the relative percentage of NL of the adhesive interface in each micrograph with the UTHSCSA ImageTool 3.0 software (University of Texas Health Science Center, San Antonio, TX, USA). The values originating from the same specimen were averaged for statistical purposes. The mean NL of all beams from the same tooth was averaged for statistical purposes.

2.4. *Degree of conversion in situ (DC)*

The adhesive interface of two resin–dentine bonded beam from each tooth was wet polished with 1500-, 2000-, and 2500-grit SiC paper for 15 s each. Then they were ultrasonically cleaned for 20 min in distilled water and stored in water for 24 h at 37 °C. The micro-Raman spectrometer (Senterra, Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany) was first calibrated for zero and then for coefficient values using a silicon specimen. The following micro-Raman parameters were employed: 20 mW Neon laser with 532 nm wavelength, spatial resolution of ≈3 μm, spectral resolution ≈5 cm^{-1}, accumulation time of 30 s with 6 co-additions, and magnification of 100× (Olympus UK, London, UK) to a ≈1 μm beam diameter. Spectra were taken at the dentine–adhesive and enamel–adhesive interface at three different sites for each specimen and an average per tooth was performed. Spectra of uncured adhesives were taken as reference. Post-processing of spectra was performed using the dedicated Opus Spectroscopy Software version 6.5 (Bruker Optik GmbH, Ettlingen, Baden Württemberg, Germany). The ratio of double-bond content of monomer to polymer in the adhesive was calculated according to the following formula:

$$\text{DC}\,(\%) = \left(1 - \frac{R_{(\text{cured})}}{R_{(\text{uncured})}}\right) \times 100$$

where "R" is the ratio of aliphatic and aromatic peak areas at 1639 cm^{-1} and 1609 cm^{-1} in cured and uncured adhesives.

2.5. *Statistical analysis*

The Kolmogorov–Smirnov test was used to assess whether the data followed a normal distribution. The Barlett's test was computed to determine if the assumption of equal variances

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Table 1 – Adhesive materials (batch number), composition and application mode of the adhesive systems used.

Materials/batch number	Composition	Application mode			
		Self-etch		Etch-and-rinse	
		Without hydrophobic resin coating	With hydrophobic resin coating	Without hydrophobic resin coating	With hydrophobic resin coating
Heliobond – HE (N37749)	bis-GMA, TEGDMA, initiators, stabilizers				
Scotchbond Universal Adhesive – SBU (448716)	1. Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide (Scotchbond Universal Etchant) 2. Adhesive: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, and silane	1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely 3. Light-cure for 10 s	1. Apply the same self-etch mode 2. Apply a very thin layer of Heliobond with a microbrush on the dental surface 3. An air blower to achieve an optimally thin layer 4. Light-cure for 10 s	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Air dry 5 s 4. Apply adhesive as for the self-etch mode	1. Apply the same etch-and-rinse mode 2. Apply a very thin layer of Heliobond with a microbrush on the dental surface 3. An air blower to achieve an optimally thin layer 4. Light-cure for 10 s
All-Bond Universal – ABU (1200002722)	1. Etchant: 35% phosphoric acid, benzalkonium chloride (SELECT HV-Etch with BAC) 2. Adhesive: MDP, bis-GMA, HEMA, ethanol, water, initiators	1. Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10–15 s per coat. Do not light cure between coats 2. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 s, there should be no visible movement of the material. The surface should have a uniform glossy appearance 3. Light cure for 10 s	1. Apply the same self-etch mode 2. Apply a very thin layer of Heliobond with a microbrush on the dental surface 3. An air blower to achieve an optimally thin layer 4. Light-cure for 10 s	1. Apply etchant for 15 s 2. Rinse thoroughly 3. Remove excess water with absorbent pellet or high volume suction for 1–2 s 4. Apply adhesive as for the self-etch mode	1. Apply the same etch-and-rinse mode 2. Apply a very thin layer of Heliobond with a microbrush on the dental surface 3. An air blower to achieve an optimally thin layer 4. Light-cure for 10 s
G-Bond Plus – GBP (1102221)	Acetone, dimethacrylate, 4 methacryloxyethyltrimellitate anhydride, phosphoric acid ester monomer, silicon dioxide, photo initiator, distilled water Note: the manufacturer does not recommend dentine conditioning with phosphoric acid	1. Apply using a microbrush 2. Leave undisturbed for 10 s after the end of application 3. Dry thoroughly for 5 s with oil free air under maximum air pressure. Use vacuum suction to prevent splatter of the adhesive 4. Light-cure for 10 s	1. Apply the same self-etch mode 2. Apply a very thin layer of Heliobond with a microbrush on the dental surface 3. An air blower to achieve an optimally thin layer 4. Light-cure for 10 s	1. Apply 34% phosphoric acid gel for 10 s (Scotchbond Universal Etchant) 2. Rinse for 5 s and gently dry 3. Apply adhesive as for the self-etch mode	1. Apply the same etch-and-rinse mode 2. Apply a very thin layer of Heliobond with a microbrush on the dental surface 3. An air blower to achieve an optimally thin layer 4. Light-cure for 10 s

HEMA, 2-hydroxyethyl methacrylate; MDP, methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol glycidyl methacrylate; TEGDMA, triethylene glycol dimethacrylate.

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Table 2 – Number (%) of specimens according to fracture pattern mode for the experimental groups.

Adhesive system	Application mode	Hydrophobic bond	Fracture pattern			
			A	C	A/M	PF
SBU	ER	Without	48 (70.6)	0 (0.0)	14 (20.6)	6 (8.8)
		With	45 (95.8)	1 (2.1)	0 (0.0)	1 (2.1)
	SE	Without	51 (83.6)	2 (3.3)	6 (9.8)	2 (3.3)
		With	55 (80.9)	4 (5.9)	1 (1.5)	8 (11.7)
ABU	ER	Without	42 (80.8)	0 (0.0)	8 (15.4)	2 (3.8)
		With	47 (92.2)	2 (3.9)	2 (3.9)	0 (0.0)
	SE	Without	50 (79.4)	0 (0.0)	10 (15.9)	3 (4.7)
		With	48 (80.0)	1 (1.7)	7 (11.7)	4 (6.6)
GBP	ER	Without	48 (77.5)	2 (3.2)	8 (12.9)	4 (6.4)
		With	50 (83.3)	1 (1.7)	8 (13.3)	1 (1.7)
	SE	Without	48 (87.3)	1 (1.8)	4 (7.3)	2 (3.6)
		With	52 (94.6)	1 (1.8)	0 (0.0)	2 (3.6)

A, adhesive fracture mode; C, cohesive fracture mode; A/M, adhesive/mixed fracture mode; PF, premature failure.

was valid. After observing the normality of the data distribution and the equality of the variances, data from μTBS, NL, and *in situ* DC were analyzed separately using two-way ANOVA having the "adhesive strategy" and "hydrophobic resin coating" as independent variables for each adhesive system. Tukey's *post hoc* test was used for pairwise comparisons at a level of significance of 5%.

3. Results

Most of the specimens showed adhesive/mixed failures. Cohesive and premature failures were observed in few specimens (Table 2).

3.1. *Microtensile bond strength (μTBS)*

When used under manufacturers' directions, higher mean μTBS were obtained with the ER mode for ABU and GBP (Table 3; $p = 0.0001$ and 0.01, respectively). The use of a hydrophobic resin coating increased the μTBS of the adhesives only in the SE mode (Table 3; $p < 0.01$).

3.2. *Nanoleakage (NL)*

None of the conditions resulted in nanoleakage-free interfaces (Table 4). Significant reductions in the NL were only observed for SBU and ABU in the ER mode when a hydrophobic resin coating was applied (Table 4; $p = 0.01$ and $p = 0.007$, respectively). For GBP, the highest NL was observed in the SE mode, which was significantly reduced with the hydrophobic resin coating (Table 4; $p = 0.001$).

3.3. *Degree of conversion (DC)*

When used as per manufacturers' instructions, GBP and SBU resulted in statistically higher DC when applied in the ER mode than when applied in the SE mode. The addition of a

Table 3 – Microtensile bond strength (μTBS) values (means ± standard deviations) of the different experimental groups.[*]

Adhesives	ER		SE	
	Without coating	With coating	Without coating	With coating
SBU	32.3 ± 3.7B	34.6 ± 4.1B	34.7 ± 5.8B	42.7 ± 6.1A
ABU	40.8 ± 5.0b	36.3 ± 4.4c	22.0 ± 5.1d	48.4 ± 5.9a
GBP	20.5 ± 3.2^{b}	22.6 ± 4.6^{b}	11.5 ± 3.3^{a}	22.0 ± 4.6^{b}

Comparisons are valid only within rows.

[*] Means identified with the identical letters are statistically similar ($p > 0.05$).

Table 4 – Nanoleakage (NL) values (means ± standard deviations) of the different experimental groups.[*]

Adhesives	ER		SE	
	Without coating	With coating	Without coating	With coating
SBU	6.2 ± 2.3B	1.3 ± 1.1A	6.7 ± 3.0B	5.9 ± 2.2B
ABU	11.2 ± 2.6c	4.3 ± 1.1a	7.3 ± 3.4a,b	10.1 ± 2.7b,c
GBP	14.8 ± 3.8^{b}	15.3 ± 2.3^{b}	39.9 ± 2.6^{c}	7.4 ± 2.3^{a}

Comparisons are valid only within rows.

[*] Means identified with identical letters are statistically similar ($p > 0.05$).

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Table 5 – Degree of conversion (DC) values (means ± standard deviations) of the different experimental groups.*

Adhesives	ER		SE	
	Without coating	With coating	Without coating	With coating
SBU	86.2 ± 4.6A,B	92.9 ± 4.3A	63.9 ± 5.2C	81.4 ± 4.6B
ABU	76.3 ± 4.1a	80.9 ± 4.1a	75.2 ± 1.9a	81.9 ± 5.1a
GBP	67.3 ± 4.5^{c}	92.1 ± 3.9^{a}	57.3 ± 4.7^{d}	87.7 ± 3.9^{b}

Comparisons are valid only within rows.

* Means identified with identical letters are statistically similar ($p > 0.05$).

hydrophobic resin coating increased the DC of GBP and SBU for either bonding strategy. However, this increase was not statistically significant for the ER strategy. The adhesive strategy and the hydrophobic resin coating did not affect the DC of ABU (Table 5; $p = 0.45$).

4. Discussion

The performance of the universal adhesives used in this study was dependent on the adhesive strategy. The results of the μTBS testing showed that two of the three adhesive systems showed improved resin–dentine bond strengths when used in the ER mode, following manufacturers' directions. The presence of the smear layer imposes a physical barrier against the penetration of resin monomers.[3] Phosphoric acid etching removes the smear layer, promotes superficial dentine demineralization[26] and increases the impregnation of resin monomers, which leads to the formation of a thick hybrid layer fully integrated with the dentine.[5,6,26–28] The findings of this study along with the improved bond strength of 1-step SE adhesives after previous phosphoric acid etching[5,6,27,28] suggests that the smear layer incorporation into the adhesive interface reduces bonding effectiveness.

We hypothesize that the demineralization by-products, as well as the smear layer remnants, may have acted as contaminants, preventing the polymer chains from getting closer during the polymerization reaction. This would explain why using the same adhesive formulation under identical light-curing protocols for two out of the three adhesive systems, the *in situ* DC was lower when the materials were used in the SE mode.

While the hydrophobic resin coating HE did not improve mean μTBS under the ER strategy, HE increased mean μTBS in the SE mode, which reached the magnitude of mean μTBS obtained in the ER approach. As HE also resulted in an increase in the *in situ* DC of adhesives applied in the SE mode (yet not statistically significant for ABU), we have to partially reject the null hypothesis. Other studies also detected higher DC and lower adhesive permeability for adhesive systems which use an extra hydrophobic resin coating.[11,29] This was also observed in a recent study, in which Peak Universal Adhesive, a 2-step SE adhesive, was superior in terms of DC than its 1-step counterparts (Scotchbond Universal, All-Bond Universal).[7]

The extra layer of hydrophobic resin adds unsolvated hydrophobic monomers to the adhesive interface, which decreases the relative concentration of retained solvents and unreacted monomers in the adhesive layer.[18] This, in turn, increases the ultimate tensile strength of the adhesive interface,[10] due to the formation of a more densely packed hybrid layers,[20] making it more resistant to the tensile forces during μTBS testing and less prone to degradation effects over time.[30,31]

Although earlier studies have reported that the permeability of the adhesive interfaces can be reduced by the application of a hydrophobic resin coating,[20] the NL of two out of the three adhesives tested in the SE mode was not significantly influenced in our study when HE was applied. This may be a result of NL being not only related to the permeability of the adhesive layer, but also to submicron defects, such as solvent/water entrapment, incomplete resin infiltration, or areas of inadequate polymerization.[25,30] For instance, the fact that only GBP applied in the SE mode resulted in a very low DC and a very high NL, lead us to speculate that excessive NL of the GBP interface might be related to incomplete polymerization of the adhesive interface. This may be the reason why the additional hydrophobic resin coating benefited GBP in SE mode, increasing the DC and simultaneously decreasing the NL.

The poorest performance of GBP in all tests in the present study, as well as an earlier study,[6] may also be attributed to the application mode. Contrary to SBU and ABU, which were applied actively, GBP was the only material for which the manufacturer does not recommend active application. It is consensual that an active adhesive application increases the resin–dentine bond strength.[32–38] Several factors may account for this effect. It is believed that an increased penetration of monomers into dentine substrates as well as higher solvent evaporation occurs under active application. Agitation also improves the polymerization efficacy through enhancing the chemical interaction with tooth substrate, mainly for acidic SE adhesives.[35,39] Additionally, unreacted acidic resin monomers from the superficial layer of the adhesive can be taken to the basal portion of dentine, increasing the substrate demineralization, monomer diffusion and better interaction with the smear layer and underlying dentine.[36–38]

Apart from the application mode, compositional differences between adhesives may play an important role on the adhesive performance. SBU and ABU contain 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP) capable to chemically interact with the hydroxyapatite by the formation of a stable nano-layering at the adhesive interface[40–42] GBP has a lower pH value (pH = 1.5) than the other adhesives (SBU = 3; ABU = 2.4).[7] The hydrolysis of the ester bond from the acid monomer results in a strong phosphoric acid that might increase the demineralization over time and explain the intense NL within the hybrid layer.[7,43]

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An overall analysis of the three adhesives shows that SBU performed generally similar when used in the ER and SE mode. As mentioned in an earlier study,[7] SBU has polyalkenoic acid copolymer and MDP in its formulation. Although both molecules may compete by binding to the calcium in hydroxyapatite,[41] they are usually associated with improved adhesive performance[44,45] which may explain the good performance of SBU in both bonding strategies.

In summary, the use of a hydrophobic resin coating may improve the performance of the universal adhesives mainly when applied as SE adhesives.

5. Conclusions

With the limitations of this study the use of an additional hydrophobic resin coating improved the adhesive performance in terms of resin–dentine bond strengths of new universal adhesives when used with the SE strategy. The additional hydrophobic resin coating also improved the DC for both the ER and the SE strategies. The reduction in NL may be more dependent on the adhesive composition than on the adhesive strategy. In spite of the improvements observed for universal adhesives *in vitro*, longevity studies should be performed to validate these initial results.

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5. DISCUSSÃO

Apesar de todos os novos sistemas adesivos universais testados compartilharem a mesma versatilidade de aplicação como CON ou AC, houve diferenças no desempenho destes materiais quando comparados com os controles o que parece estar associado às suas diferentes composições e a seus números de passos. Entretanto, quando se aplicou uma camada hidrofóbica adicional aos adesivos testados, seu desempenho tornou-se dependente principalmente da estratégia adesiva.

Com base nas semelhanças nas suas composições, esperava-se, no tempo imediato, que os adesivos universais ScAc e AIAc fossem equivalentes ao controle CSE, no entanto, isto não foi observado. Estes três materiais possuem o monômero 10-MDP em sua composição, portanto, unem-se quimicamente à dentina (Inoue et al.[66] 2005; Waidyasekera et al.[67] 2009; Peumans et al.[68] 2010). Yoshida e cols. (Yoshida et al.[69] 2012) mostraram uma eficaz interação química entre o MDP e a hidroxiapatita formando uma nanocamada estável na interface adesiva, aumentando a resistência mecânica da mesma. A deposição de sais estáveis de MDP-Ca junto à nano-camada tem permitido sua alta estabilidade (Yoshida et al.[69] 2012; Yoshihara et al.[70] 2010), a qual tem sido comprovada em pesquisas clínicas e laboratoriais (Peumans et al.[68] 2010; Toledano et al.[71] 2007; Feitosa et al.[72] 2012). O melhor comportamento em termos de RU do controle CSE, quando comparado com ScAc, pode se dever à presença do copolímero de ácido polialquenóico (PAC) na composição do ScAc. Este copolímero pode ter competido com o MDP pela união ao cálcio da hidroxiapatita prejudicando levemente a união química do MDP à dentina (Yoshida et al.[69] 2012). Além disso, o copolímero PAC poderia ter impedido a aproximação dos monômeros durante a polimerização devido a seu alto peso molecular, tendo como consequência, um menor GC deste adesivo. Adicionalmente, ScAc e AIAc são adesivos de um passo e isso, provavelmente, faz com que sua concentração de MDP seja menor que a do CSE, que tem este monômero MDP incorporado na composição tanto do *primer* quanto do *bond* (Yoshida et al.[69] 2012).

Ainda entre os adesivos AC dos experimentos 1 e 2, tanto no tempo imediato, quanto após 6 meses, AIAc mostrou o pior desempenho em termos de RU, o que poderia ser atribuído ao seu conteúdo de solvente. O adesivo AI contém maior porcentagem de solvente, quando comparado com Sc (30-60%peso e 10-15%peso, respectivamente) (Chen, Suh[73] 2013; 3MESPE[74] 2013). Isto leva a uma maior quantidade de solvente residual retido nas camadas adesiva e híbrida (Yiu et al.[9] 2005), prejudicando a formação de um polímero com alta reticulação (Ye et al.[39] 2007; Loguercio et al.[40] 2009; Paul et al.[41] 1999), portanto reduzindo o grau de conversão (Cadenaro et al.[42] 2005) e a resistência de união dente-resina (Hass et al.[43] 2011; Reis et al.[44] 2010; Takahashi et al.[45] 2002; Cho, Dickens[46] 2004) e aumentando a permeabilidade da camada adesiva após a polimerização (Malacarne et al.[47] 2006; Ito et al.[48] 2005)

Outro fator que pode ter contribuído para o comportamento inferior de AlAc foi seu modo de aplicação. No experimento 1, AlAc foi esfregado mas não aplicado vigorosamente, de acordo com nossa interpretação das instruções do respectivo fabricante, situação habitual na pratica clínica. Alguns autores apontam claramente que a aplicação ativa/vigorosa melhora o desempenho da adesão de sistemas adesivos AC à dentina (Pleffken et al.[75] 2011; Loguercio et al.[76] 2011). Acredita-se que com a aplicação ativa aumenta-se tanto a penetração dos monômeros na dentina quanto a evaporação do solvente. A agitação também melhoraria a eficácia de polimerização através da melhoria na interação química do adesivo com o substrato dentário, principalmente para adesivos AC ácidos (Wang, Spencer[50] 2003; Zhang, Wang[77] 2013). Além disso, monômeros ácidos não reagidos presentes na camada superficial do adesivo podem ser levados para uma área basal da dentina, aumentando a desmineralização do substrato e a difusão dos monômeros, melhorando a interação com a *smear layer* e a dentina subjacente (do Amaral et al.[78] 2009; do Amaral et al.[79] 2010; Miyazaki et al.[80] 1996). Assim, no experimento 2, o AlAc foi aplicado vigorosamente o que aumentou sua RU, porém, se manteve ainda como a mais baixa entre os adesivos universais. Já no experimento 3, o GBP foi o único adesivo cujo fabricante indica especificamente não esfregar e deixar em repouso e, consequentemente, apresentou uma pobre performance em todos os testes. Quando GBP foi aplicado ativamente em um recente estudo (Hanabusa et al.[29] 2012) mostrou melhores resultados, o que corrobora que uma aplicação ativa de adesivo aumenta a RU resina-dentina e a estabilidade ao logo do tempo (Pleffken et al.[75] 2011; Loguercio et al.[76] 2011; Zhang, Wang[77] 2013; do Amaral et al.[78] 2009;

Miyazaki et al.[80] 1996; Zhang, Wang[81] 2012). Futuros estudos precisam ser realizados para testar isoladamente esta hipótese com sistemas adesivos universais.

Entre todos os adesivos usados com a estratégia AC nos experimentos 1 e 2, PkAc foi o único adesivo que, no tempo imediato, apresentou valores de RU semelhantes ao controle CSE e superiores aos demais adesivos estudados, apesar de não apresentar MDP na sua composição. Tanto estudos *in vitro* (Reis et al.[62] 2008; Feitosa et al.[72] 2012; Frankenberger et al.[82] 2001; Albuquerque et al.[83] 2008) como clínicos (Reis et al.[63] 2009; Loguercio, Reis[84] 2008) tem mostrado que a aplicação de uma camada adicional melhora o desempenho de adesivos AC de 1-passo, sendo essa uma vantagem do PkAc sobre ScAc e AlAc. Em geral, adesivos AC de 1-passo apresentam um desempenho inferior aos adesivos AC de 2 passos (Van Meerbeek et al.[8] 2003; Perdigao[85] 2007). Adesivos AC de 1-passo resultam em finas camadas de adesivo, sendo propensas à inibição de polimerização por oxigênio (Nunes et al.[86] 2005). Este resultado imediato positivo do adesivo PkAc, entretanto, não foi estável ao longo de seis meses. Como essa degradação ocorreu tanto para a estratégia AC quanto para a CON, as possíveis justificativas para esse comportamento estão discutidas posteriormente.

Os adesivos com MPD normalmente são associados com um melhor desempenho de união (Van Meerbeek et al.[87] 2011; Perdigao et al.[88] 2013). No que se refere à estratégia AC, foi verificado em nossos resultados que, após 6 meses, tanto a RU quanto a NI dos adesivos CSE, AlAc e ScAc se mantiveram estáveis. Um dos fatores favoráveis desses materiais é o seu pH em uma faixa em torno de 2, que produz desmineralização parcial da dentina, deixando quantidade substancial de cristais de hidroxiapatita ao redor das fibrilas de colágeno (Tay, Pashley[89] 2001), favorecendo sua interação química com o MDP. Essa interação, juntamente com os fatores anteriormente citados, poderia explicar o comportamento dos adesivos estudados, segundo a estratégia AC.

No que diz respeito à abordagem CON, esta pareceu ser favorável no tempo imediato, pois os adesivos universais mostraram melhor RU em comparação com a estratégia AC, exceto para ScCon, que foi semelhante a ScAc. Sabe-se que a *smear layer* constitui uma verdadeira barreira física que torna extremamente difícil a conformação de uniões e formação de uma camada híbrida totalmente integrada com a dentina (Kenshima et al.[90] 2005). Após o condicionamento com ácido fosfórico, a *smear layer* é removida e a superfície dentinária é desmineralizada. Isto facilitaria a infiltração do adesivo, permitindo a criação de uma camada híbrida, como tem sido demonstrado em adesivos AC de 1-passo aplicados em dentina previamente condicionada com ácido fosfórico (Hanabusa et al.[29] 2012; Proenca et al.[31] 2007; Taschner et al.[32] 2012; Perdigao et al.[64] 2002; Kenshima et al.[90] 2005). Neste sentido, os resultados do experimento 3, mostraram para o teste de RU que dois (ABU e GBP) dos três sistemas adesivos (ABU, SBU e GBP) melhoraram seus resultados quando utilizados com a estratégia CON, embora o fabricante do GBP não recomende o condicionamento da dentina com ácido fosfórico.

Ainda abordando a RU dos adesivos, se compararmos os resultados após 6 meses do Sc (que contém MDP e PAC) com o SB (que contém somente PAC), podemos observar que a associação MDP-PAC promoveu estabilidade para ScAc e ScCon. Esta estabilidade não pode ser observada no adesivo SB. A mesma falta de estabilidade foi observada para o material AlCon (que contém apenas MDP), o qual demonstrou degradação dos valores de resistência de união após 6 meses. Alguns autores indicam que a função do PAC é melhorar a estabilidade à umidade (Van Meerbeek et al.[91] 1996; Spencer et al.[92] 2000), que torna sensível a técnica CON, quando comparada com a técnica AC (Perdigao[85] 2007).

A NI representa a localização de defeitos na interface resina-dentina que podem servir de vias para a degradação da união resina/dentina ao longo do tempo (Sano[38] 2006). O nitrato de prata é capaz de ocupar os espaços de tamanho nanométrico presentes ao redor das fibrilas de colágeno expostas, onde os monômeros não conseguiram infiltrar-se, ou onde a água residual não foi deslocada pelo adesivo ou mesmo em áreas com incompleta conversão de monômero (Sano[38] 2006). Entre os adesivos dos experimentos 1 e 2, PkCon e PkAc apresentaram as maiores percentagens de NI nas interfaces adesivas e os únicos que aumentaram após 6 meses. Situação similar no tempo imediato foi apresentada no experimento 3 para GBP na estratégia AC. O *primer* do sistema PkAc (Peak SE Primer) e o GBP possuem valores de pH muito baixos (1,2 e 1,5, respectivamente) quando comparados com os outros adesivos (SBU=3; ABU=2,4) (Munoz et al.[93] 2013) deixando-os dentro da categoria dos adesivos AC mais agressivos (Van Meerbeek et al.[8] 2003; Tay, Pashley[89] 2001). Tem sido relatado que monômeros ácidos e não polimerizados são capazes de prosseguir desmineralizando a dentina, mesmo após sua polimerização (Carvalho et al.[19] 2005; Wang, Spencer[20] 2005). Isto é provavelmente o resultado da hidrólise da união éster do monômero de ácido que resulta em um ácido fosfórico forte (Wang, Spencer[20] 2005) o que poderia explicar a intensa deposição de nitrato de prata dentro da camada híbrida destes adesivos.

Embora se pudesse esperar uma reduzida NI no interior da camada de adesivo devido ao fato de PkAc ser de 2 passos, a segunda camada de adesivo parece ser tão hidrofílica como o *primer*, uma vez que monômeros hidrofóbicos não foram listados na composição do *Bond* (Peak LC bond - Quadro 1). Embora o aumento da infiltração de monômeros possa ter ocorrido durante a aplicação de uma camada de adesivo adicional, a natureza desta infiltração é hidrofílica, o que resultou numa extensa NI na camada híbrida. No caso de PkCon, Peak LC Bond (pH=2,0) pode ter provocado um condicionamento adicional e um aumento no padrão de condicionamento do substrato dentinário. Isto provavelmente resultou num aumento da desmineralização e exposição de colágeno (Van Meerbeek et al.[8] 2003; Liu et al.[34] 2011; Brackett et al.[94] 2011), aumentando a NI (Hashimoto et al.[16] 2000; Wang, Spencer[50] 2003; Spencer et al.[92] 2000; Wang, Spencer[95] 2002) para PkCon.

Com relação à aplicação de uma camada hidrofóbica no experimento 3, esta não melhorou a média de RU para a estratégia CON, porém aumentou a média de RU na estratégia AC (atingindo magnitudes obtidas na estratégia CON), o que tem sido descrito anteriormente em vários estudos com outros adesivos AC (Reis et al.[62] 2008; Feitosa et al.[72] 2012; Frankenberger et al.[82] 2001; Albuquerque et al.[83] 2008). Assim também, resultou em um aumento no GC dos adesivos aplicados no modo AC. Outros estudos também detectaram maior GC e menor permeabilidade para os sistemas adesivos que utilizam uma camada hidrofóbica adicional (Cadenaro et al.[42] 2005; Breschi et al.[96] 2007). A camada adicional de *Bond* (HE) incorpora monômeros hidrofóbicos não solvatados na interface adesiva, o que diminui a concentração relativa de solventes retidos e monômeros não reagidos na camada adesiva (Breschi et al.[35] 2008). Isto, por sua vez, aumenta a resistência máxima à tração da interface adesiva (Loguercio et al.[40] 2009), devido à formação de uma camada híbrida mais densa ou compactada (de Andrade e Silva et al.[61] 2009), tornando-a mais resistente às forças de tração durante o teste de RU à microtração e menos propenso a efeitos de degradação ao longo do tempo (Reis et al.[97] 2007; Reis et al.[98] 2008).

Embora estudos anteriores tenham relatado que a permeabilidade da interface adesiva pode ser reduzida através da aplicação de uma camada de resina hidrófobica (de Andrade e Silva et al.[61] 2009), a NI de dois dos três adesivos universais (SBU e ABU) testados no modo AC não foi significativamente influenciada em nosso estudo quando HE foi aplicado. Isto pode ser devido à NI não só se encontrar relacionada à permeabilidade da camada adesiva, mas também a defeitos sub-micrométricos tais como, a retenção de solvente/água, infiltração incompleta de monômeros, ou áreas com polimerização inadequada (Reis et al.[97] 2007; Tay et al.[99] 2002). Assim, o fato de apenas o adesivo GBP, quando aplicado na estratégia AC, ter obtido valores muito baixos de GC e muito altos de NI, leva-nos a especular que a NI excessiva poderia estar relacionada com a incompleta polimerização da interface adesiva. Esta pode ser a razão pela qual a camada hidrofóbica beneficiou o adesivo GBP no modo AC, aumentando o GC e diminuindo a NI. O GC para o GBP foi significativamente maior quando uma camada de resina hidrofóbica foi usada. Esta diferença era esperada de acordo com resultados de outros estudos (Sadek et al.[100] 2008; Hamouda et al.[101] 2010) onde adesivos AC de 1-passo foram comparados com AC de 2-passos. No entanto, um aumento em GC não foi previamente relatado para um adesivo específico de 1-passo após a adição de uma camada de resina hidrofóbica.

Por outro lado, adesivos AC de 1-passo e CON de 2-passos podem apresentar bolhas por atração de água, produto de osmose através da camada de adesivo polimerizado (Sauro et al.[102] 2009; Tay et al.[103] 2002). Estas bolhas de osmose são observadas na zona de transição entre a camada de adesivo de AC de 1-passo ou CON de 2-passo e a resina composta (Sauro et al.[102] 2009; Tay et al.[103] 2002). Esta zona contém monômeros não polimerizados da camada de inibição por oxigênio (Van Landuyt et al.[104] 2008), o que teria resultado na diminuição do GC do GBP. Assim, a camada de resina hidrofóbica teria copolimerizado com a superfície não polimerizada deste adesivo de 1-passo, resultando em maior GC, devido ao consumo de ligações duplas residuais.

Relatos sobre a permeabilidade e sorção de água na camada híbrida e a interface adesiva mostraram uma relação com a hidrofilicidade do polímero (Tay, Pashley[105] 2003; Chersoni et al.[106] 2004). As soluções de monômeros de adesivos AC de 1-passo carecem de uma camada de resina não solvatada, que os torna membranas permeáveis (Tay et al.[65] 2002; Tay et al.[107] 2004; Tay et al.[108] 2004; Tay et al.[109] 2004). Esta estrutura permeável iria permitir uma rápida transudação de fluido dentinal através do adesivo polimerizado (Salz et al.[110] 2005), especificamente em adesivos contendo o co-monômero monofuncional 2-HEMA (Hosaka et al.[111] 2010). Teoricamente, a ausência de HEMA (como em GBP), deveria resultar em estabilidade a longo prazo da interface adesiva (Tay et al.[65] 2002; Van Landuyt et al.[104] 2008) quando comparado com adesivos contendo HEMA (Takahashi et al.[112] 2011) porque a retenção e sorção de água é maior neste último tipo de adesivos (Hosaka et al.[111] 2010), comprometendo o GC e resultando em uma degradação por hidrólise. Porém, o HEMA atua como um solvente e a sua falta pode resultar na separação de fases na interface adesiva (Van Landuyt et al.[104] 2008; Van Landuyt et al.[113] 2005; Moszner et al.[114] 2005) o que tem sido previamente observado (Van Landuyt et al.[104] 2008), podendo ser outro fator limitante para o bom desempenho do adesivo GBP.

A média de RU do GBP foi menor (AC=11,5 MPa; CON=20,5) do que as obtidas por outros grupos de pesquisa (AC=30.5 MPa; CON= 29.4 MPa) (Hanabusa et al.[29] 2012; Goracci et al.[115] 2011). Tem sido demonstrado que as propriedades mecânicas do GBP são menores do que os de outros adesivos AC de 1-passo (Hosaka et al.[111] 2010). Takahashi e cols. (Takahashi et al.[116] 2002) e Reis e cols. (Reis et al.[117] 2005), reportaram que há uma correlação positiva entre a RU à microtração em dentina e a resistência máxima à tração do adesivo, o que explicaria os baixos resultados obtidos com GBP no presente estudo. Adicionalmente, fatores relacionados à metodologia podem ter sido responsáveis por essa diferença nos valores médios de RU. Um estudo relatou valores de RU em dentina inferiores para palito da periferia quando comparados com os provenientes do centro (Loguercio et al.[118] 2005). No estudo de Hanabusa e cols. (Hanabusa et al.[29] 2012), foram utilizados nove palitos centrais de cada dente para reduzir a variabilidade do substrato, enquanto que em nosso estudo utilizamos palitos de toda a interface. Além disso, a velocidade de tração em nosso estudo foi de 0,5 mm/min, enquanto que

Hanabusa e cols. (Hanabusa et al.[29] 2012), usaram 1,0 mm/min. Um estudo comparativo relatou valores superiores de RU a microtração para Clearfil SE Bond, quando foi utilizada uma maior velocidade de tração. Um padrão mais uniforme de tempo-tensão foi observado para 1 mm/min (Poitevin et al.[119] 2008). Em outro estudo (Goracci et al.[115] 2011), embora os autores tenham utilizado a mesma velocidade de tração de nosso estudo, aplicaram GBP na dentina sob agitação, contrariamente aos nossos procedimentos (Loguercio et al.[76] 2011; do Amaral et al.[79] 2010).

Dentro das limitações do presente estudo *in vitro*, considerando os adesivos universais avaliados, apenas os que contém MDP apresentaram melhor longevidade de união à dentina, caracterizado pela manutenção da resistência de união e padrão de nanoinfiltração. Sugere-se a realização de estudos *in vitro*, com maior tempo de acompanhamento, para investigar os efeitos do uso de uma camada de resina hidrofóbica nos adesivos universais. Pode-se esperar alguma disparidade entre os resultados do presente estudo *in vitro* e aqueles obtidos clinicamente. Estudos clínicos randomizados e controlados, ou ainda, baseados na prática clínica (practice-based research), poderiam ser realizados para a comprovação dos desfechos obtidos. Além disso, estudos em outros substratos como esmalte e/ou materiais de restauração seriam necessários para investigar a versatilidade sugerida pelos fabricantes dos adesivos universais testados.

6. CONCLUSÕES

Dentro das limitações do presente estudo, foram possíveis as seguintes conclusões:

1- Os sistemas adesivos universais utilizados nas estratégias convencional e autocondicionante apresentaram um comportamento, no tempo de 24 h, inferior a seus respectivos controles (Clearfill SE Bond e Adper Single Bond 2) em pelo menos uma das propriedades avaliadas (resistência de união, nanoinfiltração e grau de conversão na camada híbrida), sendo dependentes da composição do material para ambas estratégias.

2- Após seis meses, para a estratégia adesiva AC, houve, em geral, estabilidade na resistência de união e nanoinfiltração dos adesivos com adesão química (Clearfil SE Bond, Scotchbond Universal, Allbond Universal).

3- A utilização de uma camada adicional de resina hidrofóbica melhorou o desempenho dos adesivos universais em termos de resistência de união resina-dentina quando usado com a estratégia AC. A camada adicional de resina hidrofóbica também melhorou o grau de conversão, tanto para a estratégia convencional como para a autocondicionante. A redução da nanoinfiltração foi mais dependente da composição do material que da estratégia adesiva. Apesar das melhorias *in vitro* observadas para adesivos universais, estudos de longevidade devem ser realizados para validar esses resultados iniciais.

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DE DIFERENTES PROTOCOLOS DE APLICAÇÃO DE ADESIVOS UNIVERSAIS NAS PROPRIEDADES E LONGEVIDADE DA UNIÃO À DENTINA

Pesquisador: Nara Hellen Campanha Bombarda

Área Temática:

Versão: 4

CAAE: 23656513.0.0000.0105

Instituição Proponente: Universidade Estadual de Ponta Grossa

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 631.282

Data da Relatoria: 15/04/2014

Apresentação do Projeto:

Vários fatores podem afetar a longevidade de restaurações, entretanto, a cárie adjacente as margens das restaurações ainda é um dos maiores fatores que ocasionam a troca das restaurações.(Demarco et al.2012) Spencer et al.(Spencer et al.2 2010) indicam que, esta falha deve estar relacionada a falta deselamento da interface dente/restauração, o que faz com que haja falta de resistência desta interface aosagentes internos (enzimas proteolíticas) e externos (fluidos e microorganismos, entre outros) que possam ocasionar o início da falha.(Perdigao et al.3 2013; Tjaderhane et al.4 2013) Esta interface adesiva dente/restauração se conforma pela aplicação e interação de um sistema adesivo sobre os tecido dentários tendo como mecanismo de união basicamente a substituição de minerais removidos dos tecidos dentários duros por monômeros resinosos, de tal modo que se transforma em um polímero interligado micromecanicamente com o substrato dental.(Nakabayashi et al.5 1982)Os atuais sistemas adesivos presentes no mercado podem ser classificados dentro de duas categorias segundo sua estratégia de aplicação clinica: a primeira, denominada de convencionais (CON) ou de condicionamento acido (etch-andrinse no idioma Inglês) e a segunda de autocondicionantes (AC) (self-etch no idioma Inglês.Reis et al.(Reis et al.59 2013) indicaram diferentes alternativas clínicas que podem ser usadas para diminuir a degradação da interface de união a dentina, e assim, aumentar a longevidade das restaurações. Dentre as técnicas discutidas se inclui a aplicação de uma camada adicional de resina hidrofóbica sobre o adesivo simplificado. (King et al.63 2005) Esta camada extra de resina tem como objetivo aumentar a espessura e uniformidade da interface adesiva, bem como reduzir o fluxo de fluido através dela. (king et al.63 2005, Andrade e Silva et al.64 2009) Excelente resultados in vitro e clínicos tem sido mostrados após a colocação de um revestimento de resina hidrofóbica sobre um adesivos AC de 1- passo (Reis et al.65 2008, Reis et al.66 2009), no entanto esta técnica não tem sido testado com os novos adesivos universais ou multi-mode.

Objetivo da Pesquisa:

O proposito deste estudo será avaliar o efeito nas propriedades adesivas da união a dentina de diferentes sistemas adesivos Universais aplicados segundo as estratégias adesivas convencional e autocondicionante, e modificadas com a aplicação de uma camada hidrófoba adicional.

-Objetivos específicos

1. Avaliar a resistência de união a microtração, nanoinfiltração, grau de conversão in situ de adesivos Universais aplicados em dentina de acordo as estratégias adesivas convencional e autocondicionante no tempo imediato.
2. Avaliar a resistência de união a microtração, nanoinfiltração de adesivos Universais aplicados em dentina de acordo as estratégias adesivas convencional e autocondicionante nos tempos imediato e 6 meses.
3. Avaliar a resistência de união à microtração, nanoinfiltração e grau de conversão in situ de adesivos Universais aplicados com uma camada hidrofóbica em dentina para as estratégias adesivas convencional e autocondicionante no tempo imediato.

Avaliação dos Riscos e Benefícios:

Riscos:

Não haverá riscos, pois serão utilizados dentes obtidos a partir do banco de dentes, para os pesquisadores também não haverão riscos, pois serãoutilizados todos os EPIs necessários.

Benefícios:

Melhorar as propriedades de sistemas adesivos universais com o uso de protocolos alternativos à dentina(camada hidrofobica), se rejeitada a hipótese nula desse estudo, o que será de grande beneficio para a prática clinica.

Comentários e Considerações sobre a Pesquisa:

Cento e quarenta molares humanos livres de caries serão necessários para o desenvolvimento total da pesquisa. Serão utilizados 7 tipos de materiais adesivos distribuídos nos três experimentos desenvolvidos. Trata-se, portanto, de um trabalho experimental divido em três partes:

1. Avaliação das propriedades imediatas de união de adesivos universais a dentina (40 dentes)
2. Avaliação das longevidade das propriedades de união de adesivos universais a dentina (40 dentes)
3. Influência de uma camada de resina hidrofóbica na performance adesiva de adesivos universais a dentina (60 dentes)

A metodologia é compatível aos objetivos

Considerações sobre os Termos de apresentação obrigatória:

Como se trata de estudo em dentes humanos, não há a necessidade de TCLE.
O aceite do Banco de Dentes da UEPG foi anexado
Os trabalhos in vitro da pós-graduação em Odontologia são autorizados diretamente pela Coordenção da pós-graduação (conforme justificativa anexada).
Folha de rosto preenchida adequadamente.

Recomendações:

Foi solicitado ao pesquisador alterar o grupo temático da pesquisa, tendo em vista que não há necessidade de parecer da CONEP, este projeto não é de área temática especial: Novos procedimentos terapêuticos invasivos.
Para o relator, o grupo aparece em branco.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendencias do ponto de vista ético.
Necessita confirmação do ajuste do grupo temático.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

PONTA GROSSA, 30 de Abril de 2014

Assinador por:
ULISSES COELHO
(Coordenador)