UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

MAICON SEBOLD

ESTUDOS *IN VITRO* SOBRE O DESEMPENHO DE SISTEMAS ADESIVOS UTILIZANDO DIFERENTES MÉTODOS DE CONDICIONAMENTO E COMPÓSITOS *BULK-FILL*

IN VITRO STUDIES ON THE PERFORMANCE OF ADHESIVE SYSTEMS USING DIFFERENT ETCHING METHODS AND BULK-FILL COMPOSITES

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Clínica Odontológica, na Área de Dentística.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Clinical Dentistry, in the Area of Operative Dentistry.

Orientador: Prof. Dr. Marcelo Giannini

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PROF. DR. MARCELO GIANNINI

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RESUMO

Esta tese se dividiu em três estudos, sendo o primeiro uma revisão de literatura abordando a história e os avanços tecnológicos dos adesivos odontológicos. O segundo estudo avaliou o efeito de agentes de condicionamento (ácido fosfórico 37%, adesivos autocondicionantes, solução 10:3 ─ cloreto férrico 3% em ácido cítrico 10% ─ e ácido nítrico 1,4%) na resistência de união ao esmalte por cisalhamento (24 horas) e na resistência de união à dentina por microtração (24 horas e 2 anos), cujos padrões de fratura foram classificados em microscopia eletrônica de varredura (MEV). Os adesivos Prime&Bond Universal (PBU, Dentsply-Sirona) e Gluma Bond Universal (GBU, Kulzer) foram aplicados em dentina condicionada com os agentes supracitados e seca com jato de ar ou utilizados no modo autocondicionante. A aplicação dos adesivos em dentina umedecida após condicionamento com ácido fosfórico foi empregada como controle. Foram avaliadas a atividade enzimática da camada híbrida (24 horas) e a nanoinfiltração da área de união (24 horas e 2 anos). Os testes de resistência de união empregaram 160 dentes (n=10). A nanoinfiltração (n=5) foi avaliada por MEV e a atividade enzimática por zimografia *in situ* (n=3). O terceiro artigo analisou a resistência de união à dentina, o padrão de fratura, a morfologia da área de união, a penetração do adesivo na dentina e a adaptação marginal dos adesivos Scotchbond Multipurpose (3M Oral Care), Clearfil SE Bond (Kuraray-Noritake), OptiBond All-in-One (Kavo-Kerr) e Futurabond U (Voco), em função da aplicação de compósitos *bulk-fill* (Admira Fusion x-tra, Voco; SonicFill 2, Kavo-Kerr). Cavidades classe I foram confeccionadas em terceiros molares e restauradas com os adesivos e compósitos propostos. Foram utilizados 64 dentes para a resistência de união à dentina (n=8). Padrão de fratura e adaptação marginal (n=3) foram avaliados por MEV. Morfologia da área de união e penetração dos adesivos (n=3) foram avaliadas por microscopia confocal. O primeiro estudo demonstrou que as pesquisas têm focado na modificação de formulações adesivas para simplificar sua aplicação, oferecendo ação antibacteriana, inibição enzimática e/ou remineralização. O segundo artigo mostrou não existir diferença entre os agentes de condicionamento quanto à resistência de união ao esmalte. Em 24 horas, não houve diferença na resistência de união da dentina úmida condicionada com ácido fosfórico comparada à solução 10:3 ou ao ácido nítrico, observando-se falhas adesivas e mistas. Nenhuma das abordagens de condicionamento inibiu a atividade enzimática da dentina e todos os grupos apresentaram queda na resistência de união após armazenamento. O adesivo PBU apresentou menor nanoinfiltração que o GBU. No terceiro artigo, não houve diferença na resistência de união entre os adesivos para a SonicFill 2; a resistência de união da Admira Fusion x-tra foi menor com o Clearfil SE Bond em comparação ao Scotchbond Multipurpose e os compósitos não diferiram entre si. Camada híbrida espessa foi obtida com Scotchbond Multipurpose. O adesivo Futurabond U apresentou altas taxas de desadaptação. Em geral, concluiu-se que os aspectos determinantes da qualidade e longevidade da adesão dentinária foram influenciados pelo tipo de agente de condicionamento e adesivo, mas não pelo tipo de compósito *bulk-fill*.

Palavras-chave: Dentina. Adesivos dentinários. Condicionamento ácido do dente. Cloreto férrico. Ácido nítrico. Inibidores de metaloproteinases de matriz. Resinas compostas. Ultrassom. Cerâmicas modificadas organicamente.

ABSTRACT

This thesis was divided into three studies. The first study was a narrative review about the history and the technological advancements of dental adhesives. The second study addressed the effect of etchants (37% phosphoric acid, adhesives in self-etch mode, 10-3 solution ─ 3% ferric chloride dissolved in 10% citric acid ─ and 1.4% nitric acid) on enamel shear bond strength (24 hours), and dentin microtensile bond strength (24 hours and 2 years), from which samples were classified regarding failure mode by scanning electron microscopy (SEM). The Prime&Bond Universal (PBU, Dentsply-Sirona) and Gluma Bond Universal (GBU, Kulzer) adhesives were applied on air-dried dentin previously etched with the abovementioned agents or used in self-etch mode. Application of the adhesives on phosphoric acid-etched, blot-dried dentin was used as control. Enzymatic activity within the hybrid layer (24 hours), and nanoleakage of the bonding interface (24 hours and 2 years) were evaluated. Bond strength tests used 160 teeth (n=10). Nanoleakage (n=5) was evaluated by SEM, whereas enzymatic activity was analyzed by *in situ* zymography (n=3). The third study investigated dentin bond strength, failure mode, morphology of the bonding interface, adhesive diffusion into dentin, and marginal adaptation of the adhesives Scotchbond Multipurpose (3M Oral Care), Clearfil SE Bond (Kuraray-Noritake), OptiBond All-in-One (Kavo-Kerr), and Futurabond U (Voco) coupled with bulk-fill composites (Admira Fusion x-tra, Voco; Sonic Fill 2, Kavo-Kerr). Class-I cavities were prepared in third molars and restored with the proposed adhesives and composites. Sixty-four teeth were used for the dentin bond strength test (n=8). Failure mode and marginal adaptation (n=3) were evaluated by SEM. Bonding interface morphology and adhesive diffusion (n=3) were analyzed by confocal microscopy. The first study demonstrated research has focused on the modification of adhesive composition to simplify their application, while offering antibacterial effect, enzymatic activity inhibition, and/or remineralization. The second article showed there was no difference among etchants concerning enamel bond strength. At 24 hours, there was no difference in dentin bond strength comparing the phosphoric acid-etched, blot-dried group with 10-3 solution or nitric acid, and mostly adhesive and mixed failures were observed. None of the etching approaches could inhibit dentin enzymatic activity, and all groups presented bond strength reduction after storage. PBU showed less nanoleakage than GBU. In the third article, there was no difference in bond strength among adhesives for SonicFill 2; the bond strength of Admira Fusion x-tra was lower when Clearfil SE Bond was used in comparison with Scotchbond Multipurpose; and the tested composites did not differ between each other. A thick hybrid layer was achieved by Scotchbond Multipurpose. The Futurabond U adhesive led to higher rates of marginal gaps. Overall, the present thesis concludes the determining aspects linked to dentin bonding quality and longevity were influenced by the type of etchant and adhesive system, but not by the type of bulk-fill composite.

Keywords: Dentin. Dentin-bonding agents. Acid etching, dental. Ferric chloride. Nitric acid. Matrix metalloproteinase inhibitors. Composite resins. Ultrasonics. Organically modified ceramics.

RIASSUNTO

Questa tesi è suddivisa in tre studi. Inizialmente, è stata effettuata una revisione della letteratura sulla storia e i progressi degli adesivi dentali. Il secondo studio ha valutato l’effetto di mordenzanti (acido fosforico 37%, adesivi automordenzanti, soluzione 10:3 ─ cloruro ferrico 3% in acido citrico 10% ─ e acido nitrico 1,4%) sulla forza di adesione al taglio dello smalto (24 ore) e microtensile della dentina (24 ore e 2 anni), di cui le modalità di fallimento sono state classificate tramite microscopia elettronica a scansione (MES). Gli adesivi Prime&Bond Universal (PBU, Dentsply-Sirona) e Gluma Bond Universal (GBU, Kulzer) sono stati applicati su dentina mordenzata con i diversi mordenzanti e asciugata con getto d'aria, oppure utilizzati nella modalità self-etch. L’applicazione degli adesivi su dentina umida dopo mordenzatura con acido fosforico è stata impiegata come controllo. Sono state valutate la nanoinfiltrazione dell’interfaccia adesiva (24 ore e 2 anni) e l’atività enzimatica dello strato ibrido. Per verificare la forza di adesione sono stati utilizzati 160 denti (n=10). La nanoinfiltrazione (n=5) è stata valutata da MES e l’attività enzimatica da zimografia in situ (n=3). Il terzo studio ha analizzato la forza di adesione alla dentina, la modalità di fallimento, la morfologia dell’interfaccia adesiva, la diffusione nella dentina e l’adattamento marginale degli adesivi Scotchbond Multipurpose (3M Oral Care), Clearfil SE Bond (Kuraray-Noritake), OptiBond All-in-One (Kavo-Kerr) e Futurabond U (Voco), in base all’uso di compositi bulk-fill (Admira Fusion x-tra, Voco; SonicFill 2, Kavo-Kerr). Cavità di I classe sono state preparate su terzi molari e riempite utilizzando gli adesivi e compositi proposti. Sono stati utilizzati 64 denti per il test di forza di adesione (n=8). Modalità di fallimento e adattamento marginale (n=3) sono stati valutati. La morfologia dell’interfaccia adesiva e la diffusione degli adesivi (n=3) sono state valutate tramite microscopia confocale. Il primo studio ha dimostrato che la ricerca si è concentrata sulla semplificazione degli adesivi, includendo delle potenziali azioni antibatteriche, inibizione enzimatica e/o remineralizzazione. Il secondo articolo ha mostrato che non c’è differenza tra i mordenzanti quanto la forza di adesione allo smalto. In 24 ore, non c’è stata differenza sulla forza di adesione alla dentina umida mordenzata con acido fosforico in confronto alla soluzione 10:3 oppure all’acido nitrico, osservando fallimenti adesivi e misti. Nessun mordenzante è stato in grado di inibire l’attività enzimatica della dentina, e tutti i gruppi hanno presentato forza di adesione ridotta dopo invecchiamento. L’adesivo PBU ha presentato nanoinfiltrazione inferiore dello GBU. Nel terzo articolo, non c'è stata differenza nella forza di adesione tra gli adesivi per SonicFill 2; la forza di adesione di Admira Fusion x-tra è stata più bassa quando Clearfil SE Bond è stato utilizzato a confronto con Scotchbond Multipurpose. I compositi non hanno avuto differenza tra di loro. Uno strato ibrido spesso è stato ottenuto con Scotchbond Multipurpose. L'adesivo Futurabond U ha presentato elevato disadattamento marginale. In generale, si è concluso che gli aspetti determinanti della qualità e longevità della adesione sulla dentina sono stati influenzati dal tipo di mordenzante e adesivo, ma non dal tipo di composito bulk-fill.

Parole chiave: Dentina. Adesivi dentinali. Attacco acido dello smalto. Cloruro ferrico. Acido nitrico. Inibitori delle metalloproteinasi della matrice. Compositi. Ultrasuono. Ceramiche modificate organicamente.

SUMÁRIO

1 INTRODUÇÃO

O uso de compósitos resinosos como materiais restauradores diretos, aliado aos sistemas adesivos tem aumentado desde a década de sessenta, devido às suas propriedades mecânicas favoráveis, aos resultados estéticos satisfatórios e ao concomitante reforço da estrutura dental remanescente (Demarco et al., 2012; Dietschi et al., 2019). Neste contexto, os adesivos odontológicos e as técnicas de aplicação destes tem se tornado um ponto de interesse bastante relevante na pesquisa científica. O mecanismo de união à dentina compreende a penetração dos monômeros adesivos por entre as fibrilas colágenas expostas em decorrência do condicionamento com ácido fosfórico ou da aplicação de adesivos autocondicionantes (Martins et al., 2008; Pashley et al., 2011; Giannini et al., 2015; Breschi et al., 2018).

Diversos fatores relacionados à técnica de adesão podem afetar a união dos compósitos com a estrutura dental, dentre eles: o agente de condicionamento dentinário empregado (Kong et al., 2015; Rodrigues et al., 2017; Sebold et al., 2017; Sebold et al., 2019), o grau de umidade residual da dentina condicionada (Reis et al., 2012; da Silva et al., 2013; Sugimura et al., 2019), o método de aplicação (Reis et al., 2007; Zander-Grande et al., 2011) e a composição do sistema adesivo utilizado (Van Landuyt et al., 2007; Sebold et al., 2020). Já em relação aos compósitos, a união criada pelo sistema adesivo deve ser capaz de suportar as tensões induzidas pela contração de polimerização do material resinoso, ajudando a reduzir potenciais consequências, como sensibilidade pós-operatória, manchamento e desadaptação marginais e infiltração bacteriana ao longo do tempo (Fronza et al., 2015).

Os sistemas adesivos podem ser classificados segundo a forma como interagem com o substrato dental e o número de passos clínicos necessários para a sua aplicação (Van Meerbeek et al., 1998). Há basicamente dois tipos de adesivos: os convencionais ou *etch-and-rinse*, que geralmente apresentam três ou dois passos de aplicação, e os autocondicionantes, comumente aplicados em dois passos ou em passo único (Sebold et al., 2020). Os agentes de união convencionais requerem um passo de condicionamento com ácido fosfórico em concentração de 30 a 40% antes da sua aplicação para remover a lama dentinária (*smear layer*) e desmineralizar superficialmente a dentina (Milia et al., 2012). Os adesivos autocondicionantes, por outro lado, não precisam de condicionamento prévio da dentina com ácido fosfórico, visto que eles contêm monômeros ácidos capazes de desmineralizar e infiltrar a dentina simultaneamente, além de reagir quimicamente com o substrato, ligando-se ao cálcio da hidroxiapatita (Van Meerbeek et al., 2011). Há aproximadamente uma década, adesivos chamados “universais” foram introduzidos no mercado odontológico, sendo desenvolvidos para aplicação tanto pela técnica de condicionamento ácido prévio, quanto no modo autocondicionante, de acordo com a preferência do profissional (Ruschel et al., 2018).

No caso de sistemas adesivos convencionais, a superfície dentinária deve ser mantida levemente umedecida após o condicionamento com ácido fosfórico para a aplicação do adesivo, de modo a formar camada híbrida adequada (Kanca, 1992). Isso se deve ao fato de que a secagem excessiva da dentina condicionada leva à rápida formação de pontes de hidrogênio entre as fibrilas colágenas expostas, ocasionando o colapso da rede de colágeno, o que prejudica a difusão dos monômeros adesivos e a formação da camada híbrida (Manso et al., 2008). Todavia, a manutenção de uma superfície úmida prejudica a reação de polimerização do adesivo, comprometendo a longevidade deste polímero e a integridade das fibrilas colágenas, que serão deixadas expostas (Brackett et al., 2011; Lopes et al., 2016). A presença de água na área de união durante a polimerização do adesivo resulta em um polímero com propriedades mecânicas comprometidas (Reis et al., 2012) e maior susceptibilidade à degradação precoce das cadeias poliméricas por hidrólise (Boushell, 2013). O processo de hidrólise é ainda intensificado pela sorção de água promovida por monômeros hidrofílicos presentes na formulação de adesivos simplificados (Kong et al., 2015).

Outro problema reportado quando utilizada a técnica de adesão à dentina úmida é a inerente limitação de difusão dos monômeros adesivos nas camadas mais profundas da dentina condicionada, onde fibrilas de colágeno são, consequentemente, deixadas expostas e desprotegidas (Pioch et al., 2001; Tay et al., 2002; Breschi et al., 2008). Essas fibrilas colágenas insuficientemente envolvidas pela resina adesiva e desprovidas de minerais se tornam suscetíveis à degradação por enzimas colagenolíticas, gelatinolíticas, dentre outras; tanto aquelas liberadas por bactérias, quanto as proteases endógenas dentinárias, como as metaloproteinases da matriz (MMPs) ou as cisteíno catepsinas (CCs) (Tjaderhane et al., 2013; Breschi et al., 2018).

O uso de ácido fosfórico para condicionar a dentina provoca a exposição e a ativação de MMPs latentes (De Munck et al., 2009; Breschi et al., 2010), além de danificar as fibrilas colágenas e modificar seu arranjo molecular, tornando mais fácil a interação das cadeias polipeptídicas destas com o sítio catalítico das MMPs (Bertassoni et al., 2012). Consequentemente, as fibrilas colágenas da camada híbrida são submetidas à degradação hidrolítica, o que pode ocasionar redução da resistência de união à dentina ao longo do tempo (Stape et al., 2012; Maravic et al., 2018). Portanto, a associação entre condicionamento com ácido fosfórico e técnica de adesão à dentina úmida cria condições desfavoráveis para a durabilidade da união dente-restauração (Pashley et al., 2011; Abedin et al., 2015). Por isso, ácidos mais brandos, como os ácidos cítrico, maléico, oxálico e etilenodiamino tetracético (EDTA) foram propostos como potenciais substitutos para o ácido fosfórico 35-40% tradicionalmente utilizado (Camps et al., 1995; Vaidyanathan e Vaidyanathan, 2009; Sebold et al., 2017; Sebold et al., 2019).

Dentro deste contexto, uma mistura de cloreto férrico 3% dissolvido em ácido cítrico 10% (solução 10:3), assim como diluições do ácido nítrico foram sugeridas para o condicionamento da dentina (Nakabayashi et al., 1982; Asmussen e Bowen, 1987; Schumacher et al., 1997). Relatos da literatura demonstram resistência de união adequada e clara formação de camada híbrida pelo tratamento dentinário com solução 10:3 (Takagaki et al., 2009; Nurrohman et al., 2012). Os íons ferro presentes nesta solução também se mostraram capazes de evitar o colapso da rede de colágeno na ausência de água, o que se deve provavelmente à formação de agregados insolúveis destes íons com os polieletrólitos da dentina (Piemjai et al., 2003). Isso possibilita a difusão dos monômeros adesivos e a hibridização mesmo quando a dentina condicionada é seca com jato de ar (Sebold et al., 2019). Adicionalmente, soluções contendo ferro podem inibir a atividade das MMPs -2 e -9 (Kato et al., 2010) e a ação da catepsina-K (Rodrigues et al., 2017). O ácido nítrico, por sua vez, interage com a dentina de maneira similar ao ácido fosfórico (Yoshioka et al., 2002), removendo a *smear layer* após aplicação por 10 segundos, o que permite a profunda penetração do adesivo e a formação de *tags* de resina (Blosser, 1990; Marshall et al., 1993). Em estudo mais recente, o uso de ácido nítrico 1,4% como agente de condicionamento para adesão à dentina seca com jato de ar promoveu resistência de união equiparável à técnica tradicional de adesão com ácido fosfórico quando um adesivo simplificado à base de acetona foi aplicado. Além disso, a resistência de união também se mostrou estável após um ano de envelhecimento das amostras (Sebold et al., 2019).

Atualmente, além das variáveis envolvidas no condicionamento e na técnica de adesão dentinários, também tem se tornado essencial selecionar adequadamente o compósito restaurador a ser empregado. Esta preocupação advém da popularização dos chamados compósitos *bulk-fill*, que possuem menor contração volumétrica produzida pela reação de polimerização. Estes compósitos foram obtidos em decorrência de modificações na matriz monomérica ou no sistema fotoiniciador dos compósitos convencionais, além de mudanças na quantidade de monômeros, formato, tamanho e/ou tratamento superficial das partículas de carga (Ilie e Hickel, 2011). Consequentemente, o cirurgião-dentista consegue confeccionar restaurações diretas por meio da inserção e fotoativação de incrementos de resina com 4 a 5 mm de espessura, devido à alta reatividade do compósito (Leprince et al., 2014). Além disso, os compósitos *bulk-fill* podem reduzir a incorporação de defeitos no corpo das restaurações, possibilitar maior rapidez no preenchimento cavitário com o material restaurador (Park et al., 2008; Campos et al., 2014) e diminuir os riscos associados às tensões de contração na área de união dentina-adesivo, como sensibilidade pós-operatória e falhas na união que levam à formação de fendas ou *gaps* (Gerula-Szymanska et al., 2020).

O compósito Admira Fusion x-tra (Voco GmbH, Cuxhaven, NI, Alemanha), pertencente à classe dos compósitos *bulk-fill*, se baseia na tecnologia de cerâmicas organicamente modificadas (ormocers), as quais são polímeros híbridos caracterizados por uma rede de siloxano seletivamente modificada pela incorporação de grupos orgânicos (polisiloxanos com metacrilatos fotopolimerizáveis ligados covalentemente à sílica) (Manhart et al., 2000). Teoricamente, essa formulação peculiar aumentaria o tamanho das moléculas de monômero e reduziria a contração de polimerização, o desgaste e a lixiviação do material (Bottenberg et al., 2007).

Outro representante dos compósitos *bulk-fill* é o material restaurador SonicFill 2 (Kavo Kerr, Orange, CA, EUA), a segunda geração de uma resina *bulk-fill* de alta viscosidade que utiliza dispositivo ultrassônico para aplicação. A energia fornecida ao material pela ativação sônica produz uma redução significativa na sua viscosidade, o que melhoraria sua adaptação às paredes e margens cavitárias (Gamarra et al., 2018). Quando a energia sônica é removida, o material retorna gradualmente para sua viscosidade inicial, o que garantiria propriedades mecânicas adequadas para a restauração (Monterubbianesi et al., 2016). Adicionalmente, a aplicação ultrassônica também reduziria a formação de porosidades no corpo do material (Hirata et al., 2018).

Considerando as limitações relacionadas à atual técnica de adesão à dentina úmida utilizando ácido fosfórico, assim como o surgimento e a rápida evolução dos compósitos resinosos, é extremamente importante o estudo da influência do método de tratamento dos tecidos dentais e dos materiais restauradores na adesão dentinária. Dessa forma, novas abordagens para a união das restaurações adesivas à estrutura dental, que simplifiquem os procedimentos clínicos e melhorem a longevidade das restaurações em resina composta, podem ser propostas. Concomitantemente, a análise dos efeitos de diferentes compósitos *bulk-fill* frente às técnicas de adesão atualmente disponíveis pode facilitar o processo de seleção e uso desses materiais. Portanto, a presente tese de doutorado se dividiu em três artigos distintos, cada um com objetivos específicos que visam um melhor entendimento sobre a influência de dois fatores-chave que determinam o sucesso da adesão dentinária: o condicionamento do substrato e o tipo de compósito utilizado.

O primeiro artigo abordou, de maneira cronológica, a história e os atuais avanços tecnológicos dos sistemas adesivos dentinários por meio de uma revisão da literatura. O segundo artigo teve como objetivo avaliar a viabilidade da solução 10:3 e do ácido nítrico 1,4%, usados como agentes de condicionamento, na resistência de união ao esmalte em 24 horas e nas características da área de união dentina-adesivo (resistência de união em 24 horas e 2 anos, padrão de fratura em 24 horas e 2 anos, atividade enzimática na camada híbrida em 24 horas e nanoinfiltração em 24 horas e 2 anos) obtida por dois adesivos “universais” contendo solventes distintos (álcool ou acetona). Por fim, o terceiro artigo buscou analisar a influência de diferentes sistemas adesivos (convencional de 3 passos, convencional de 2 passos ou autocondicionante/universal de passo único) nas áreas de união criadas em cavidades classe I preenchidas por compósitos *bulk-fill* com diferentes composições e modos de aplicação (Admira Fusion x-tra e SonicFill 2).

2 ARTIGOS

2.1 Chronological history and current advancements of dental adhesive systems development: a narrative review

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ABSTRACT

This literature review describes the history, and discusses important aspects regarding bonding mechanisms, clinical performance, and current status of different types of dental adhesives. The development of dental bonding has remarkably changed concepts and procedures related to Restorative Dentistry. Buonocore’s report on enamel acid-etching, followed by the description of the hybrid layer by Nakabayashi and his co-workers, and the use of the total-etch protocol were some of the scientific breakthroughs responsible for changing paradigms in dental practice. Also, the synthesis of functional monomers that can chemically bond to dental hard tissues has helped increase clinicians trust on Adhesive Dentistry. According to the mechanism of action and mode of use, past and present (available in the market) dental adhesives can be divided in generations. Currently, user-friendly, simplified products and restorative techniques have become popular among clinicians, who require products that do not just bond to the tooth, but also present antibacterial, remineralizing and/or enzymatic-inhibitory effects that can increase the longevity of adhesive procedures. Therefore, due to the great range of available bonding agents,

clinicians must understand how these products work, and how their composition and mode of use influence the clinical outcomes of adhesive procedures, in order to decide what is the best adhesive system for each clinical application.

KEYWORDS: Dental adhesives; hybrid layer; total-etch; adhesion by chemical bonding; antibacterial adhesives

1. The beginning of dental bonding and acid-etching

In the late 1940s, Oskar Hagger, a Swiss chemist who worked for DeTrey/Amalgamated Dental Company, developed the first dental adhesive product, which was called Sevriton Cavity Seal [1]. This bonding agent contained glycerolphosphoric acid dimethacrylate, and its action was based on the revolutionary approach of chemical bonding to dental structures [2]. A study published by Kramer and McLean in 1952 suggested the glycerolphosphoric acid dimethacrylate improved dentin adhesion through surface penetration [3]. However, this pioneering product presented poor clinical results due to the high interfacial stress and thermal expansion caused by the methacrylate-based composites available at the time [4].

In 1955, Buonocore published a paper in which he reported an improvement in the bond between acrylic resin and enamel after surface treatment with 85% phosphoric acid [5]. The idea of enamel acid-etching derived from Buonocore’s observations that phosphoric acid, and preparations containing it, were used industrially for treating metallic surfaces, with the aim of achieving better adhesion of ink or resin coats to the surfaces [6]. The improved bond between enamel and acrylic resin was attributed to the micromechanical retention caused by an increase in surface roughness (Figure 1), and a potential chemical alteration of the enamel surface due to acid-etching, making it more reactive to the resin [7].

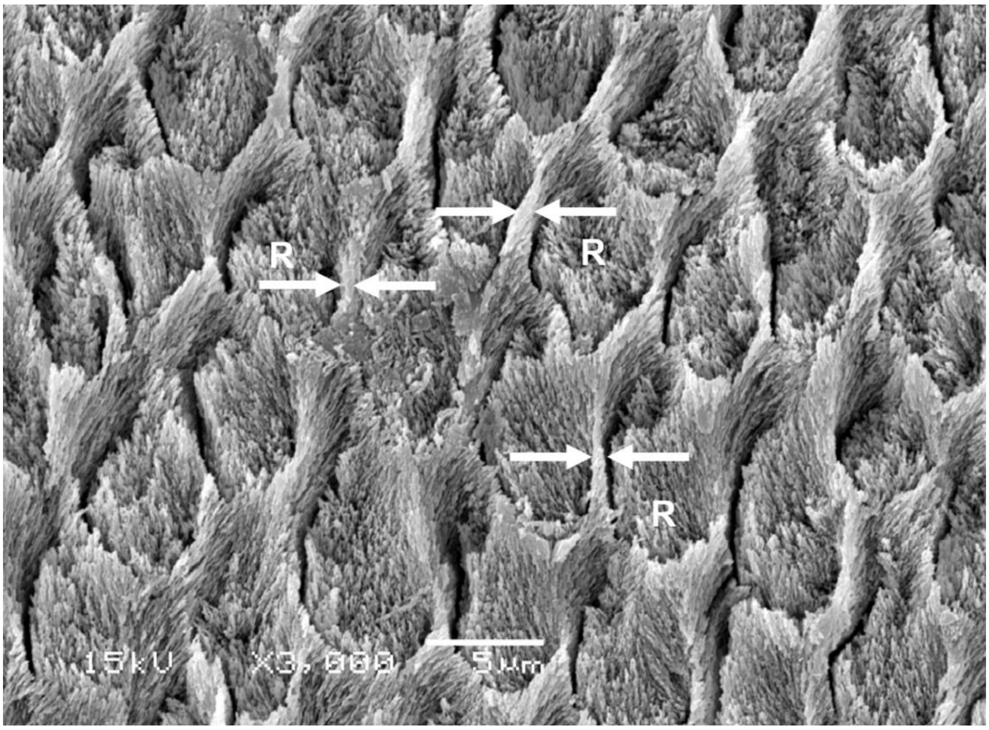


Figure 1. Scanning electron microscopy image of the enamel surface etched with 37% phosphoric acid. R: enamel rods; arrows indicate the interrod area.

Enamel acid-etching would become widely accepted only in the mid-1970s, after researchers and practitioners with experience on enamel bonding showed their findings at the International Symposium on Enamel Etching (1974) [2]. The information from this event was published and distributed to academic institutions, helping spread the concept of enamel bonding [8].

2. Early attempts at dentin bonding

Considering the product developed by Hagger in the 1940s, Brudevold et al. developed, in 1956, a primary dentin adhesive composed by a methyl methacrylate-based resin containing phosphoric acid dimethacrylate [9]. This adhesive was believed to promote bonding by means of an interaction between its bifunctional molecules and the calcium ions from hydroxyapatite (HAp) [10]. Nonetheless, the highest bond strength values obtained by this bonding agent were approximately 2.6 MPa when applied on untreated dentin, and 5.4 MPa when 7% hydrochloric acid was used previous to its application. Also, bond strengths were drastically reduced under water immersion [9].

Still in the 1950s, Bowen synthesized the Bis-GMA (bisphenol glycidyl methacrylate) monomer, which would later become the basis of current composites

[11]. Because of this discovery, Bowen started putting effort into the study of dentin adhesives, with the aim of developing a resin system capable of bonding to dentin [12–16]. However, effective dentin bonding would be much more challenging to achieve than enamel bonding [17], as dentin presents some inherent features and issues: (1) mineralized dentin has few channels that allow monomer diffusion, except for the dentinal tubules; (2) oxygen and water in the dentinal fluid cannot be completely removed, which interferes with the polymerization of adhesive resins; (3) due to potential pulp damage, polymerization reactions must occur at body temperature, which hinders the physical properties of polymers and copolymers; (4) tooth preparation leads to smear layer formation, causing a decrease in dentin permeability [18].

In 1965, Bowen developed a surface-active comonomer from the reaction between N-phenylglycine (NPG) and glycidyl methacrylate (GMA) [19]. This compound, named NPG-GMA, was introduced as a potential dentin bonding agent [13]. NPG-GMA was believed to react with dental hard tissues, leading to the formation of a new compound, which could bond to enamel and dentin HAp crystals at one end, through ionic and chelation bonds, and copolymerize with the restorative material at the other end [20,21]. However, this theory was never really proven, since the required technology to evaluate and show the chemical bonding between NPG-GMA and HAp was not available at the time.

3. First and second-generation adhesive systems

The introduction of the supposed chemical bonding mechanism of NPG-GMA originated the first generation of dental adhesives. The main commercial product of this generation was the Cervident system (SS White Burs Inc., Lakewood, NJ, USA), a resin composite which used an NPG-GMA-based primer before its application [22]. These materials showed low bond strengths (1.5 to 4.5 MPa) in laboratory studies [13]. and poor clinical performance (Figure 2) [23].

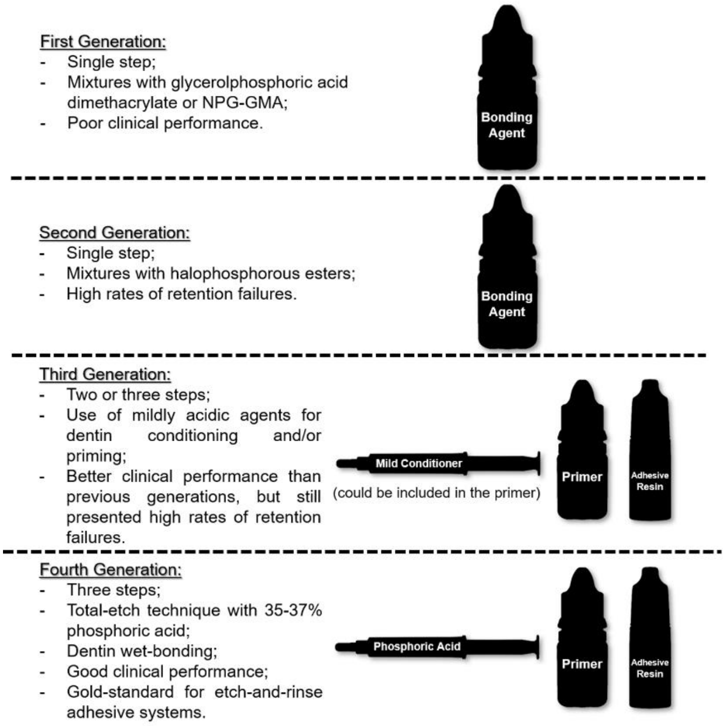


Figure 2. Schematic representation of adhesive systems from the first to the fourth generation.

Consequently, in the late 1970s, new materials were developed to improve the performance of first-generation adhesives. Most second-generation products comprised a hydroxyethyl methacrylate (HEMA) or Bis-GMA-based resin composite mixed with halophosphorous esters, without filler particles [10]. These adhesive systems were believed to form ionic bonds between its halophosphorous groups and the calcium from Hap, and to copolymerize its carbon-carbon double bonds with the composite matrix [24]. Second-generation products, similarly to the first generation, resulted in low bond strength values *in vitro* [25,26], and very high rates of retention failures in clinical trials (Figure 2) [27,28].

Simultaneously to the development of the first generations of dental adhesives, researchers started showing interest regarding the effects of rotary cutting/abrasive instruments on the morphological features of dental substrates [29]. In 1961, the evolution of electronic microscopy enabled detailed observation of the surfaces of cut enamel and dentin replicas: while the prismatic structure of enamel could be easily identified, dentin was irregular and showed no evidence of its tubular nature [30]. Later, Boyde et al. described the presence of an organic layer containing apatite particles on the surface of abraded enamel, which they called smear layer [31]. This layer was also identified on the dentin surface [32].

The smear layer can be defined as a thin, amorphous layer composed by degraded collagen, bacteria, and inorganic debris from enamel and dentin [33] that is left on dental surfaces due to the use of cutting or abrasion rotary instruments [34]. Since first and second-generation adhesives were applied over the smear layer, they could not diffuse through dentin adequately, resulting in low bond strengths [35]. These bonding agents did not interact directly with the dentin substrate, but with a layer of debris that presented low cohesive and dentin bond strengths, which limited their performance [36]. Researchers would soon realize the smear layer had to be removed or modified/surpassed somehow to allow direct interaction of adhesive primers and composites with dentin [37].

4. Dentin acid-etching and the concept of the hybrid layer

In 1979, Fusayama et al. demonstrated that dentin treatment with 40% phosphoric acid for 60 s could increase the tooth-restoration bond strength [38]. The results from this study were met with great skepticism by the scientific community, as the use of acidic agents on dentin was believed to cause pulpal inflammation, or even necrosis [39–41]. Nevertheless, during this time, Clearfil Bond System-F (Kuraray Noritake Dental Inc.), the first etch-and-rinse system, was developed (1978). It contained phenyl-P (2-methacryloxyethyl phenyl hidrogenphosphate) as the adhesive resin monomer, and it was applied after enamel and dentin phosphoric acid-etching [38]. Anyhow, previous investigations had already suggested toxic effects from dentin acidic pre-treatment on the dental pulp were very unlikely [42,43], and in 1986 Chan and Jensen reported low penetration of hydrogen ions through 0.4 mm-thick dentin specimens after short exposure (up to 1 min) to 37% phosphoric acid [44]. Later studies concluded the pulpal reactions previously reported were probably caused by the infiltration of cytotoxic bacterial byproducts instead of dentin acid-etching itself [36], since the adhesive systems available at the time had not been designed to be used on etched dentin, resulting in poor-quality tooth-restoration bonds [45,46].

In 1982, Nakabayashi et al. presented an important discovery that helped consolidate the idea of dentin acid-etching. A 4-META (4-methacryloxyethyl trimellitate anhydride)-based adhesive was applied on dentin treated with a solution containing citric acid and ferric chloride, and the adhesive interface was evaluated by scanning electron microscopy. Adhesive resin monomers diffused into intra and intertubular dentin and formed an acid-resistant layer beneath the bonding interface [47].

Acid-etching can remove the mineral content from the superficial layers of dentin and expose the matrix collagen fibrils [48]. Adhesive monomers penetrate between collagen fibrils and create a resin-impregnated dentin zone after polymerization, which is called the hybrid layer (Figure 3) [49], a term that had been coined by Nakabayashi in his work of the early 1980s [47]. The mechanical interlocking created by the hybrid layer is responsible for bonding restorations to dental hard tissues, and for dentin sealing, which prevents hypersensitivity [50]. Following such discoveries, in 1984, phenyl-P was replaced by the MDP monomer (phenyl phosphate derivative of methyl methacrylate called methacryloyloxydecyl dihydrogen phosphate), and therefore the Japanese company Kuraray created the Clearfil New Bond, an etch-and-rinse, self-curing adhesive system. However, dentin bond strength was still low (although higher than Clearfil Bond System-F), which was explained by the low calcium remaining in dentin after phosphoric acid-etching [51,52]. Later, in 1987, with the introduction of catalysts for visible light-curing, Clearfil Photo Bond, another etch-and-rinse MDP-containing dental adhesive, was developed, resulting in a slight dentin bond strength improvement [52].

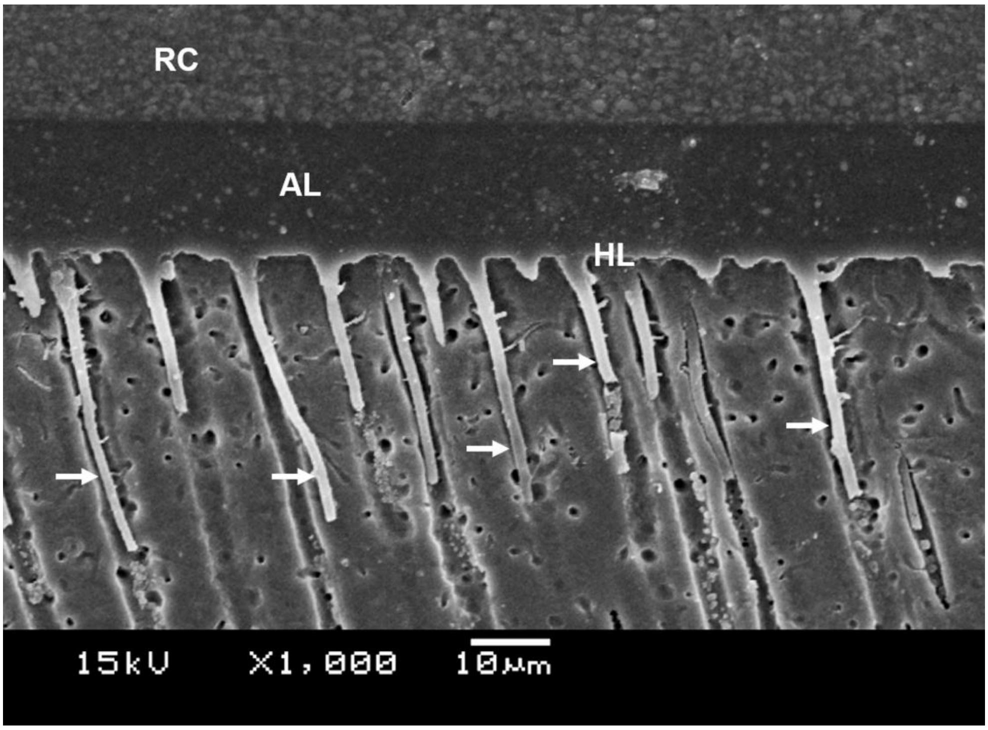


Figure 3. Scanning electron microscopy image of the dentin-adhesive bonding interface obtained by a fifth-generation adhesive system applied on acid-etched dentin. RC: resin composite; AL: adhesive layer; HL: hybrid layer; arrows indicate resin tags.

5. Third-generation adhesive systems

Despite the findings of Fusayama et al. (1979) and Nakabayashi et al. (1982) to argue in favor of dentin etching, dental professionals were still hesitant to treat dentin with phosphoric acid [53]. Therefore, third-generation dentin adhesives were introduced in the market in the mid-1980s. The bonding mechanism of these systems was based on the modification or partial/total removal of the smear layer, which led to improved surface wettability and adhesive resin diffusion [54]. During this period, several mild acidic solutions were used to remove or modify the layer of debris left by cavity preparation, namely solutions containing HEMA and organic acids (Scotchbond 2, 3M ESPE, St. Paul, MN, USA; All Bond, Bisco Inc., Schaumburg, IL, USA), aluminum oxalate mixed with nitric acid (Tenure, Den-Mat Holdings, LLC, Lompoc, CA, USA), 10% citric acid containing 20% calcium chloride (Clearfil Liner Bond System, Kuraray Noritake Dental Inc., Tokyo, Japan), ethylenediaminetetraacetic acid (Gluma, Bayer AG, Dormagen, DE-NW, Germany), and primers composed by a mix of dipentaerythritol pentaacrylate (PENTA), HEMA, and ethanol (Prisma Universal Bond 3, Dentsply Sirona, Milford, DE, USA) [55–58].

Third-generation adhesives considerably increased the complexity of restorative procedures [59]. Each one of these adhesive systems had a dentin primer composed by hydrophilic monomers, which improved dentin surface wettability and promoted bonding between the hydrophobic adhesive resin and the substrate [60]. Thus, the clinical procedure was divided into two or three steps: (1) acid-etching, (2) primer application (depending on the adhesive system, this step could be combined in a single bottle with the conditioner), and (3) application of an adhesive resin without filler particles [4].

The third generation of dentin adhesives represented a technological advancement compared to the first ones [61]. Although the *in vitro* performance of these products was still unpredictable, showing great variation regarding bond strength values [60,62–67], and poor results in microleakage studies [54,62,68]. Clinical trials reported retention rates over 90% at 6 months, and over 80% after 2 years (Figure 2) [69,70]. These findings might be related to poor adhesive resin infiltration through the smear layer, suggesting that bond strength results were actually based on the cohesive strength of smear layer particles [10,71].

However, as a consequence of the development of third-generation bonding systems, a self-curing adhesive resin (4-META/MMA-TTB resin) consisting of 4-META (4-methacryloyloxyethyl trimellitate anhydride), MMA (methyl methacrylate), and PMMA (poly methyl methacrylate) has been widely used and clinically successful since 1982. At that time, the recommendation before placing a 4-META/MMA-TTB resin was to pre-treat dentin with 10-3 solution, which contained 3% ferric chloride dissolved in 10% citric acid [72].

6. Dentin wet-bonding and the fourth generation of adhesive systems

The next generation of bonding agents, introduced in the early 1990s, focused on the incorporation of the acid-etching or total-etch technique [73]. First described by Fusayama et al. in vitro in 1979 [38], and later applied in a clinical protocol by Kanca (1989) [74] and Fusayama (1992) [75], this technique recommends phosphoric acid application on enamel and dentin simultaneously for 20 s, with the aim of completely removing the smear layer before bonding agent application [74]. Complete removal of the smear layer allows direct interaction between the adhesive system and the superficially demineralized dentin substrate [76]. Adhesive resin diffuses into the etched intertubular dentin and replaces the apatite crystals that were demineralized and removed by phosphoric acid, producing a resin-impregnated dentin layer [75].

In 1992, Kanca and Gwinnett reported, in two different publications, that dentin bond strength could be improved if the dentin surface was kept visibly moist during adhesive procedures [77,78]. Air-drying of acid-etched dentin causes the collapse of the exposed collagen network, and, consequently, loss of spaces between collagen fibrils [48]. Absence of the interfibrillar spaces hinders the diffusion of adhesive monomers between collagen fibrils, resulting in poor hybridization and low bond strength [79]. Conversely, if etched dentin is left slightly moist during adhesive procedures, the interfibrillar nanospaces will be preserved, which will improve the diffusion of primer and adhesive monomers into the substrate, and will favor the hybridization process [80,81].

Fourth-generation adhesive systems consolidated the three-step approach adopted in the previous generation: (1) acid-etching; (2) primer application; and (3) adhesive resin application [82]. The primer, a mixture of hydrophilic monomers with an organic solvent, functions by displacing water from etched dentin, leading to a reduction of dentin moisture, and an increase of dentin surface energy [83]. This process facilitates the infiltration of adhesive resinous monomers between the collagen fibrils exposed by acid-etching [81]. The adhesive resin contains hydrophobic, high-molecular weight monomers coupled with diluent monomers of lower molecular weight [84]. This hydrophobic material can copolymerize with the primer, creating the hybrid layer, and it is the last component applied on dentin during the adhesive procedure [85].

In 1984, Davidson et al. reported the shrinkage stress caused by resin composite polymerization inside a class V cavity was approximately 20 MPa [86]. Two years later, supporting the findings of Davidson et al., Komatsu and Finger suggested dentin adhesives with shear bond strengths around 20 MPa were effective to prevent the formation of marginal gaps [87]. This finding, however, needs to be carefully interpreted owing to the fact that the polymerization stress of a composite depends on its composition, curing conditions, as well as cavity compliance [88]. Still in the same decade, a correlation between bond strength and marginal gap width was proposed: to obtain gap-free restorations properly adapted to the cavity walls, adhesive systems should achieve minimal bond strengths of 23 MPa for the tensile strength tests, and 17 MPa for the shear strength tests [89]. Nonetheless, a correlation between microleakage or marginal gaps and hypersensitivity or marginal caries formation was not found in studies both *in vivo* and *in situ* [90].

During the decade of 1990, several studies reported shear bond strength values close to or higher than the 17-20 MPa range, showing the fourth-generation adhesives could produce adequate dentin bonds [91–93]. In fact, the bonding agents from this generation present excellent bond strength results, even in laboratory studies with updated methodologies [84,94–96]. Additionally, the retention rates found in longitudinal clinical trials with follow-ups of more than 10 years testify the optimal performance of these adhesive systems (Figure 2) [97–99]. Meanwhile, also in the 1990s, filled adhesive resins were introduced, with varying proportions of glass filler particles [100]. Initially, the purpose of adding glass fillers to adhesives was to obtain thicker adhesive layers [90], seeking to modify their viscosity to provide relief of contraction stresses produced by composite restorations [101]. Adding fillers to dental adhesives is also suggested to fortify the adhesive layer, and the size of the filler particles should be less than 20nm in order for them to be able to infiltrate the demineralized collagen network [101].

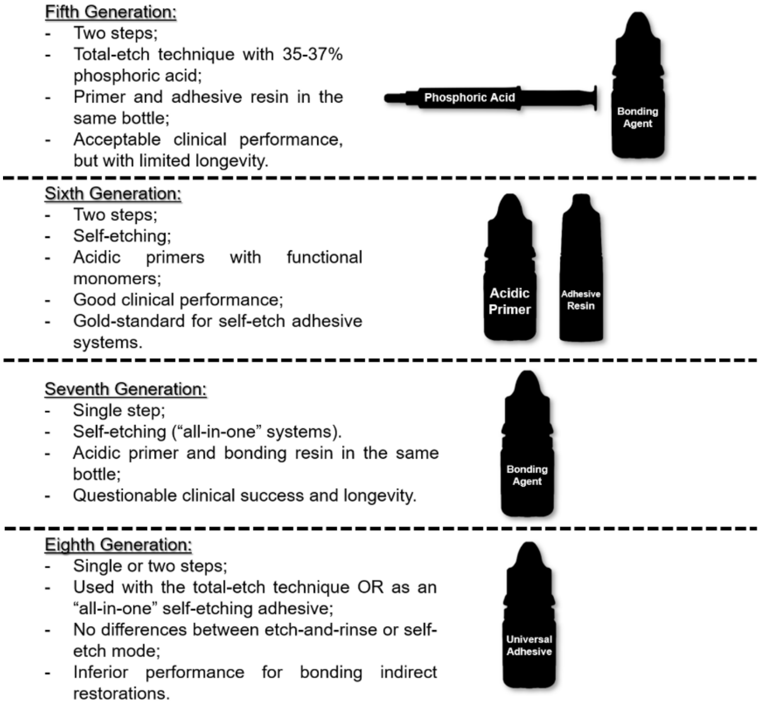
In 2014, Loguércio et al. evaluated the mechanical and bonding properties of four etch-and-rinse, three-step adhesive systems. Optibond FL (Kerr Corporation, Orange, CA, USA), which is considered the main representative of the fourth-generation adhesive systems, presented the best results for all properties analyzed when compared to the other systems, including: higher microtensile bond strength, lower nanoleakage, higher degree of conversion and *in situ* degree of conversion, higher ultimate tensile strength, and lower water sorption and solubility [84]. This study confirms the literature consensus that Optibond FL is the gold standard for etch-and-rinse dental adhesives.

7. Fifth-generation simplified adhesive systems

Due to the perception of some professionals that three-step bonding systems were too complicated and hard to use, manufacturers tried simplifying their products by merging application steps [102]. Thus, single-bottle systems that combined primer and adhesive resin in the same bottle were released to facilitate clinical use [103]. Simplification of dentin bonding systems is the key aspect of the fifth-generation adhesive products.

Merging primer and adhesive resin in a single application step required the addition of higher concentrations of hydrophilic components to the adhesive formulations to improve their ability to diffuse into the collagen network of etched dentin [104]. The HEMA monomer is frequently used in the composition of simplified adhesive systems, since its hydrophilicity makes it an excellent adhesion promoter that can increase dentin wettability, and significantly improve bond strength [103]. Water-based solvents, ethanol and/or acetone are also added to these formulations, with the aim of dissolving monomers, which helps reduce viscosity and improves adhesive resin wettability, facilitating its diffusion into dentin [101].

There are conflicting reports regarding the *in vitro* performance of fifth-generation ‘adhesive primers’. While some studies report dentin bond strength values comparable to fourth-generation, three-step systems [105–107], other studies show lower results [108–110]. However, even the investigations that show lower microtensile bond strengths for simplified adhesives compared to more complex systems present values exceeding 20 MPa, which, as aforementioned, can be considered the minimum bond strength required to resist the polymerization shrinkage of composites. On the other hand, clinical trials report unfavorable long-term performance for fifth-generation adhesive systems (Figure 4) [111–113]. These results can be explained by the higher hydrophilicity and reduced hybridization potential of simplified bonding agents [114].



Fifth Generation:
- Two steps;
- Total-etch technique with 35-37% phosphoric acid;
- Primer and adhesive resin in the same bottle;
- Acceptable clinical performance, but with limited longevity.

Sixth Generation:
- Two steps;
- Self-etching;
- Acidic primers with functional monomers;
- Good clinical performance;
- Gold-standard for self-etch adhesive systems.

Seventh Generation:
- Single step;
- Self-etching (“all-in-one” systems).
- Acidic primer and bonding resin in the same bottle;
- Questionable clinical success and longevity.

Eighth Generation:
- Single or two steps;
- Used with the total-etch technique OR as an “all-in-one” self-etching adhesive;
- No differences between etch-and-rinse or self-etch mode;
- Inferior performance for bonding indirect restorations.

Figure 4. Schematic representation of adhesive systems from the fifth to the eighth generation.

8. First steps towards the development of self-etch adhesives (sixth generation)

Following generations of bonding agents were characterized by products that do not require a separate etching step, as they contain acidic monomers capable of etching and diffusing into dentin simultaneously [103]. These so-called self-etch adhesive systems demand a smaller number of application steps, and their use is much simpler compared to previous bonding agents, rendering the hybridization procedure faster and less sensitive to potential technical mistakes [115]. Furthermore, some specific functional monomers in the composition of self-etch adhesives promote additional bonding through chemical interaction with the dental substrate [116,117]. Consequently, reliable and long-lasting bonds can be achieved [118].

The concept of self-etching was first introduced in a publication by Watanabe and Nakabayashi (1993), in which the authors evaluated the durability of bovine dentin bonds attained by an experimental adhesive comprising phenyl-P dissolved in triethyleneglycol dimethacrylate (TEGDMA) [119]. In that study, the adhesive was applied in a single step, without any separate dentin treatment, followed by a composite filling. Bond strength values were unfavorable, ranging from 6.7 MPa after 24 h of storage, to 2.8 MPa past 12 months of storage. The low results were explained by the limited capability of this experimental formulation to penetrate the smear layer and interact with the underlying mineralized dentin [119].

Based on the approach of Watanabe and Nakabayashi, the first adhesive system of the sixth generation was released in the early 1990s under the name of Clearfil Liner Bond 2 (Kuraray Co., Osaka, Japan) [120]. This bonding system consisted of a phenyl-P-based acidic primer and an adhesive resin, both delivered in separate bottles. The phenyl-P monomer presents a functional monohidrogenphosphate group that can dissociate into one proton (H^+) [101], allowing a chemical interaction by ionic bonding with calcium from HAp. However, the calcium-phenyl-P bond can be easily disrupted in aqueous medium, leading to a PO_4^{-3} and OH^- supersaturated condition, which results in HAp demineralization (approximately 1 mm deep) around collagen fibrils [121]. Hence, although phenyl-P allows adhesive resin diffusion between collagen fibrils, due to its demineralization capability, its chemical interaction with the dental structure is limited and hydrolytically unstable.

9. The adhesion-decalcification concept applied to self-etch adhesive systems

In 2001, Yoshida et al. envisioned the adhesion-decalcification concept (AD-concept), which explained how molecules that contain carboxylic groups interact with HAp, and why some of them will rather bond to than decalcify dental hard tissues, regardless of their low pH [122]. According to the AD-concept, a two-phase process takes place when acidic agents (organic and inorganic acids or carboxylic acid- and phosphate-based monomers) are applied to dentin [123,124]. In the first phase, the acid will form ionic bonds to calcium at the HAp surface, while simultaneously extracting PO_4^{-3} and OH^- ions from the outer layers of the tissue. In the second phase, depending on the dissolution rate of their respective calcium salts, acids will either remain attached to HAp, causing limited decalcification, or debond, leading to significant demineralization [122]. The AD-concept can be applied to all human hard tissues, since it does not depend on the crystallinity of HAp [123].

The most significant advancement in self-etch adhesive technology occurred in 1981, when the Japanese company Kuraray synthesized and patented the functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) [125,126]. The 10-MDP molecule has a methacrylate group at one end, which copolymerizes with other resin monomers [126], and functional phosphate groups at another end, which present a twofold role in dentin bonding: demineralization and chemical interaction [121]. These distinct groups (methacrylate and phosphate) are connected by a long hydrophobic carbon structure called the spacer chain [127]. The main representative of the sixth generation of adhesive systems is a 10-MDP-based product called Clearfil SE Bond (Kuraray Noritake Dental Inc., Tokyo, Japan). This bonding agent presents great clinical performance, with low postoperative sensitivity rates [118,128,129], being regarded as the gold-standard for self-etch adhesives (Figure 4).

As previously mentioned, the interaction of phosphate-based monomers, like 10-MDP, with dental hard tissues fits into the AD-concept [115]. Adhesive formulations comprising 10-MDP bond to dentin through micromechanical interlocking and chemical adhesion [130]. Hydrogen phosphate groups from 10-MDP ionically bond to calcium at the HAp surface [116], forming a regularly spaced, nanolayered structure composed by two 10-MDP molecules with their methacrylate groups facing each other, and their phosphate groups facing opposite directions [131]. During this process, different species of calcium salts are deposited between the phosphate groups of the nanolayers [132]. Simultaneously, superficial HAp dissolution (≈1 mm) is caused by the attack of hydronium (H_3O^+) ions, removing PO_4^{-3} and OH^- from the surface [121,124], which ensures enough demineralization to create a thin hybrid layer of 0.5–1.5 mm [133,134]. Conversely, one study has challenged the AD-concept by 10-MDP-containing adhesives, due to the complete elution of phosphoric acid ester monomers from mineralized dentin powder when column chromatography was performed [135].

Besides 10-MDP, glycerol phosphate dimethacrylate (GPDM) is another functional monomer that has been used for a long time in commercial dental adhesives. It was pioneered by the manufacturer Kerr, and first used for dental bonding by Hagger (1949) [72,101,136]. Despite presenting an acidic functional group and two polymerizable methacrylate groups in its molecule, GPDM was initially used in etch-and-rinse adhesives (Optibond FL, Kerr, among other), and later incorporated into self-etch systems. Although good *in vitro* and *in vivo* results have been reported for self-etch adhesives containing GPDM (Optibond XTR, Kerr), as well as for those containing 10-MDP (Clearfil SE Bond, Kuraray Noritake Dental Inc.), a recent study found contrasting chemical-interaction efficacy between these two monomers [136].

Another important feature of self-etch adhesives is their pH, which is intimately related to their etching aggressiveness: self-etch adhesives can be subdivided into strong ($pH \leq 1$), intermediary strong ($pH \approx 1.5$), mild ($pH \approx 2.0$), or ultra-mild ($pH \geq 2.5$) systems [137,138]. This information is important since high concentrations of acidic monomers in the composition of these adhesives favor their permeable membrane-like behavior by promoting non-stable chemical interactions with the exposed collagen fibers, which compromises the durability of the bonding interface [115,139].

10. Simplification of the self-etch approach: from all-in-one, self-etch to universal bonding systems (seventh and eight generations)

Simplified versions of self-etch adhesive systems have also been introduced in the market in order to reduce the number of clinical steps involved in adhesive procedures, optimizing speed and efficiency in clinical practice [140,141]. These simplified adhesives combine both self-etch primer and bonding resin in a single bottle (or two separate components that need to be mixed), along with water or organic solvents [140], and they represent the seventh generation of dentin bonding agents. The concentration of acidic monomers was increased in all-in-one, self-etch bonding systems to allow simultaneous etching of dentin and enamel [142]. Also, significant amounts of water are required to render these systems more acidic [140,141]. An inverse correlation between the hydrophilicity of adhesives and the stability of the bonding interface was previously demonstrated, as unpolymerized acidic and aggressive monomers could continue to etch dentin after bonding [140–142]. Their formulations were more prone to hydrolysis and chemical breakdown [143,144]. Also, excess water can affect the adhesive resin polymerization [145], and without an additional hydrophobic resin layer, these bonding agents can behave as permeable membranes after polymerization [35,139]. As a result, the clinical success and longevity of these all-in-one systems is questionable (Figure 4) [140–142].

The addition of HEMA into one-bottle self-etch adhesives avoided phase separation of their components; however, it also adversely affected the mechanical strength of dental adhesives [146]. Some concerns regarding the use of HEMA in adhesive formulations were that it could cause lower cross-linking, higher hydrophilicity, decreased polymerization degree, and plasticization of the polymer, and also the possibility of allergic reactions, oxidative stress induction and cytotoxicity in pulp cells depending on the remaining dentin thickness [147–150]. Consequently, some manufactures removed HEMA from one-bottle self-etch adhesives, which led to the phase separation of the components, resulting in the formation of droplets within the adhesive resin. However, phase separation has also been suggested to have a positive effect in HEMA-free one-step self-etch adhesives, as it would require strong air-blowing during bonding, which could help remove water more effectively, since this type of bonding agent requires higher amounts of water for optimal ionization [147]. Clinically, similar performance was achieved for both HEMA-free and HEMA-containing adhesives in non-carious cervical lesions [151].

Following the ongoing trend of simplifying adhesive procedures, multi-mode universal bonding agents were introduced a few years ago. These eighth-generation adhesive systems are designed to be used with both etch-and-rinse or self-etch techniques, depending on the specific clinical situation and personal preferences of the operator [152,153]. Selective etching of enamel is often recommended, and universal adhesives can be applied to dry or wet dentin [154,155]. Some manufactures also claim universal adhesives can be used for indirect restorations as adhesive primers for zirconia, alloys, ceramics, and composites [153,156]. The expiration of Kuraray's 10-MDP patent (around 2003) enabled the development of universal adhesives, and the first product of this class was released in 2011 (Scotchbond Universal, 3M ESPE, St. Paul, MN, USA), containing 10-MDP in its composition, which was followed by many other adhesive systems produced by different manufacturers [153]. Universal adhesives may also contain silane in the same bottle, suggesting an improvement of their bonding to silica-based ceramics [157]. Nevertheless, indirect restorations still presented better bonding performance when silane and bonding resin were applied separately, resulting in superior bond strength to etched glass-based ceramic substrate after one year of water storage when compared to two universal adhesives [157].

Bonding to non-silica-based substrates, namely metal or zirconia, is obtained by a chemical interaction between the hydrophilic phosphate terminal of 10-MDP and oxides from the internal surface of restorative materials [157,158]. On the other hand, the reaction between silane and 10-MDP lays the foundation for bonding to silica-based materials, as feldspathic, leucite-based or lithium disilicate glass ceramics. This mechanism can be further explained by the formation of hydrogen bonds of free silanol groups with hydroxyl groups from silica-based restorations [157]. Other phosphate ester monomers, such as dipentaerythritol penta-acrylate phosphate (PENTA) and GPMD are also found in universal adhesives [153,154].

Results regarding dentin bonding of universal adhesives were analyzed in a systematic review, which showed there is sufficient scientific evidence to support the hypothesis that bonding performance does not significantly vary whether the etch-and-rise or self-etch mode is used (Figure 4) [159]. Another recently published systematic review reports that dentin bond strengths of universal adhesives were dependent on their pH under *in vitro* conditions. Mild universal adhesives presented better bonding stability in both etch-and-rise and self-etch strategies (ultra-mild, pH ≥ 2.5; mild pH ≈ 2; or intermediately strong, pH ≈ 1.5) [160].

11. The development of the antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB)

MDPB was developed and first incorporated into composite materials to provide them with antibacterial activity [161,162]. MDPB is a derivative of quaternary ammonium synthesized by combining this antibacterial agent with a methacryloyl group, and it is able to copolymerize with other monomers, leading to the immobilization of the antibacterial group in the polymer chain, which might provide long-term antibacterial activity to composites [163,164]. The effects of quaternary ammonium compounds rely on cationic binding to bacterial cell wall components, which disturbs the membrane function and induces cytoplasmic material leakage [165]. Later, this monomer was incorporated to a dentin primer and antibacterial results were obtained regardless of whether the material tested was cured or not [163,166].

Clearfil SE Protect (Kuraray Noritake Dental Inc., Tokyo, Japan), which contains MDPB in the primer solution and fluoride in the adhesive resin, was the first antibacterial adhesive system to be commercialized. The incorporation of MDPB in the primer solution did not adversely affect dentin bond strength or the curing performance of the adhesive [167–169]. Several studies have shown *in vitro* antibacterial effects of MDPB when it was incorporated in the primer solution [163,165,166,169–171]. However, just a few investigations were performed *in vivo* [172,173], as well as *in situ* [174, 175]. Antibacterial effect in infected cavities of dogs' teeth was reported when MDPB was included into an experimental primer [172]. Another study showed a reduction on enamel demineralization around orthodontic brackets, with significant effect even after 30 days, indicating the use of this antibacterial monomer-containing adhesive for bonding orthodontic brackets [173]. *In situ* studies indicate Clearfil SE Protect is capable of controlling caries progression in enamel at the tooth-restoration interface under high cariogenic challenge [174,175]. In addition, a lower risk of MDPB-produced drug resistance is suggested, as *Enterococcus faecalis* and *Streptococcus mutans* were not able to adapt to MDPB [176]. After the development of MDPB, several other monomers based on quaternary ammonium have been synthesized and incorporated into dental materials as antibacterial agents [177–179] or antibacterial agents with combined metalloproteinase (MMP) inhibition effects [180]. Also, fluoride was added to other dental adhesives as a fluoride release source, including: Clearfil Universal Bond Quick (Kuraray Noritake Dental Inc.), Clearfil S3 Bond Plus (Kuraray Noritake Dental Inc.), One Up F Bond Plus (Tokuyama Dental Corporation Inc.), Futurabond NR (Voco GmbH).

12. Addition of therapeutic agents to dental adhesives

Besides fluoride and MDPB, the addition of therapeutic agents into restorative materials, such as antibacterial, remineralizing, or collagen cross-linking agents (MMP inhibitors), could bring benefits in terms of enhanced durability of composite restorations [181,182]. Antibacterial agents may avoid the replacement of fillings by reducing biofilm accumulation [169,182], especially in areas as the interproximal and cervical regions of the tooth. Besides Clearfil SE Protect, other dental adhesives containing antibacterial agents, such as Gluma 2Bond (Kulzer GmbH, Hanau, DE-HE, Germany) and Peak Universal Bond (Ultradent Products, Inc., South Jordan, UT, USA), are commercially available. Gluma 2Bond contains 5% glutaraldehyde and Peak Universal Bond contains 0.2% chlorhexidine diacetate [169,170]. Even though the main goal of these substances was not to obtain antibacterial activity, some studies have reported *in vitro* antibacterial effects when they were incorporated to dental adhesives [170,183–185].

Several experimental antibacterial agents have been incorporated into dental adhesives by mixing the substances with the adhesive resin, adding doped fillers (usually nanoparticles), nanocapsules, or even synthetizing new monomers in order to promote therapeutic effects [177,182,186,187]. Some of these materials include antibacterial agents with broad antimicrobial spectra [186–191], lantibiotics [192], protein-repellent [193–195], and compounds isolated from natural products [182,196–199]. Silver nanoparticles are the most notorious and widely explored of these antibacterial agents, which exhibit a wide antimicrobial spectrum and can be added to different dental materials [200], including dentin adhesives [188–190]. The suggested mechanism of antibacterial action of silver nanoparticles consists on the rupture of cell membranes by contact with the silver metal surface, the uptake or ion penetration into the nucleus, leading to formation of reactive oxygen species, and the inhibition of cell reproduction by interaction with DNA [189,201,202].

Addition of substances with broad antimicrobial spectra in restorative materials arises some concerns about potential interferences in the oral health resident bacteria (resulting in the promotion of bacterial resistance) [182], its biocompatibility, toxic or allergenic effects [199]. Hence, alternative products, such as compounds derived from natural sources, are considered a potential alternative approach to the current chemotherapeutic strategies [203,204]. A recent study incorporated two anti-caries agents isolated from Brazilian propolis (apigenin and *tt*-farnesol) into commercial dental adhesives containing fluoride in their composition [182]. Both compounds target the main virulence factors of *S. mutans* biofilm, the insoluble polysaccharides and intracellular polysaccharides [182], affecting *S. mutans* ability to colonize the tooth surface, become the dominant bacteria, and express its virulence [205].

Following the same trend of incorporating natural products with antibacterial properties into dental adhesives, chitosan, epigallocatechin-3-gallate (EGCG), and essential oil (*Butia capitata* fruits) were also investigated [196,198,199]. The interaction between the positively charged chitosan and the negatively charged bacteria cell surface leads to the rupture of the cell membrane [196,206]. Antibacterial effects were reported when chitosan was added to dental adhesives [196,197]. EGCG is a flavonoid produced by the *Camellia sinensis* plant (green tea), and it might be capable of suppressing glycosyltransferases B, C, and D gene expression, disrupting *S. mutans* biofilm formation. In some concentrations, this compound was able to express antibacterial activity when incorporated to a dental adhesive [198]. An experimental self-etching adhesive containing essential oil from *B. capitata* presented antimicrobial effect in a microcosm biofilm, with physical and mechanical properties similar to the other adhesives tested [199]. Natural products were also added in dental adhesives in order to promote matrix MMPs inhibition [207,208].

MMP inhibitors-containing dentin adhesives are also considered as an approach to improve the longevity of restorative procedures [207,208]. Host-derived MMPs have been reported to be involved in hybrid layer degradation [209]. Activated MMPs may progressively degrade the collagen fibrils that were not fully enveloped by the adhesive resin, and, consequently, may increase the water content within the hybrid layer, leading to deterioration of the dentin-restoration bond [210]. Several studies have incorporated MMP inhibitors into dentin adhesives with promising results [210–214].

Another promising therapeutic effect regards the ability of remineralization by dental adhesives containing bioactive glasses [181,215–217]. The ability of bioactive glasses within resin-based materials to release calcium or phosphate ions that will subsequently crystallize into hydroxyapatite induces the remineralization and preservation of the resin-dentin interface [181,217]. Some studies have also reported simultaneous therapeutic effects for experimentally modified dental adhesives [180,181], encouraging future research on restorative materials with therapeutic agents. Despite the promising findings described, clinical studies are still necessary in order to validate the clinical efficacy of these modified dental adhesives when exposed to a more complex environment, and the long-term effect of either commercially available materials, experimental monomers or therapeutic incorporations.

13. Current classification of dental adhesives based on mode of action and clinical steps

Although the generational classification is based on chronological history, some developments in dental bonding were achieved within a short period of time or even simultaneously, making it difficult to classify each generation accurately. Therefore, the most practical and rationale classification for adhesive systems nowadays is based on their bonding mechanism and number of clinical steps. After the introduction of ‘universal’ adhesives, bonding agents can be classified in etch-and rinse, self-etch, or ‘universal’, according to their bonding mechanism. Subsequently, each bonding mechanism can be subdivided according to the number of clinical steps it requires: three-steps (acid-etching + primer + adhesive resin), two-steps (acid-etching + adhesive primer or acidic primer + adhesive resin), or one-step (all-in-one). This classification, first introduced by van Meerbeek et al. [218], is summarized in Figure 5.

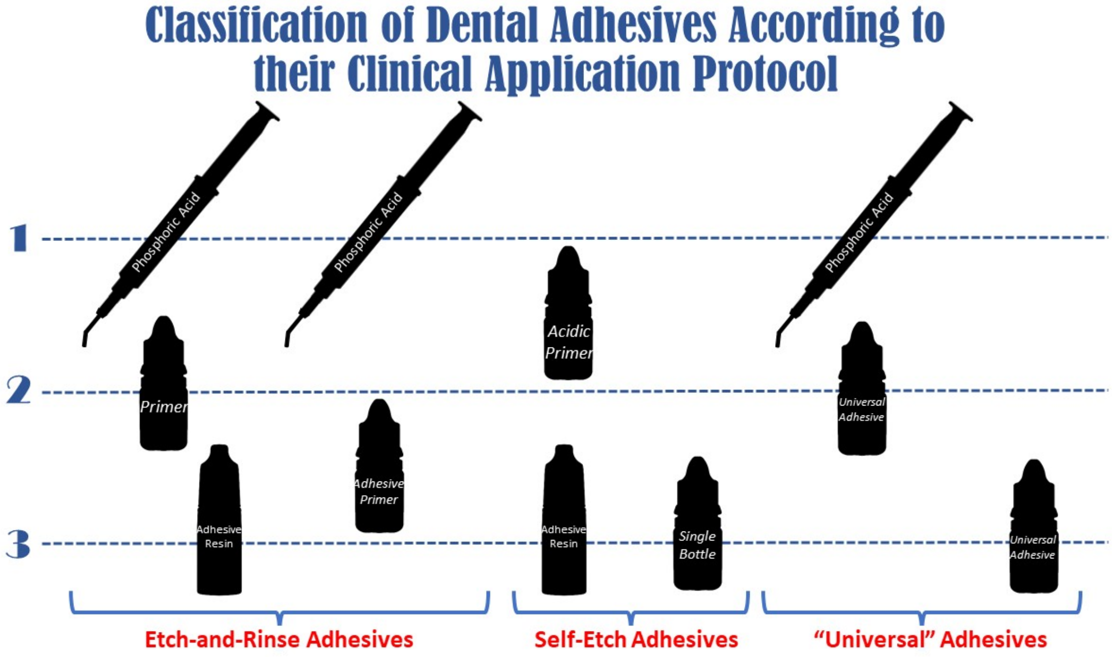


Figure 5. Classification of dental adhesive systems according to their protocol of clinical application (etch-and-rinse, self-etch or “universal”; and one, two, or three steps).

14. Conclusions

Dental adhesives have been around for over six decades now, and these products have gone through multiple iterations, with fairly significant modifications. Despite changes, self-etch and universal bonding agents have come full circle from Hagger’s avant-garde approach of chemically bonding to dentin. Considering the scientific events that led to the current status of dental adhesion, some breakthroughs should be regarded as essential for dental bonding technology development, namely the description of enamel acid-etching by Buonocore, which was later extended to dentin in the form of the etch-and-rinse technique; the discovery of the hybrid layer by Nakabayashi and his co-workers; the use of the wet-bonding adhesive technique, first described by Kanca; and the synthesis of 10-MDP by Kuraray.

Bonding agents have moved towards technique simplification throughout the years, as shown by the recent popularity of all-in-one, single-bottle adhesive systems among clinicians, although there is still room for improvement in these products. Moreover, manufacturers and researchers seem to be driven by the current multitasking mentality, as adhesive systems cannot simply bond to enamel and/or dentin anymore, they also have to present additional features, such as antibacterial effects, enzymatic inhibition, remineralizing properties, and so on. However, as technological development runs at a fast pace, looking at the past is extremely important to ensure new bonding agents and clinical procedures are strongly based on sound foundations. Otherwise, practitioners might end up with products that promise too much, but only deliver average results.

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2.2 Bonding interface and dentin enzymatic activity of two universal adhesives applied following different etching approaches

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HIGHLIGHTS

- Prime&Bond Universal performed well on phosphoric acid-etched, blot-dried dentin.
- Dry-bonding to phosphoric acid-etched dentin is not advised for either adhesive.
- Long-term storage caused significant dentin bond strength reduction.
- Ten-three solution and nitric acid could not inhibit dentin proteases *in situ*.
- Nanoleakage within the hybrid layer seemed to be adhesive-dependent.

ABSTRACT

Objectives: Etching approaches [37% phosphoric acid, self-etching, 10-3 solution (3% ferric chloride dissolved in 10% citric acid), or 1.4% nitric acid] were evaluated regarding enamel shear bond strength (24 hours), dentin microtensile bond strength (24 hours and 2 years), failure mode, enzymatic activity of the hybrid layer, and nanoleakage (24 hours and 2 years) of Prime&Bond Universal (PBU, Dentsply-Sirona) and Gluma Bond Universal (GBU, Kulzer). *Methods:* Adhesives were applied on blot-dried (wet-bonding, positive control) or air-dried (remaining groups) dentin after acid-etching (15 s) or in self-etch mode. Enamel and dentin bond strength tests used 160 human teeth (n=10). Failure mode of tested samples and nanoleakage within the dentin-adhesive interface (n=5) were analyzed by scanning electron microscopy. Dentin enzymatic activity was investigated by *in situ* zymography (n=3). *Results:* Enamel bond strengths did not differ statistically among groups. Wet-bonding with 37% phosphoric acid showed similar dentin bond strength compared to 10-3 solution or 1.4% nitric acid at 24 hours for both adhesives. None of the etchants inhibited enzymatic activity, and all groups showed dentin bond strength reduction after 2-year storage. GBU showed higher nanoleakage. Experimental etchants did not affect enamel bond strength. Dentin bond strength was not stable after 2 years, despite promising 24-hour results. *Significance:* This study suggests multiple etching approaches to optimize and achieve stable dentin bonding, while also offering in-depth information about the performance of recently released universal adhesive systems.

KEYWORDS: ferric chloride; nitric acid; matrix metalloproteinase inhibitors; dentin-bonding agents; dental etching.

1. Introduction

Air-drying acid-etched dentin leads to the collapse of collagen fibrils, hampering adhesive resin diffusion and hybrid layer formation [1]. However, the maintenance of a moist surface considerably increases the complexity of dentin bonding [2]. Water can be incorporated into the bonding interface during polymerization, which impairs the quality of dentin hybridization. Therefore, the resin polymer chains of the hybrid layer will be more susceptible to hydrolytic degradation [3], particularly for hydrophilic, monomer-rich, simplified bonding agents [4]. Also, water can interfere with polymerization, resulting in a polymer of compromised mechanical properties [5].

Additionally, the limited diffusion of adhesive monomers can create microporosities below and inside the hybrid layer [6], leaving exposed collagen fibrils at its deepest regions [7]. These collagen fibrils will then become susceptible to degradation by endogenous proteases, namely matrix metalloproteinases (MMPs) and cysteine cathepsins (CCs) [8, 9]. Dentin etching with phosphoric acid activates latent MMPs, and, consequently, exposed collagen fibrils undergo hydrolytic degradation, which might decrease bond strength over time [10].

The aggressive phosphoric acid etching not only increases dentin MMP activity [11, 12], but it also modifies the molecular arrangement of collagen fibrils, facilitating the interaction between polypeptide chains and catalytic domains of MMPs [13]. Milder, alternative etchants, such as citric, maleic, oxalic, and ethylenediaminetetraacetic acids could reduce this effect, and therefore they have been studied to replace the traditional 35-40% phosphoric acid gel [14-16].

In the 1980s, 3% ferric chloride dissolved in 10% citric acid (10-3 solution) was suggested for dentin etching [17]. Adequate dentin bond strength with evidence of hybrid layer formation has been reported as a result of dentin treatment with 10-3 solution [16, 18]. Iron ions from 10-3 solution supposedly form insoluble aggregates with dentin polyelectrolytes, which could avoid the collapse of the collagen network in the absence of water [19]. Furthermore, iron-containing solutions can inhibit MMP-2, -9, and cathepsin-K activity *in vitro* [20, 21], and iron bonds to collagen are suggested to improve resin infiltration due to the modification of dentin surface tension [16].

Studies from the 1980s/1990s also reported the use of nitric acid as a dentin etchant [22, 23]. This acid interacts with dentin following the adhesion-demineralization concept [24], and it achieved dentin bond strength values comparable to the bond between resin and enamel reported at that time by using a mixture of 2.4% nitric acid and 5.7% N-phenylglycine [22]. Nitric acid has been used for dentin etching, followed by dry-bonding [16], and the results showed no significant difference in dentin bond strength at baseline or after 12-month aging compared to the traditional wet-bonding technique with phosphoric acid using a simplified adhesive system.

Ten-three solution and nitric acid have the potential to make adhesive procedures more straightforward and less technique-sensitive, as these etchants could allow dry-bonding to dentin, minimizing the impact of water on the quality and durability of hybridization. Furthermore, enzymatic degradation of collagen at the hybrid layer might also be prevented or reduced with the use of these etchants. Hence, the present study aimed to evaluate enamel shear bond strength (at 24 hours), dentin microtensile bond strength and its resulting failure mode (at 24 hours and after 2 years of storage), nanoleakage of the dentin adhesive interface (at 24 hours and after 2 years of storage), and *in situ* enzymatic activity of the hybrid layer (at 24 hours) after dentin etching through different approaches (37% phosphoric acid gel, self-etch mode on dentin, 10-3 solution, or 1.4% nitric acid), followed by bonding with two universal adhesives (alcohol or acetone-based).

The null hypotheses were: (1) there would be no significant differences in enamel shear bond strength between the selected adhesive systems or among the tested etchants; (2) there would be no significant differences in dentin bond strength among the investigated groups, regardless of etching strategy, adhesive system, or aging; (3) the percentages of occurrence of the studied failure modes would not differ significantly among groups at 24 hours or 2 years; (4) no differences among dentin etchants regarding enzymatic activity would be observed for any of the tested adhesive systems.

2. Materials and Methods

2.1. Teeth selection and preparation of the experimental etchants

One hundred and seventy-six extracted, sound, human third molars obtained under a protocol approved by the Ethics Committee in Research of the Piracicaba Dental School - University of Campinas (CAAE 87550318.0.0000.5418) were selected for the study. All teeth were subjected to manual scaling, polished with a paste of pumice, and stored in thymol solution (Labsynth, Diadema, SP, Brazil) at 4 °C for no longer than three months.

Three etchants were tested: 37% phosphoric acid gel (Condicionador Dental Gel, Dentsply-Sirona, Pirassununga, SP, Brazil); 10-3 solution (3% ferric chloride dissolved in 10% citric acid); and 1.4% nitric acid aqueous solution. The 37% phosphoric acid gel had a pH of 0.2, according to the manufacturer’s safety data sheet (MSDS). Ten-three and 1.4% nitric acid solutions were prepared following a previous publication [16]. Both experimental solutions had a pH of 0.6 and were stored in lightproof vials kept at room temperature.

Two universal adhesive systems were selected for the tests performed in the present study: Prime&Bond Universal (PBU, Dentsply-Sirona, Milford, DE, USA), and Gluma Bond Universal (GBU, Kulzer GmbH, Hanau, Germany). The composition and batch number of the materials used in this study, as well as the pH of the selected adhesive systems, are shown in Table 1.

Table 1. Manufacturer, composition, batch number, and pH of the materials used in this study

Material	Manufacturer	Composition	Batch number	pH
37% Phosphoric Acid Gel	Dentsply-Sirona	Phosphoric acid; surfactant; aerosil 200; deionized water; dye.	218582I	0.2
Prime&Bond Universal (PBU)	Dentsply-Sirona	Bisacrylamide 1; propan-2-ol; 10-methacryloyl-oxydecyl-dihydrogenphosphate; bisacrylamide 2; dipentaerythritol pentaacrylate phosphate; 4-(dimethylamino)benzonitrile.	1709000808	2.5
Gluma Bond Universal (GBU)	Kulzer GmbH	4-methacryloxyethyl trimellitate anhydride; methacrylate monomer; acetone; 10-methacryloyl-oxydecyl-dihydrogenphosphate.	K010031	1.6-1.8
Spectra Smart	Dentsply-Sirona	Glass powder; silica; hydrophobic colloid; dimethacrylate; benzophenone III; ethyl 4-dimethylamine benzoate; flublau concentrate; camphorquinone; butylated hydroxytoluene; yellow iron oxide; red iron oxide; black iron oxide; titanium dioxide.	356416K	NA
SureFil SDR flow+	Dentsply-Sirona	Barium-alumino-fluoro-borosilicate glass; strontium alumino-fluoro-silicate glass; ytterbium trifluoride glass; silicon dioxide; modified urethane dimethacrylate resin; polymerizable dimethacrylate resin; polymerizable trimethacrylate resin; triethyleneglycol dimethacrylate; camphorquinone; photoaccelerator; butylated hydroxyl toluene; UV stabilizer; titanium dioxide; inorganic iron oxide; fluorescing agent; inorganic filler particles.	1612144	NA

NA: not applicable

2.2. Enamel shear bond strength test (at 24 hours)

Sixty human third molars were embedded in acrylic resin (Vertex-Dental B. V., Zeist, The Netherlands) and their buccal coronal surfaces were ground down with 180-grit silicon carbide paper to expose a flat enamel surface. A piece of tape (tesa Werk Hamburg GmbH, Hamburg, Germany) with a punch hole of 2.34 mm of diameter was attached to the flattened surfaces to limit the bonding area. Then, specimens were randomly assigned to six groups (n=10): A1- 37% phosphoric acid gel + PBU; A2- 10-3 solution + PBU; A3- 1.4% nitric acid + PBU; A4- 37% phosphoric acid gel + GBU; A5- 10-3 solution + GBU; and A6- 1.4% nitric acid + GBU.

Enamel surfaces were treated for 30 s with one of the three etchants and rinsed for the same time. Considering manufacturers of the selected adhesives did not advise the self-etch approach for enamel, the use of bonding agents without prior enamel etching was not tested. The etched surfaces were dried with an oil-free air spray for 30 s at a distance of approximately 10 cm. Adhesive systems were applied and light-cured (single-peak LED Demi Plus with 1,100 mW/cm^2 irradiance, Kerr Corporation, Orange, CA, USA) following manufacturers' instructions.

A plastic, cylindrically-shaped mold with 2.34 mm diameter and 3 mm height of internal space (Ultradent Products Inc., South Jordan, UT, USA) was placed over the bonded surfaces, and a high-viscosity resin composite (shade A2, Spectra Smart, Dentsply-Sirona, Pirassununga, SP, Brazil) was inserted into it and light-cured for 20 s. The same light-curing unit previously mentioned was used, placed parallel and in contact with the surface of the mold. Restored specimens were kept in relative humidity at 37 °C for 24 hours. Subsequently, each specimen was placed in a mounting jig with the bonded enamel surface parallel to the notched crosshead contact blade of the testing device (UltraTester Bond Strength Testing Machine, Ultradent Products Inc., South Jordan, UT, USA). Shear bond strength was tested at a constant speed of 1.0 mm/min until failure of the bonding interface, and the peak load of each specimen was divided by the cross-sectional area to calculate the stress at fracture (in MPa).

2.3. Dentin microtensile bond strength test (at 24 hours and 2 years)

One hundred human third molars were randomly assigned to ten experimental groups (n=10), which are described in Table 2.

Table 2. Experimental groups for the dentin microtensile bond strength test

Group	Etchant	Dentin	Adhesive System
G1	37% Phosphoric Acid Gel	Blot-dried	Prime&Bond Universal
G2	37% Phosphoric Acid Gel	Air-dried	Prime&Bond Universal
G3	37% Phosphoric Acid Gel	Blot-dried	Gluma Bond Universal
G4	37% Phosphoric Acid Gel	Air-dried	Gluma Bond Universal
G5	None	Air-dried	Prime&Bond Universal
G6	None	Air-dried	Gluma Bond Universal
G7	10-3 Solution	Air-dried	Prime&Bond Universal
G8	10-3 Solution	Air-dried	Gluma Bond Universal
G9	1.4% Nitric Acid	Air-dried	Prime&Bond Universal
G10	1.4% Nitric Acid	Air-dried	Gluma Bond Universal

The bond strength test was carried out according to the guidelines of the Academy of Dental Materials [25]. The occlusal enamel of the teeth was removed with a diamond saw (Buehler Ltd., Lake Bluff, IL, USA), under water-cooling, to expose middle-depth dentin. Dentin surfaces were abraded with 600-grit silicon carbide paper for 5 s, under water-cooling, to standardize the smear layer and flatten the substrate. Afterwards, dentin surfaces were treated with the corresponding etchant of each group, passively, for 15 s. After etching, teeth were rinsed for 15 s with air-water spray. Dentin was blot-dried in the positive control groups (G1 and G3) before adhesive application. In the remaining groups, dentin surfaces were air-dried with oil-free air spray for 30 s at a distance of approximately 10 cm [26]. The selected universal adhesives were then applied and light-cured following manufacturers' instructions. For the self-etch mode of application, manufacturers' instructions were also followed, slightly agitating the adhesive resin against sound dentin surface for 20 s.

Following dentin treatment and adhesive application, a composite block of approximately 4 mm height was built-up using 2 mm-thick composite layers (shade A2, Spectra Smart), which were separately light-cured for 20 s. A multiple-peak LED light-curing unit (Valo, Ultradent Products Inc., South Jordan, UT, USA) was used for all materials. The light irradiance of the device was periodically checked with a radiometer (Demetron, Kerr Corporation, Orange, CA, USA) during the entire experiment to ensure a minimum value of 500 mW/cm^2 was always delivered to the polymerizable materials [25].

Restored teeth were kept at relative humidity at 37 °C for 24 hours. Subsequently, teeth were serially sectioned in buccal-lingual and mesial-distal directions to obtain 8-16 stick-shaped specimens per tooth, with a cross-section area around 1.0 mm^2. Half of the specimens was tested after 24 hours, while the other half was tested after 2 years of storage in deionized water at 37 °C. For the long-term storage protocol, deionized water was removed and replaced every month [16, 27].

Each specimen was attached to a microtensile device of a universal testing machine (EZ Test, Shimadzu, Kyoto, Japan) using cyanoacrylate-based glue (Super Bonder Gel, Henkel/Loctite, Diadema, SP, Brazil), and tested at a crosshead speed of 1 mm/min, until the bonding interface was disrupted. The peak tensile load of each specimen was divided by the cross-sectional area to calculate the stress at the moment of failure (in MPa). A single value of failure stress was then calculated for each tooth at both times by averaging the values of the specimens.

2.4. Failure mode analysis under scanning electron microscopy (SEM)

The fractured surfaces of each specimen from the dentin microtensile bond strength test were analyzed by SEM regarding failure mode. Samples were attached to metallic stubs using carbon tape, with the surfaces involved in fracture facing upwards. Then, specimens were sputter-coated with gold (SDC 050 Sputtercoater, Baltec, Balzers, Liechtenstein) and observed under SEM (JSM 5600LV, Jeol, Tokyo, Japan) at magnifications of 100x and 400x. Failure modes were descriptively evaluated according to the structures seen in the micrographs [15]: cohesive failure of composite (type 1); adhesive failure between composite and adhesive resin (type 2); adhesive failure between dentin and adhesive resin (type 3); mixed failure showing the different components of the bonding interface (type 4); cohesive failure within the adhesive layer (type 5); cohesive failure within the hybrid layer (type 6); and cohesive failure of dentin (type 7). The type of fracture was not classified as mixed only if a single specific failure mode prevailed on 70% or more of the evaluated surface area.

2.5. Nanoleakage evaluation of the dentin-adhesive interface under SEM

Ten sound human third molars were used for SEM nanoleakage analysis. Preparation and evaluation of samples followed the protocol described by Pereira et al. (2019) [28]. Teeth were sectioned in halves and randomly divided among the groups from Table 2. Each two tooth halves were used to produce ten specimens per group, from which five were tested at 24 hours and the other five at 2 years, after storage in deionized water at 37 °C (n=5). Bonding and restorative protocols followed the same procedures of the dentin microtensile bond strength test. Restored teeth were stored in relative humidity at 37 °C for 24 hours. Afterwards, teeth were vertically sectioned in buccal-lingual and mesial-distal directions, resulting in stick-shaped specimens. Samples were immersed in an ammoniacal silver nitrate solution and kept in the dark, at 37 °C, for 24 hours. Subsequently, samples were rinsed with running water for 2 minutes, submerged in a developer solution (D-19, Eastman Kodak Company, Rochester, NY, USA) and kept under fluorescent light for 8 hours. After this period, samples were rinsed with deionized water and embedded in epoxy resin (EpoxiCure 2, Buehler Ltd., Lake Bluff, IL, USA).

Later, the embedded samples were polished with silicon carbide paper (600-grit for 2-4 minutes, 1,000-grit for 5 minutes, and 2,000-grit for 5 minutes), and cloths coupled with diamond pastes of decreasing particle sizes (6 µm, 3 µm, 1 µm, and ¼ µm). Samples were cleaned through 10-minute ultrasonic baths in-between steps and at the end of the polishing procedure. Specimens were demineralized in 85% phosphoric acid solution for 10 s, rinsed with deionized water, and submerged in 2% sodium hypochlorite solution for 10 minutes. Then, specimens were taken to a final ultrasonic bath for 10 minutes, followed by a dehydration protocol in increasing concentrations of ethanol (20%, 50%, 70%, 90%, and absolute ethanol). Finally, samples were carbon-coated and observed under SEM (JSM 5600LV, Jeol, Tokyo, Japan) at magnifications of 100x and 500x.

Micrographs from the nanoleakage evaluation by SEM were descriptively analyzed by the identification of silver ion deposits within the hybrid layers of the specimens. The general appearance of the bonding interfaces from the five repetitions of each group was used to characterize trends observed in different groups and at distinct evaluation times.

2.6. *In situ* zymography of the hybrid layer

The occlusal enamel of six human third molars was removed using a low-speed diamond saw (Isomet 5000 Buehler Ltd, Lake Bluff, IL, USA). Two 1 mm-thick dentin slabs were obtained from the middle portion of each tooth. Each slab was transversally sectioned into four pieces, and each piece was assigned to the following groups (n=3): 37% phosphoric acid gel (blot-dried); self-etch mode (adhesives applied on sound dentin); 10-3 solution (air-dried); and 1.4% nitric acid (air-dried). This procedure assured all four groups for each adhesive system were tested within the same tooth, reducing the effect of teeth variability in the results. Experimental procedures were conducted following the methods introduced by Mazzoni et al. (2012) [29], and later described by Mazzoni et al. (2014) [30].

Specimen preparation, bonding, and restorative procedures were carried out following the same steps described above for the microtensile bond strength test, except that a 1 mm-thick layer of flowable composite (universal shade, SureFil SDR flow+, Dentsply-Sirona, Milford, DE, USA) was placed on bonded dentin instead of multiple layers of a high-viscosity composite. The Demi Plus LED light-curing unit (Kerr Corporation, Orange, CA, USA) was used for all materials with a minimum light irradiance of 1,100 mW/cm^2.

Bonded samples were then vertically sectioned into 1-mm slabs (one for each tooth piece) to expose the resin-dentin bonding interface, glued to glass slides, and ground down to obtain approximately 50 µm-thick specimens. *In situ* zymography was performed with self-quenched fluorescein-conjugated gelatin as the MMP substrate (E-12055, Molecular Probes, Eugene, OR, USA) [31]. Fifty µl of the fluorescent gelatin mixture was placed over each specimen and covered by a coverslip. Slides were protected from light and incubated in humidifying chambers at 37 °C for 24 hours. The hydrolysis process of the quenched fluorescein-conjugated gelatin substrate was assessed by a confocal laser scanning microscope (excitation, 488 nm; and emission, lp530 nm; Nikon A1-R, Tokyo, Japan). For each assembled specimen, a series of images was taken at 1-µm intervals, and the integrated density of fluorescent signal (IntDen) was assessed by the ImageJ 1.52p software (U. S. National Institutes of Health, Bethesda, MD, USA). Relative differences among the tested groups were quantified from the images as evidence of the degree of dentin enzymatic activity.

2.7. Statistical analysis

Data from the dentin microtensile and the enamel shear bond strength tests passed the Shapiro-Wilk normality test, as well as Levene's test for equality of variances. Therefore, enamel shear bond strength results were submitted to a two-way ANOVA (1- etchant; 2- adhesive system), followed by Bonferroni’s test ($\alpha = 0.05$). Dentin microtensile bond strength results were analyzed by a three-way mixed ANOVA (between-subjects factors: 1- etchant and 2- adhesive system; within-subjects factor: 3- aging), and Bonferroni’s test ($\alpha = 0.05$). Conversely, data from the analysis of IntDen of fluorescent signal did not present a normal distribution. Thus, *in* *situ* zymography results were submitted to the non-parametric independent samples Kruskal-Wallis test, followed by Dunn's pairwise comparison between groups. These analyses were performed using the IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0, IBM Corp., Armonk, NY, USA).

Regarding failure mode, data was descriptively analyzed by means of percentages of frequency. Pearson's chi-squared test was applied to analyze the association between the tested groups and the observed failure modes at both evaluation times (24 hours and 2 years). Then, adjusted Pearson residuals were calculated to estimate the relative contribution of each cell to the result of the chi-squared test. Since multiple comparisons among the groups were performed, Bonferroni correction was applied. The described analyses were all carried out by the R software (R Foundation for Statistical Computing, Vienna, Austria), with a significance level of 5%.

3. Results

3.1. Enamel shear bond strength test (at 24 hours)

Statistical analysis showed enamel bond strength was not influenced by the different enamel etchants ($p = 0.387$) or by the type of adhesive system ($p = 0.269$). There was also no significant interaction between enamel etchants and type of adhesive system ($p = 0.680$). Table 3 presents the results of enamel bond strength.

Table 3. Mean (± standard deviation) enamel shear bond strength (in MPa) according to etchant and adhesive system

Etchant	**Adhesive System**	
	Prime&Bond Universal	**Gluma Bond Universal**
37% Phosphoric Acid	18.9 (± 5.0)	16.0 (± 5.7)
10-3 Solution	17.7 (± 4.0)	17.5 (± 3.9)
1.4% Nitric Acid	16.3 (± 5.1)	15.1 (± 4.9)

No statistically significant difference ($p > 0.05$) between means in rows (comparing the same etchant for different adhesive systems) or among means in columns (comparing the same adhesive system for different etchants) was detected.

3.2. Dentin microtensile bond strength test (at 24 hours and 2 years)

Analysis of variance showed dentin bond strength was influenced by etching approach ($p = 0.009$) and aging ($p < 0.001$), but it was not influenced by type of adhesive system ($p = 0.163$). Additionally, the interaction between etchant and adhesive system was significant ($p = 0.003$), as well as the interaction between aging and adhesive system ($p = 0.003$). However, there was no significant interaction between the three study factors ($p = 0.1$). Table 4 presents the dentin bond strength results of the tested groups, according to the etching approach utilized with each adhesive system at both aging times.

Table 4. Mean (± standard deviation) and percentages of reduction after storage of dentin microtensile bond strength (in MPa), according to etching approach, adhesive system, and aging (24 hours and 2 years)

Aging	**Etching Approach**	**Adhesive System**			
		Prime&Bond Universal		**Gluma Bond Universal**	
		Bond Strength	**Reduction**	**Bond Strength**	**Reduction**
24 hours	Phosphoric Acid (blot-dried)	45.9 (± 7.6) Aa		40.9 (± 5.1) Ba	
	Phosphoric Acid (air-dried)	37.3 (± 6.3) Ab		40.8 (± 4.2) Aa	
	Self-etch Mode	37.9 (± 6.2) Ab		39.4 (± 4.5) Aa	
	10-3 Solution	40.5 (± 6.1) Aab		44.3 (± 5.5) Aa	
	1.4% Nitric Acid	42.3 (± 4.9) Aab		42.6 (± 3.4) Aa	
2 years	Phosphoric Acid (blot-dried)	§ 38.2 (± 4.7) Aa	16.8%	§ 24.6 (± 8.2) Ba	39.9%
	Phosphoric Acid (air-dried)	§ 28.4 (± 7.2) Ab	23.9%	§ 23.0 (± 8.2) Aa	43.6%
	Self-etch Mode	§ 24.9 (± 6.3) Ab	34.3%	§ 27.5 (± 7.0) Aa	30.2%
	10-3 Solution	§ 25.9 (± 7.8) Ab	36.1%	§ 24.2 (± 6.6) Aa	45.4%
	1.4% Nitric Acid	§ 27.5 (± 5.4) Ab	35.0%	§ 27.5 (± 3.7) Aa	35.5%

(§) indicates statistically significant difference ($p \leq 0.05$) between a mean value at 2 years compared to its respective mean value at 24 hours for the same adhesive system and etchant. Means followed by distinct uppercase letters represent significant statistical difference ($p \leq 0.05$) in rows (comparing adhesive systems for the same etchant and within the same aging time). Means followed by distinct lowercase letters represent significant statistical difference ($p \leq 0.05$) in columns (comparing etchants for the same adhesive system and within the same aging time).

Regarding the 24-hour results of PBU, the positive control (phosphoric acid-etched, blot-dried dentin) group presented higher mean bond strength than the negative control (phosphoric acid-etched, air-dried dentin) group ($p = 0.008$) and the adhesive used in self-etch mode ($p = 0.015$). Dentin etching with 10-3 solution or 1.4% nitric acid demonstrated comparable bond strengths as the application of the adhesive in self-etch mode or both bonding techniques with phosphoric acid (blot-dried or air-dried dentin). After 2 years of storage in deionized water, PBU showed a significant reduction in dentin bond strength for all the tested etchants. Its application on phosphoric acid-etched, blot-dried dentin resulted in a mean bond strength higher than the other groups ($p \leq 0.05$), which did not differ among themselves ($p > 0.05$).

For the GBU adhesive system, bond strength means were not influenced by different etching approaches evaluated at either 24 hours or 2 years. A significant reduction in bond strength was also observed for all GBU groups after 2 years of storage.

With respect to the comparison between the tested adhesive systems, PBU resulted in higher bond strength compared to GBU when dentin was etched with phosphoric acid and blot-dried before adhesive resin application, which was observed at both aging times ($p = 0.044$ for 24 hours, and $p < 0.0001$ for 2 years). As for the remaining dentin etchants, there was no statistical difference between adhesives, regardless of aging time.

3.3. Failure mode analysis

The frequencies of occurrence of the different failure modes observed at 24 hours are depicted in Figure 1. There was a significant association between test group and failure mode [χ^2 (45 degrees of freedom)] = 136.66; $p < 0.0001$]. Significantly lower frequency of occurrence was observed for type-4 failures (mixed) when PBU was applied in self-etch mode (G5) compared to the remaining groups (adjusted Pearson residual < -3.30), while a significantly higher frequency of occurrence was seen for type-2 failures (between composite and adhesive resin) in this group. Type-3 failures (between dentin and adhesive resin) were more common for the negative control of GBU (G4), and type-5 failures (cohesive within the adhesive layer) occurred more often in the negative control and 10-3 solution groups of PBU (G2 and G7, respectively) than in the other groups (adjusted Pearson residual < 3.30).

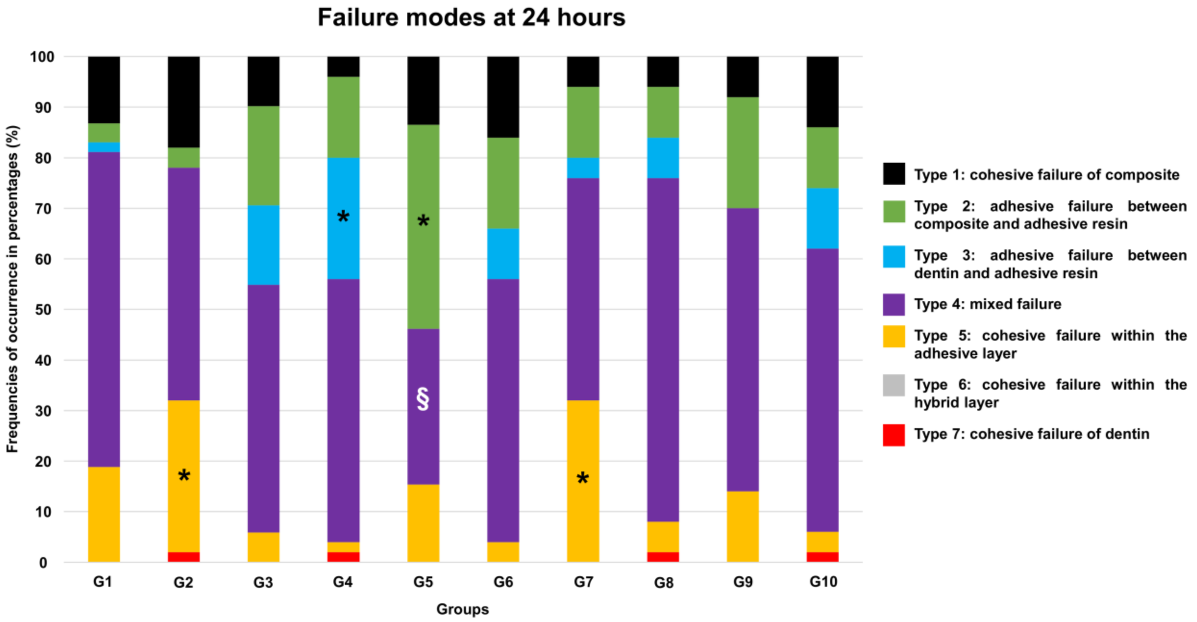

Figure 1. Distribution of failure modes at 24 hours according to the tested groups. (*) Represents a higher percentage of a certain failure mode compared to the other groups ($p<0.05$). (§) Represents a lower percentage of a certain failure mode compared to the other groups ($p<0.05$). G1: phosphoric acid-etched, blot-dried dentin + PBU; G2: phosphoric acid-etched, air-dried dentin + PBU; G3: phosphoric acid-etched, blot-dried dentin + GBU; G4: phosphoric acid-etched, air-dried dentin + GBU; G5: PBU in self-etch mode; G6: GBU in self-etch mode; G7: 10-3 solution + PBU; G8: 10-3 solution + GBU; G9: nitric acid + PBU; G10: nitric acid + GBU.

Failure mode analysis at 2 years is presented in Figure 2. Similarly to the 24-hour results, there was a significant association between test group and failure mode [χ^2 (54 degrees of freedom)] = 349.23; $p < 0.0001$]. GBU applied in self-etch mode (G6) presented a significantly lower frequency of occurrence of type-3 failures, while PBU used in self-etch mode (G5) or with 1.4% nitric acid (G9) had a significantly lower occurrence of type-4 failures compared to the other tested groups (adjusted Pearson residual < -3.28). Moreover, between-group comparisons showed type-1 failures were more commonly found when PBU was applied in self-etch mode (G5) or with 1.4% nitric acid (G9); type-2 failures were the main fractures resulting from the application of GBU in self-etch mode (G6); and type-4 failures were often observed in the positive control of PBU (G1) (adjusted Pearson residual < 3.28).

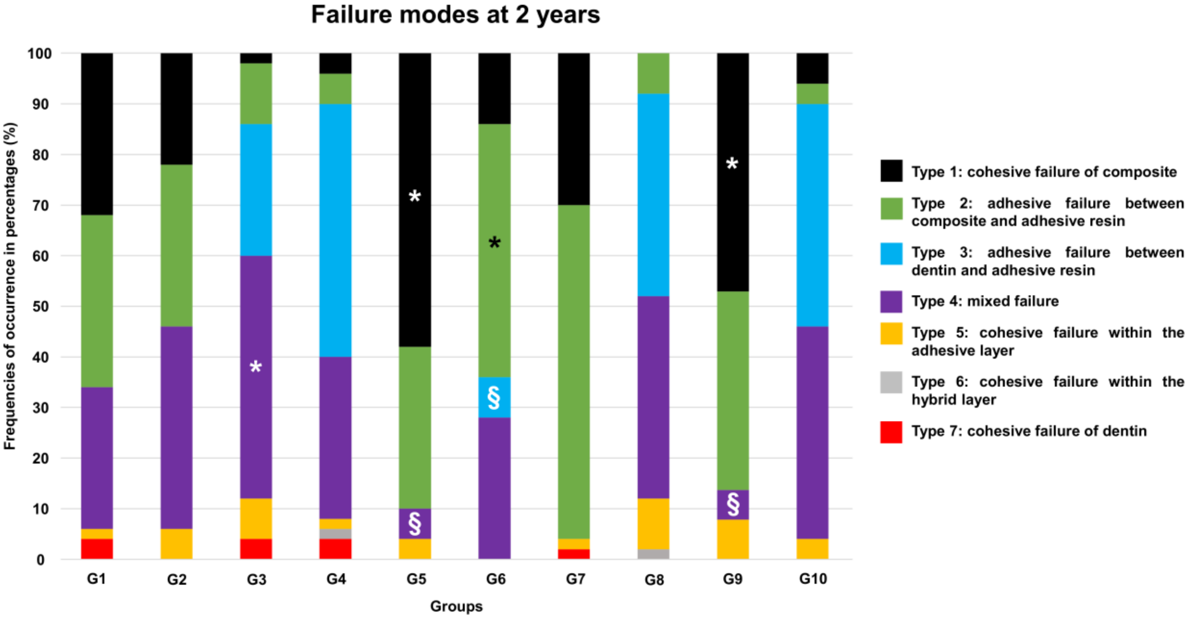

Figure 2. Distribution of failure modes at 2 years according to the tested groups. (*) Represents a higher percentage of a certain failure mode compared to the other groups ($p<0.05$). (§) Represents a lower percentage of a certain failure mode compared to the other groups ($p<0.05$). G1: phosphoric acid-etched, blot-dried dentin + PBU; G2: phosphoric acid-etched, air-dried dentin + PBU; G3: phosphoric acid-etched, blot-dried dentin + GBU; G4: phosphoric acid-etched, air-dried dentin + GBU;

G5: PBU in self-etch mode; G6: GBU in self-etch mode; G7: 10-3 solution + PBU; G8: 10-3 solution + GBU; G9: nitric acid + PBU; G10: nitric acid + GBU.

3.4. Nanoleakage evaluation of the dentin-adhesive interface

Figures 3 and 4 show representative micrographs of the nanoleakage (silver impregnation) analysis at 24 hours and after 2 years of water storage, respectively. These micrographs are backscattered electron images obtained in compositional mode by SEM. The 24-hour evaluation clearly demonstrated a material-dependent trend in silver deposition within hybrid layers. All groups bonded with PBU presented no to little deposition of silver, except for its negative control (G2), which showed a reasonable amount of silver impregnation. However, GBU led to remarkably high amounts of silver deposits within the bonding interfaces, regardless of aging time. When the SEM analysis was repeated after 2 years of storage, the groups G1, G5, G7, and G9 (PBU) maintained a trend of low silver deposition, while all the remaining groups experienced a substantial increase in nanoleakage.

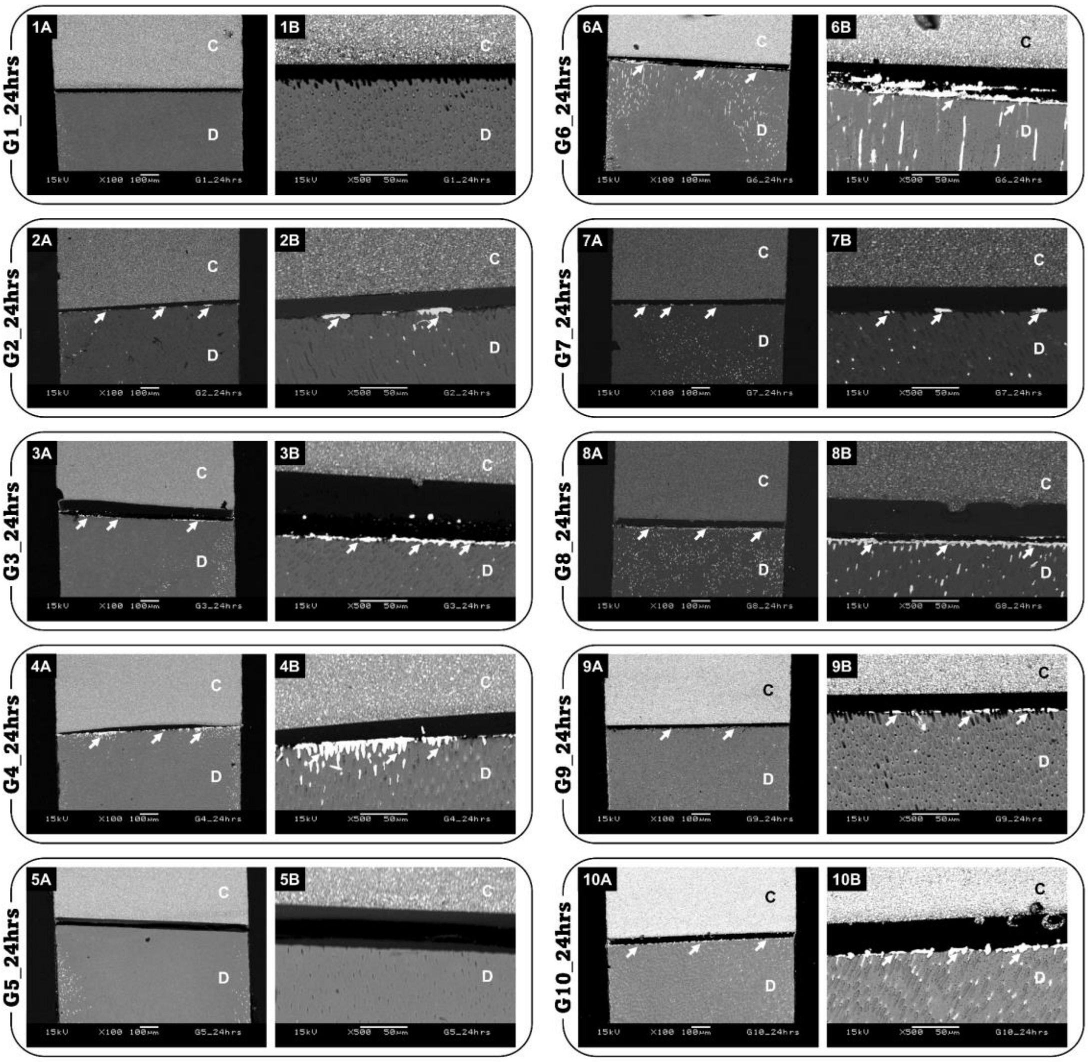

Figure 3. Representative micrographs of nanoleakage evaluation by scanning electron microscopy, according to the groups tested at 24 hours. Each pair of micrographs represents a single test group. Right-hand images (1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B, 9B, and 10B) correspond to a magnification of 500x of the left-hand images (1A, 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A, and 10A), taken at 100x. Arrows: silver deposition within the bonding interface; C: composite; D: dentin. G1: phosphoric acid-etched, blot-dried dentin + PBU; G2: phosphoric acid-etched, air-dried dentin + PBU; G3: phosphoric acid-etched, blot-dried dentin + GBU; G4: phosphoric acid-etched, air-dried dentin + GBU; G5: PBU in self-etch mode; G6: GBU in self-etch mode; G7: 10-3 solution + PBU; G8: 10-3 solution + GBU; G9: nitric acid + PBU; G10: nitric acid + GBU.

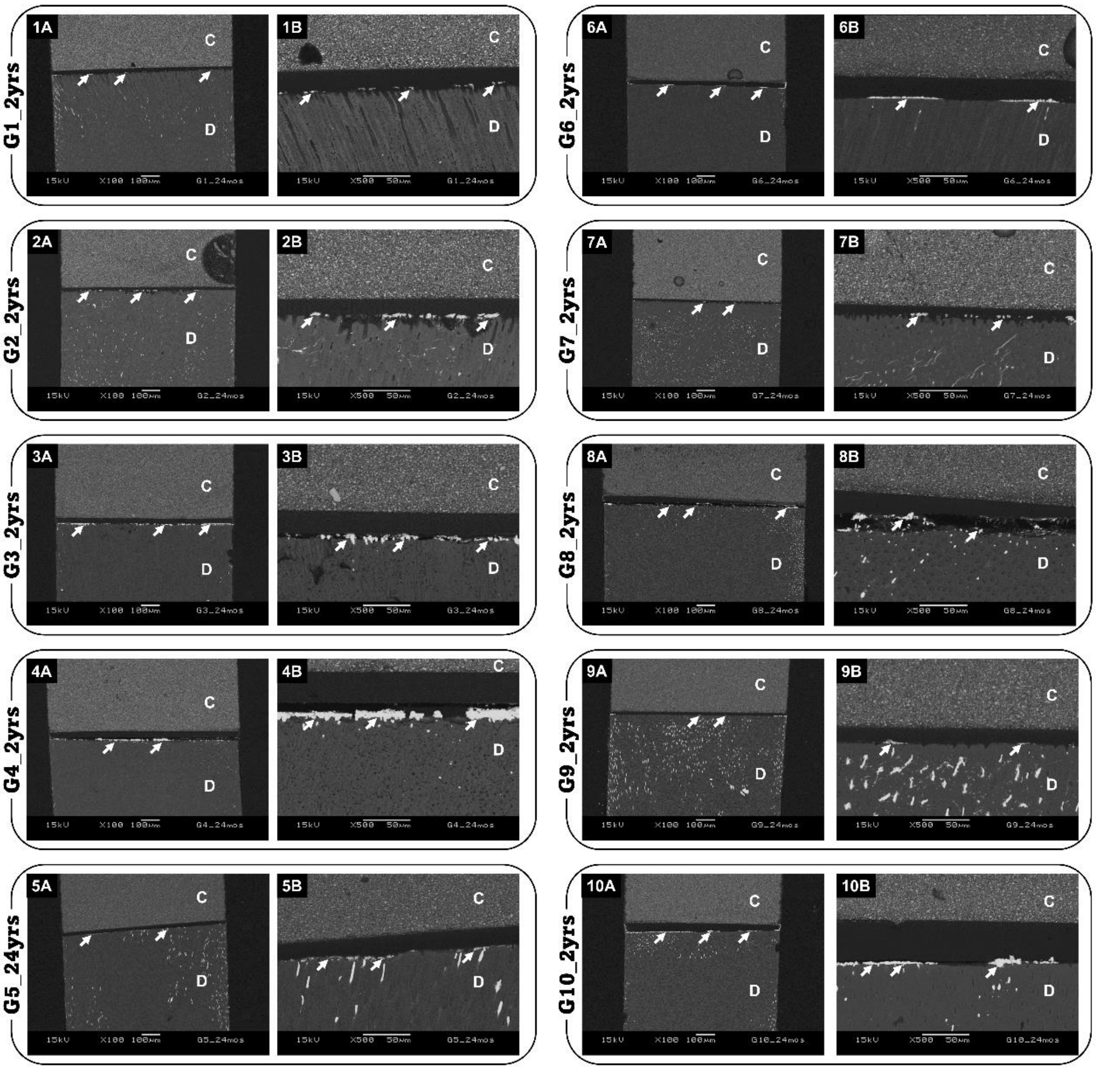

Figure 4. Representative micrographs of nanoleakage evaluation by scanning electron microscopy, according to the groups tested at 2 years. Each pair of micrographs represents a single test group. Right-hand images (1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B, 9B, and 10B) correspond to a magnification of 500x of the left-hand images (1A, 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A, and 10A), taken at 100x. Arrows: silver deposition within the bonding interface; C: composite; D: dentin. G1: phosphoric acid-etched, blot-dried dentin + PBU; G2: phosphoric acid-etched, air-dried dentin + PBU; G3: phosphoric acid-etched, blot-dried dentin + GBU; G4: phosphoric acid-etched, air-dried dentin + GBU; G5: PBU in self-etch mode; G6: GBU in self-etch mode; G7: 10-3 solution + PBU; G8: 10-3 solution + GBU; G9: nitric acid + PBU; G10: nitric acid + GBU.

3.5. *In situ* zymography of the hybrid layer

3.5.1. Prime&Bond Universal adhesive system

Micrographs of the *in situ* zymography analysis for each dentin etching approach used with PBU are shown on the upper half of Figure 5, indicating endogenous enzymatic activity. These micrographs are a result from the overlap between the image acquired in the green channel (showing fluorescence in dentin tubules and within the hybrid layer), and the image of differential interference contrast (showing the optical density of the resin-dentin interface). A graphical representation of the IntDen quantification for PBU is presented at the bottom of Figure 5. *In situ* zymography demonstrated a significant influence of the tested etchants on the results ($p = 0.001$). PBU applied in self-etch mode showed higher IntDen than 10-3 solution ($p = 0.025$, adjusted using the Bonferroni correction) and 1.4% nitric acid ($p = 0.001$, adjusted using the Bonferroni correction), which did not differ statistically from each other ($p = 1.000$, adjusted using the Bonferroni correction). IntDen for 37% phosphoric acid did not differ statistically from any of the other groups ($p > 0.05$, adjusted using the Bonferroni correction).

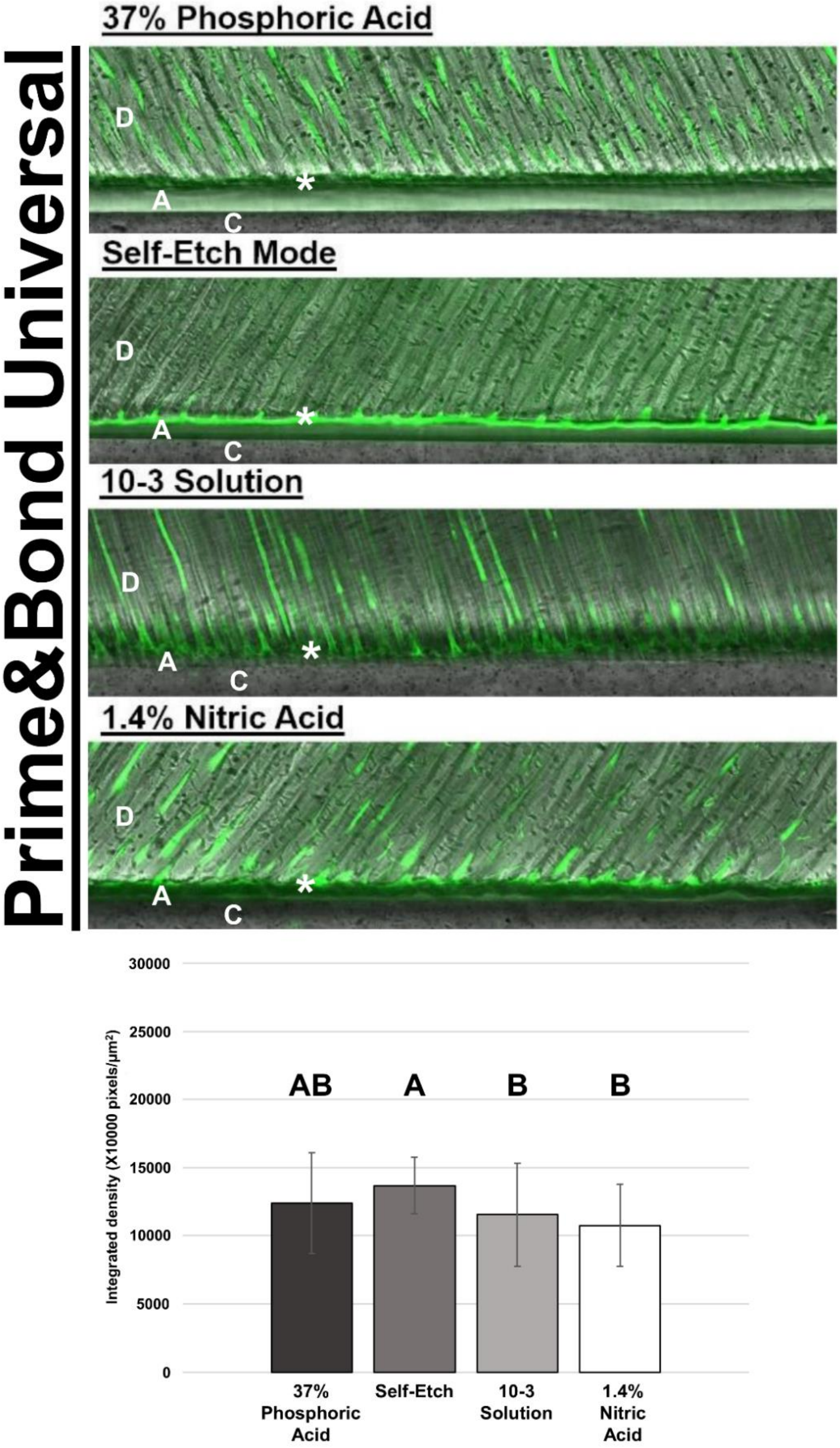

Figure 5. Micrographs after 24-hour incubation with quenched fluorescein-labeled gelatin, and quantification of gelatinolytic activity for the adhesive-dentin interfaces of PBU, according to the tested etchants. Groups marked with the same uppercase letters did not differ statistically ($p > 0.05$). (*) indicates the hybrid layer. D: dentin; A: adhesive layer; C: composite.

3.5.2. Gluma Bond Universal adhesive system

Figure 6 (upper half) illustrates the *in situ* zymography analysis for each dentin etchant used with GBU, indicating dentin endogenous enzymatic activity. Images were acquired as described above for PBU. A graphical representation of the IntDen evaluation for GBU is also presented in Figure 6 (bottom half). The zymography assay showed a significant influence of the tested etchants on the IntDen results ($p < 0.001$). GBU used in self-etch mode had lower IntDen than 37% phosphoric acid ($p < 0.001$, adjusted using the Bonferroni correction), 10-3 solution ($p < 0.001$, adjusted using the Bonferroni correction), and 1.4% nitric acid ($p = 0.001$, adjusted using the Bonferroni correction), which did not differ significantly among themselves ($p = 1.000$ for 37% phosphoric acid x 10-3 solution, $p = 1.000$ for 37% phosphoric acid x 1.4% nitric acid, and $p = 0.820$ for 10-3 solution x 1.4% nitric acid, adjusted using the Bonferroni correction).

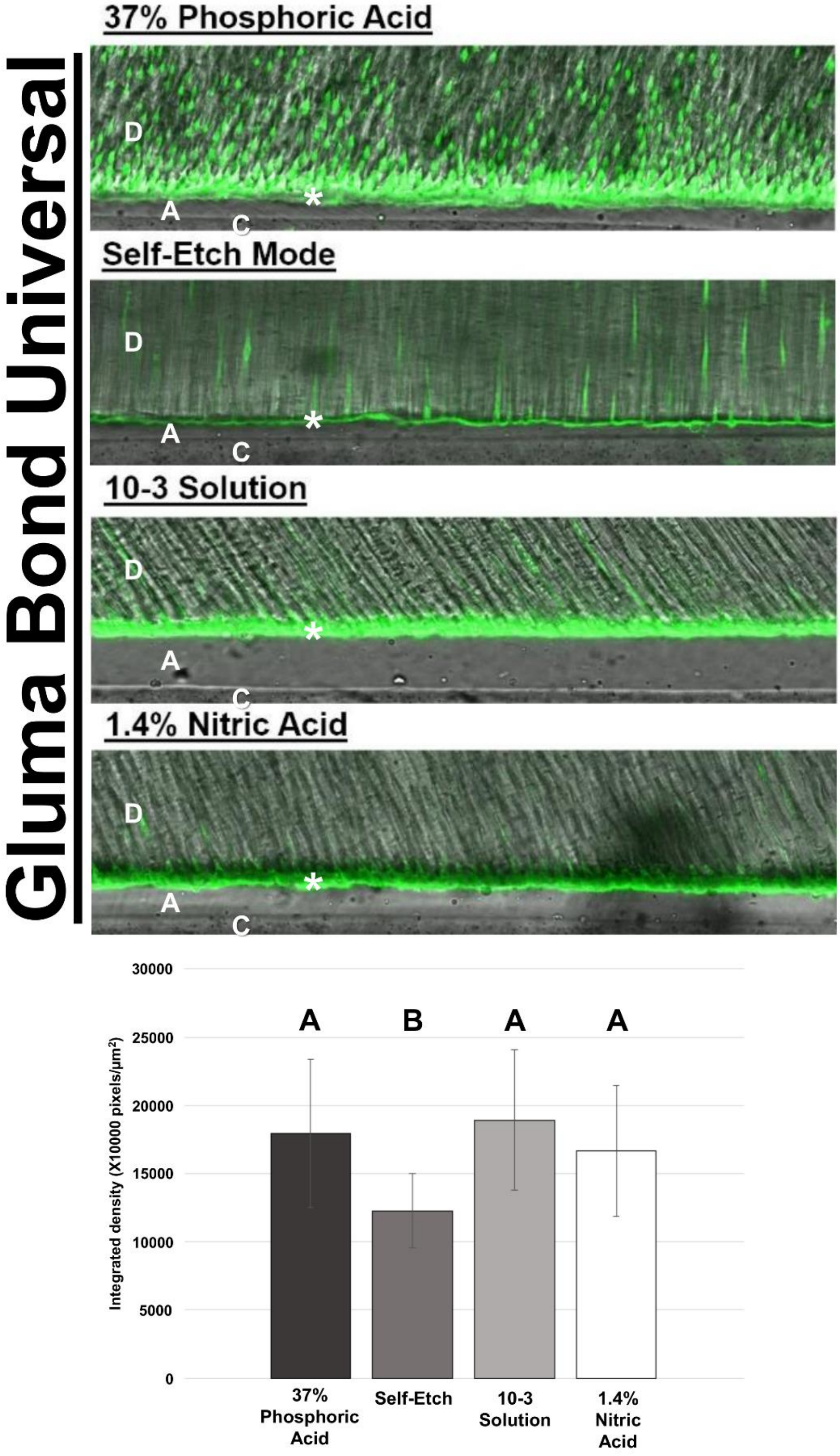

Figure 6. Micrographs after 24-hour incubation with quenched fluorescein-labeled gelatin, and quantification of gelatinolytic activity for the adhesive-dentin interfaces of GBU, according to the tested etchants. Groups marked with the same uppercase letters did not differ statistically ($p > 0.05$). (*) indicates the hybrid layer. D: dentin; A: adhesive layer; C: composite.

4. Discussion

The main goal of this study was to find alternative etchants to replace phosphoric acid in dental bonding procedures. Hence, enamel and dentin bond strengths were evaluated to determine whether the experimental etchants would provide adequate bonding to both tooth tissues. Furthermore, dentin endogenous enzymatic activity after treatment with the alternative etchants was assessed, since it could influence hybrid layer longevity.

Results from the enamel shear bond strength test (Table 3) revealed no significant difference in bond strength means between any of the investigated groups ($p > 0.05$). Therefore, the first null hypothesis was accepted. The similar results among etchants regarding enamel bonding were expected, since citric and nitric acids have been shown to interact with human hard tissues following the adhesion-demineralization concept, akin to phosphoric acid [24]. Additionally, similar performance of the tested adhesives might be due to their proper interaction with etched enamel, despite having different monomeric composition. Both adhesives contain the functional monomers 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), besides dipentaerythritol pentaacrylate monophosphate (PENTA) within PBU, and 4-methacryloyloxyethyl trimellitate anhydride (4-META) within GBU, which can chemically interact with calcium from hydroxyapatite in the predominantly inorganic enamel tissue (96% w/w) [32, 33].

Conversely, achieving effective dentin bonding is much more challenging, technique-sensitive, and dependent on the type of adhesive, because of the higher organic content, mineral distribution in a non-prismatic structure, and moisture of dentin compared to enamel [34]. The present study evaluated 10-3 solution and diluted nitric acid as alternative dentin etchants that could allow dry-bonding and potentially inhibit MMP activity [16]. When dentin was etched by 10-3 solution prior to the application of PBU, a 24-hour bond strength mean similar to dry- or wet-bonding with phosphoric acid and self-etching was obtained (Table 4), along with a low degree of nanoleakage (Figure 3). Likewise, there was no difference between the bond strengths of 10-3 solution and phosphoric acid-etching, either blot- or air-dried, by switching the adhesive to GBU, although high silver deposition was observed (Figure 3). Regardless of the tested bonding agent, the 24-hour dentin microtensile bond strength means of 10-3 solution were always above 40 MPa and accompanied by high rates of mixed failures (Figure 1).

The combination of nitric acid-based etchant with PBU performed similarly to wet-bonding with phosphoric acid or the adhesive in self-etch mode concerning dentin bond strength at 24 hours, with few nanoleakage spots along the bonding interface (Figure 3). The same behavior was observed after the application of GBU on nitric acid-etched dentin, although silver deposition was greatly increased (Figure 3). High 24-hour bond strengths around 42 MPa, mainly characterized by mixed failures (Figure 1), were achieved by nitric acid for both tested universal adhesives. Results about the experimental etchants led to the rejection of the second null hypothesis.

A previous publication showed 10-3 solution and diluted nitric acid could be viable etchants for dry-bonding to dentin using simplified, etch-and-rinse adhesives [16]. The good performance of the adhesives following dry-bonding might be attributed to the iron ions in 10-3 solution, which form insoluble aggregates with dentin polyelectrolytes that avoid the collapse of the collagen network due to air-drying [19, 35], while also modifying surface energy. Interactions between dissociated ions from nitric acid and exposed collagen on dentin surface have also been suggested to help stabilize the collagen network during air-drying [16]. However, in spite of promising results at 24 hours, none of the experimental etchants (10-3 solution or nitric acid) could circumvent the reduction in dentin bond strength after 2 years of storage, which was likely related to the lack of *in situ* MMP-inhibiting effect of these solutions and adhesive resin degradation (Figures 5 and 6).

The microtensile bond strength data was also analyzed taking into account the two different adhesive systems. When dentin bond strength data of both adhesive systems are set side by side, considering only the positive control group (phosphoric acid-etched, blot-dried), the influence of adhesive type can be observed. At both aging times (24 hours and 2 years), PBU performed better than GBU only on blot-dried dentin, showing higher bond strength means with less nanoleakage within the hybrid layer (Table 4, Figures 3 and 4).

One of the main differences between the selected adhesive systems is the organic solvent in their composition. PBU has an alcohol-based solvent (propan-2-ol), whereas GBU contains acetone. Previous literature has suggested acetone-based adhesives are highly sensitive to moisture [36], as they demand a higher amount of water on the etched dentin surface for optimal hybridization [1], which might explain the significant difference observed between adhesives for the wet-bonding technique.

Long-term evaluation showed a significant decrease in dentin microtensile bond strength for all groups using phosphoric acid as etchant. Reduction rates ranged from 16.8% to 23.9% for PBU, and from 39.9% to 43.6% for GBU, according to the bonding technique (wet- versus dry-bonding, respectively - Table 4). These findings further supported the rejection of the second null hypothesis. The exposure of MMPs by phosphoric acid etching [29, 37], and their activation due to buffering induced by the application of higher-pH resin monomers and solvents [38], results in the degradation of collagen fibrils left unprotected mainly at the bottom of the hybrid layer. Acid etchants, such as phosphoric acid, might also remove the enzyme-bound MMP inhibitors TIMP-1 and TIMP-2 [39], as well as facilitate the interaction between MMPs and collagen by exposing the catalytic binding site of the α2 chain of tropocollagen [13]. The harmful effect of phosphoric acid on dentin bonding longevity caused by these mechanisms was clearly observed in this study, since the tested adhesives showed prominent dentin endogenous enzymatic activity (Figures 5 and 6), followed by significant bond strength reduction and silver deposition at the bottom of the hybrid layer after 2 years of water storage.

Judging by the results, one could imply PBU benefited from the etch-and-rinse approach with 37% phosphoric acid, since lower bond strength and similar nanoleakage within the bonding interface were observed for the self-etching group at both aging times (Table 4, Figures 3 and 4). Ultra-mild self-etch adhesives like PBU have only limited, nanometric interaction with dentin [40, 41], at most causing a slight demineralization of smear plugs [42], without removing the smear layer. A systematic review and meta-analysis of *in vitro* studies demonstrated dentin microtensile bond strength of ultra-mild universal adhesives was improved by the etch-and-rinse strategy, probably due to better hybridization and resin tags formation provided by phosphoric acid etching [43].

Moreover, the solvent employed in PBU (propan-2-ol) has a lower dielectric constant than mostly used ethanol [44]. Salz et al. (2006) suggested a decrease in the dielectric constant of a solvent could increase the pKa value of its solvated acidic monomer [45], reducing the presence of hydrolyzed species from the functional monomer, which could hinder its interaction with calcium from dentin. Another problematic characteristic of the bonding interface produced by self-etching with PBU was the significantly higher incidence of adhesive failures between adhesive resin and composite at 24 hours. This raises questions about the compatibility between the tested adhesive and composite, since type-2 failures have been linked to chemical reactions of acidic adhesive monomers with tertiary amines from the overlying composite [46]. Thus, the third null hypothesis was rejected.

Conversely, GBU in self-etch mode could reach bond strength means similar to blot-dried, phosphoric acid-etched dentin, although significant nanoleakage was registered at both evaluation times (Table 4, Figures 3 and 4). GBU is a more acidic universal adhesive, which promotes a deeper demineralization effect on dentin [42]. Interestingly, despite the high bond strength mean obtained by self-etching with GBU, there was still prominent silver uptake within the bonding interface, which was also the case for all groups using this universal adhesive (Figures 3 and 4). The higher nanoleakage could be related to the phase separation during solvent evaporation of bonding agents containing acetone [6], which can impair the diffusion of adhesive resin into the bottom region of the etched surface. Besides, the highly hydrophilic nature of GBU, coupled with its low pH, might have left poorly polymerized acidic monomers and oligomers on dentin surface, which could continue to etch the substrate well after bonding, and consequently favor silver deposition [47].

Nonetheless, the *in situ* enzymatic activity analysis for both universal adhesive systems using the alternative etchants led to gelatinolytic activity similar to phosphoric acid, with no effective reduction in MMP activity. Therefore, the fourth null hypothesis was rejected. The lack of MMP inhibition found in the present study for the experimental etchants might be explained by inherent characteristics of dentin bonding when performing *in situ* zymography: surface area, rinsing of the etchants with water, and time of treatment. Gelatin zymography uses fine dentin powder as substrate, which is incubated with the etchants to be tested for 10 minutes, at 4 °C, under constant agitation [39, 48]. In this study, *in situ* zymography utilized a flat, mineralized dentin surface as substrate, and the experimental solutions were applied for 15 s, followed by complete rinsing to simulate clinical application [16, 29]. Hence, the short clinical application time of 15 s on a mineralized flat surface, coupled with rinsing the solutions off dentin surfaces, might have hindered the interaction of ferric chloride or nitrate ions with MMPs in the *in situ* zymography approach. Although Rodrigues et al. (2017) have reported iron binding to the collagen matrix after a 15-s exposure, completely demineralized dentin slabs were used for energy-dispersive Xray spectroscopy analysis in that study [21], which might not apply to a dentin surface with a considerable amount of mineral content.

The use of the tested universal adhesive systems without a previous, separate etching step did not avoid the *in situ* activation of dentin MMPs (Figures 5 and 6). These results agree with previous findings of increased enzymatic activity caused by self-etch adhesives, considering their acidic monomers can expose dentin proteases and activate latent MMPs through the cysteine-switch mechanism [39, 49]. Nonetheless, different patterns of MMP activity were observed for the investigated adhesives: PBU in self-etch mode presented similar *in situ* MMP activity when compared to its use with phosphoric acid, while self-etching with GBU led to lower MMP activation than its combination with phosphoric acid, although still at a significant degree.

The key to explaining this difference might lay on the pH values of these universal adhesives. Nishitani et al. (2006) suggested if one-step, relatively weak (pH ≈ 2.4) self-etch adhesives were used, MMP activation far beyond baseline levels would be reached with no denaturation of enzymes [50]. Also, single-step, self-etch bonding agents have been shown to offer strong buffering capacity when mixed with dentin powder, elevating pH values around 6.3-7.11 [51]. Thus, we hypothesize that even though PBU would not etch dentin at the same depth or expose as much endogenous proteases as phosphoric acid, it could still result in relevant MMP activity, since the pH of etched dentin would get closer to the optimal pH for MMPs [52]. As for GBU, a recent study reported a similar trend for an intermediately strong, 4-MET-based, self-etch adhesive, that is high dentin MMP activity and extracellular matrix solubilization by endogenous proteases, yet lower than the phosphoric acid-treated group [12]. Hence, the reduction of dentin bond strength over storage time found in this study is understandable, given the proteolytic activity and less efficient dentin-adhesive interaction created by the tested universal adhesives used in self-etch mode.

Nevertheless, it seems promising that bond strength values after treatment with the experimental etchants were comparable to those after conventional phosphoric acid etching, while enabling a dry-bonding technique, a much less technique-sensitive clinical option. Further studies are necessary to investigate if the tested etchants or their derived ions could still be incorporated into dentin primers or adhesive resins in order to optimize their effects on bonding.

5. Conclusions

The following conclusions can be drawn from the results presented by this study:

1. The experimental etchants (10-3 solution and 1.4% nitric acid) could provide enamel shear bond strengths comparable to phosphoric acid.
2. When the tested universal adhesives were applied following the traditional wet-bonding technique using phosphoric acid, Prime&Bond Universal presented higher bond strength than Gluma Bond Universal.
3. None of the tested universal adhesive systems should be used following a dry-bonding protocol on dentin after etching with phosphoric acid.
4. The etch-and-rinse strategy led to more reliable dentin bonding when Prime&Bond Universal was used, whereas etching approach and dentin residual moisture did not seem to influence the results for Gluma Bond Universal.
5. After long-term storage for 2 years, all of the investigated etching approaches presented a significant reduction in dentin bond strength for both adhesive systems.
6. Despite promising 24-hour results regarding dentin bonding, the experimental etchants did not inhibit *in situ* MMP activity or avoided bonding interface degradation.
7. Nanoleakage within the dentin-adhesive interface seemed to be material-dependent, showing more silver deposition in those samples bonded with the acetone-based universal adhesive.

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2.3 Microtensile bond strength, bonding interface morphology, adhesive resin infiltration, and marginal adaptation of bulk-fill composites placed using different adhesives

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ABSTRACT

Purpose: This study evaluated dentin bond strength, failure mode, interface morphology, adhesive infiltration into dentin, and marginal adaptation of bulk-fill composites used with different adhesives. *Materials and Methods:* Third molars received occlusal class I cavities (4 mm x 4 mm x 4 mm) that were bulk-filled with Admira Fusion x-tra (Voco) or SonicFill 2 (Kerr) using four adhesives (Scotchbond Multipurpose, 3M Oral Care; Clearfil SE Bond, Kuraray Noritake; OptiBond All-In-One, Kerr; Futurabond U, Voco). Scotchbond was used with acid-etching, while the remaining adhesives were applied in self-etch mode. Sixty-four teeth were selected for the microtensile bond strength test (n = 8). Failure modes were analyzed with scanning electron microscopy (SEM). Interface morphology and adhesive infiltration (n = 3) were investigated using confocal laser scanning microscopy (CLSM). Marginal adaptation (n = 3) was also evaluated using SEM. Bond strength, failure mode, and adhesive infiltration data were analyzed for distribution and homocedasticity, followed by appropriate statistical analyses ($\alpha = 0.05$). *Results:* Regarding bond strength, no differences were found among adhesives for SonicFill; Clearfil showed a significantly lower mean value than Scotchbond ($p \leq 0.05$) for Admira; the two composites did not differ. Adhesive and mixed failures were observed for all groups. Scotchbond led to thicker hybrid layers with deeper adhesive infiltration as opposed to Futurabond. The groups Admira+Futurabond, SonicFill+Clearfil, and SonicFill+Futurabond presented the highest marginal discontinuity. *Conclusion:* The tested bulk-fill composites did not affect dentin bonding. Scotchbond and Clearfil seem to be reliable for bonding SonicFill 2 to

dentin. Futurabond presented questionable performance, given its poor-quality interface and higher percentages of marginal gaps.

KEYWORDS: dentin, composite resin, ultrasonics, dental etching, dental bonding, adhesive.

INTRODUCTION

The use of resin-based composites as direct restorative materials for posterior teeth has increased in recent decades.[2,12] The clinical success of direct restorations made with composites depends on several factors, namely patient selection, cavity location and size, material choice, composite placement technique, and light-curing.[47] Although the available dental literature reports long-term clinical service for composite restorations, undesirable polymer-inherent side-effects, such as polymerization shrinkage and low wear resistance of some types of composites, still limit the use of these materials.[12] In fact, polymerization shrinkage-induced stresses at the tooth-restoration interface may result in enamel cracks, postoperative sensitivity, poor marginal adaptation, marginal discoloration, microleakage (which can lead to recurrent carious lesions), and ultimately, failed restorations.[18]

Some modifications in the composition of resin composites have been suggested, such as alterations in monomeric composition and photoinitiators, as well as changes in the amount of monomers, shape, type, and/or superficial treatment of filler particles.[26] Consequently, low polymerization-shrinkage composites, known as bulk-fill, were developed. These materials enable clinicians to perform direct restorations by placing a single composite layer up to 4-5 mm thick (depending on manufacturer’s instructions) all at once, which is due to their ability to generate lower shrinkage stress, while presenting higher reactivity to light-curing.[32] Among the advantages of bulk-fill composites, reduced incorporation of defects into restorations and faster clinical restorative procedures are particularly interesting.[8,41]

Admira Fusion x-tra (Voco; Cuxhaven, Germany) is a bulk-fill composite based on organically modified ceramics (ormocers). Ormocers are hybrid polymers characterized by a siloxane network selectively modified by the incorporation of organic groups (polysiloxanes with light-curable methacrylates covalently bonded to silica).[35] This special composition renders the monomer molecules larger and, consequently, might reduce polymerization shrinkage, wear, and leaching.[7]

According to the manufacturer, this ormocer-based material would reduce the volumetric shrinkage to an extremely low level (1.25% by volume), causing very low shrinkage stress (3.87 MPa).[54] SonicFill 2 (Kerr; Orange, CA, USA) is the second generation of a high-viscosity bulk-fill composite that requires the use of an ultrasonic handpiece for application. The energy delivered to the material by sonic activation should produce a significant reduction in its viscosity, turning it into a flowable composite-like material, which would improve its adaptation to the cavity walls and margins.[20] Once the sonic energy is removed, the composite would gradually return to its initial high viscosity, assuring adequate mechanical properties for the restoration.[38] Additionally, ultrasonic application should also reduce void formation in the bulk material.[25]

Similar to non-bulk-fill composites, the hybridization process is responsible for bonding bulk-fill composites to the dentin substrate.[40,59] This process is characterized by the interdiffusion of adhesive monomers into dentin, creating a hybridization zone (layer of resin-reinforced dentin) that bonds the restorative material to the substrate.[36] Conventionally, in order to create a hybrid layer, the dentin surface needs to be etched with phosphoric acid to expose collagen fibrils.[43] Hence, the adhesive resin will infiltrate into the collagen-fibril network, creating micromechanical interlocking to retain the restoration in position.[39]

Adhesives can be classified regarding how they interact with the substrate and their number of clinical application steps.[61] There are etch-and-rinse adhesives, which usually involve two or three application steps, and self-etch adhesives, commonly applied in one or two steps.[53] Etch-and-rinse bonding agents require a separate etching step with phosphoric acid before their application to remove the smear layer and demineralize the dentin surface.[37] Conversely, self-etch adhesives do not need previous etching with phosphoric acid, as they contain acidic monomers capable of mildly etching and infiltrating dentin simultaneously, in addition to chemically reacting and bonding with calcium from dentin hydroxyapatite.[62] Recently, “universal” adhesives, designed to be used in either etch-and-rinse or self-etch approaches, depending on the preference of the operator, have also been introduced in the market.[49]

The bond strength created by the adhesive should be able to withstand the polymerization shrinkage stress of the composite, helping ameliorate its consequences. In fact, a recent publication demonstrated the bonding strategy (etch-and-rinse or self-etch) significantly influenced the mean values of shrinkage vectors in class-I cavities, which might have occurred because of the lower bond strength achieved with the self-etch approach, according to the authors.[29] Furthermore, the rapid rise and evolution of several types of bulk-fill composites, as well as the paucity of studies dealing with the interaction between these restorative materials and the dentin bonding approaches currently available (3-step etch-and-rinse, 2-step self-etch, and 1-step self-etch) should be considered. Thus, this study aimed to analyze the influence of different adhesives on the bonding interfaces created in class I cavities filled with two currently available bulk-fill composites (Admira Fusion x-tra and Sonic-Fill 2). In order to achieve this objective, dentin microtensile bond strength, failure mode, morphology of the bonding interface, adhesive resin infiltration, and marginal adaptation were evaluated.

The null hypotheses were: 1. there would be no statistically significant differences in dentin bond strength between the tested composites when the same adhesive was used; 2. there would be no statistically significant differences in dentin bond strength between the tested adhesives when the same composite was used; 3. there would be no statistically significant differences in the proportion of failure mode types within each of the tested groups; 4. marginal discontinuity along the cavity walls would not differ between the investigated composites or between the tested bonding agents; 5. the morphology of the bonding interface would not differ between the tested bonding agents; 6. there would be no statistically significant differences in adhesive resin infiltration between the tested bonding agents.

MATERIALS AND METHODS

Teeth Selection and Preparation

One hundred twelve sound human third molars were collected and utilized according to a protocol approved by the Ethics Committee in Research of the Piracicaba Dental School – University of Campinas (CAAE #86272518.3.0000.5418). All teeth were cleaned by hand scaling with a periodontal curette (SS White Duflex; Juiz de Fora, MG, Brazil), and polished with a paste of pumice and water. Afterwards, they were stored in thymol solution (Labsynth; Diadema, SP, Brazil) at 4°C for no longer than three months.

Dentin Microtensile Bond Strength (μTBS) Test

Sixty-four teeth were selected and randomly assigned into eight groups (n = 8) according to bonding strategy (three-step etch-and-rinse, two-step self-etch, or one-step self-etch) and bulk-fill composite (Admira Fusion x-tra, Voco; SonicFill 2 Single-Fill Composite System, Kerr). Table 1 describes the materials used in this study, and Table 2 presents the different experimental groups and their corresponding acronyms. Using a diamond saw (Buehler; Lake Bluff, IL, USA) under water cooling, the teeth were sectioned 2 mm below the cementoenamel junction to remove their roots, and the occlusal enamel was flattened by grinding the teeth down on silicon carbide paper (600-grit). Then, standardized 4 mm x 4 mm x 4 mm occlusal class I cavities were prepared with a diamond bur (#1016, KG Sorensen; Barueri, SP, Brazil) by a single operator (MRS) who had been previously trained and calibrated by one of the authors with extensive clinical experience (MG).

Table 1. Commercial name (manufacturer), shade, composition, and batch number of the materials used in the present study.

Material (manufacturer), shade	Composition	Batch number
Admira Fusion x-tra Nano-hybrid ORMOCER restorative material (Voco GmbH), shade A2	Organically modified silicic acid, barium-aluminum-silica-glass, silica nanoparticles	1840062
SonicFill 2 Single-Fill Bulk Fill Dental Composite System (Kerr Corporation), shade A2	Silicon dioxide, glass, oxide, chemicals, Poly(oxy-1,2-ethanediyl), α,α'-[(1-methylethylidene)di-4,1-phenylene]bis[ω-[(2-methyl-1-oxo-2-propen-1-yl)oxy]-, ytterbium trifluoride, 2,2'-ethylenedioxydiethyl dimethacrylate	6964456
Adper Scotchbond Multi-Purpose Adhesive (3M Oral Care)	Primer: water, 2-hydroxyethyl methacrylate, copolymer of acrylic and itaconic acids Adhesive: bisphenol A diglycidyl ether dimethacrylate, 2-hydroxyethyl methacrylate	N876946
Clearfil SE Bond (Kuraray Noritake Dental Inc.)	Primer: 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, water, accelerators, dyes Bond: bisphenol A diglycidylmethacrylate, 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate, hydrophobic aliphatic dimethacrylate, colloidal silica, dl-camphorquinone, initiators, accelerators	CMo165
OptiBond All-In-One Single Component Self-Etch Dental Adhesive (Kerr Corporation)	Acetone, 2-hydroxyethyl methacrylate, ethanol, 2-hydroxy-1,3-propanediyl bismethacrylate	LL01577
Futurabond U Dual-curing universal adhesive (Voco GmbH)	Liquid 1: bisphenol A diglycidyl ether dimethacrylate, 2-hydroxyethyl methacrylate, 1,6-hexanediylbismethacrylate, acidic adhesive monomer, urethanedimethacrylate, catalyst Liquid 2: ethanol, initiator, catalyst	1748169
Ultra-Etch Etchant (Ultradent Products Inc.)	Phosphoric acid, dimethicone	BFDJ8

Table 2. Experimental groups.

Group Acronym	Composite	Adhesive	
		Commercial name	**Classification**
ADM + SB	Admira Fusion x-tra	Scotchbond Multipurpose	3E&R
ADM + CF	Admira Fusion x-tra	Clearfil SE Bond	2SE
ADM + OB	Admira Fusion x-tra	OptiBond All-In-One	1SE
ADM + FU	Admira Fusion x-tra	Futurabond U	U
SF + SB	SonicFill 2	Scotchbond Multipurpose	3E&R
SF + CF	SonicFill 2	Clearfil SE Bond	2SE
SF + OB	SonicFill 2	OptiBond All-In-One	1SE
SF + FU	SonicFill 2	Futurabond U	U

Abbreviations: 3E&R (three-step etch-and-rinse); 2SE (two-step self-etch); 1SE (one-step self-etch); U (universal).

Four different adhesives were used: a three-step etch-and-rinse bonding agent (3E&R, Adper Scotchbond Multipurpose, 3M Oral Care; St Paul, MN, USA); a two-step self-etch bonding agent (2SE, Clearfil SE Bond, Kuraray Noritake; Osaka, Japan); a one-step self-etch bonding agent (1SE, Optibond All-in-One, Kerr), and a universal bonding agent (U, Futurabond U, Voco). Although Adper Scotchbond Multipurpose is not considered the gold standard for etch-and-rinse adhesives, it is still a literature-proven, widely available, three-step etch-and-rinse system. Many reports have shown Adper Scotchbond Multipurpose provides adequate dentin bonding,[13,17,34] and therefore its use in the present study as a control group was very unlikely to hinder the results. Clearfil SE Bond, on the other hand, was chosen because it is the gold standard for self-etch adhesives, and one of the main objectives of this study was to compare its performance with the simplified self-etch/universal bonding agents recommended by each manufacturer of the tested bulk-fill composites. Optibond All-in-One and Futurabond U were selected not only because they are both self-etch products recommended by the manufacturers of the tested composites, but also because they present a similar composition, which is based on a mixture of methacrylates and bis-methacrylates.

For the 3E&R adhesive, enamel and dentin were both etched with 35% phosphoric acid gel (Ultra-Etch, Ultradent; South Jordan, UT, USA) for 30 and 15 s, respectively. In regard to the remaining adhesives, selective enamel etching was carried out for 30 s with the same phosphoric acid gel mentioned above, while dentin was treated using the self-etch approach. All adhesives were applied and light-cured according to their respective manufacturers' instructions. Subsequently, the cavities were bulk-filled by the selected composites using single 4-mm-thick increments, which were light-cured with a multiple-peak LED light-curing unit (Valo, Ultradent) for 20 s. Before starting the experimental procedures, the light-curing unit was checked using a spectroradiometer (MARC-PS, BlueLight Analytics; Halifax, NS, Canada) to ensure the delivery of a radiant exposure of at least 16.8 J/cm^2.[50] Admira Fusion x-tra was applied using an insertion instrument, while SonicFill 2 was placed into the cavities by means of unidose capsules attached to an ultrasonic applicator. Similar to cavity preparation, a single operator (BOS), previously trained and calibrated by the more experienced author (MG), performed all adhesive and restorative procedures to avoid inter-operator variability. Then, the restored teeth were stored at 37°C for 24 h in sealed vials containing water-soaked cotton at the bottom in order to keep them in a humid environment without submerging the composite material in water.

Thereafter, the teeth were serially sectioned in lingual-buccal and mesial-distal directions using a high-speed diamond saw (Buehler; Lake Bluff, IL, USA) under water-cooling to obtain stick-shaped specimens with a cross-sectional area of approximately 1 mm^2. Each restored tooth resulted in 4-9 specimens, and therefore a total of 294 specimens was obtained.

Specimens were tested in a microtensile device attached to a universal testing machine (EZ Test, Shimadzu; Kyoto, Japan). Each specimen was fixed to the device with a cyanoacrylate-based glue (Super Bonder Gel, Henkel/Loctite, Diadema; SP, Brazil), and submitted to the μTBS test at a constant speed of 1 mm/min until failure. After testing, specimens were measured with a digital caliper (Mitutoyo; Kanagawa, Japan) to determine their cross-sectional area. The peak tensile load at the moment of fracture was divided by the cross-sectional area of each specimen to obtain the dentin μTBS in MPa. Then, the mean bond strength values for each group were registered using each tooth as an experimental unit. Pre-test failures were recorded, but not included in calculating the μTBS means.

Scanning Electron Microscopy (SEM) Analysis of Failure Mode

The failure mode of each specimen submitted to the μTBS test was analyzed by SEM (JSM 5600LV, JEOL; Tokyo, Japan). The tested samples were mounted on metal stubs with the fractured surfaces facing upwards, sputter-coated with gold (SDC 050 Sputtercoater, Bal-Tec; Balzers, Liechtenstein), and submitted to microscopic analysis at 100X and 400X magnifications. Specimens were classified into seven categories, according to a previously published study:[52] type I: cohesive failure in resin composite; type II: adhesive failure between resin composite and bonding agent; type III: adhesive failure between dentin and bonding agent; type IV: mixed failure (dentin, bonding agent, and resin composite can be observed on the same fractured surface); type V: cohesive failure in the bonding agent; type VI: cohesive failure in the hybrid layer; and type VII: cohesive failure in dentin.

Confocal Laser Scanning Microscopy (CLSM) Analysis of Adhesive-Dentin Bonding Interface Morphology

The occlusal enamel of 24 third molars was ground-down using 600-grit silicon carbide paper, and standardized 4 mm x 4 mm x 4 mm occlusal class I cavities were prepared. Rhodamine B dye (Sigma-Aldrich; St. Louis, MO, USA), which has a pinkish-red color, was added to the adhesives at a concentration of 0.1 wt%.[30] Teeth were then randomly assigned to the experimental groups described in Table 2 (n = 3), and restorative procedures were carried out as described above for the μTBS test. Samples were stored in vegetable oil at 37°C for 24 h to avoid water loss and/or dye dissolution. Afterwards, teeth were sectioned into 1 mm-thick slices. These slices were manually polished with 2000-grit silicon carbide paper for 30 s.

Subsequently, samples were analyzed by CLSM (TCS SP5, Leica Microsystems; Mannheim, Germany). The excitation energy provided by the argon (488 nm) and He-Ne (453 nm) lasers and the photomultipliers amplification were constant throughout the whole investigation. A layer approximately 10 μm below the surface of the sample was observed, and the CLSM micrographs were obtained in fluorescent and transmission modes with an oil-immersion objective (63X magnification, 3X zoom, numeric aperture of 1.3, pinhole of 5.5 μm). At least three sets of micrographs were obtained for each group, which comprised (1) an image of dye detection in fluorescent mode, (2) a gray-scale image of the sample surface in transmission mode, and (3) an image formed by overlapping the micrographs of fluorescent and transmission modes.

For the bonding interface morphology analysis by CLSM, visual differences among experimental groups were analyzed regarding the presence and thickness of the hybrid layer, and resin tags formation. The general appearance of the slices from the three repetitions of each group was used to characterize trends observed in different test conditions. Additionally, a quantitative analysis of adhesive resin infiltration into dentin was carried out using the ImageJ software (National Institute of Health; Bethesda, MD, USA). Three measurements of the integrated density (pixels/μm^2) of the fluorescent signal observed in the micrographs were obtained for each image, from the top of the hybrid layer to 15 μm into the dentin substrate.

Internal Marginal Adaptation Analysis by SEM

Additional teeth (n = 3) were prepared for each group as described above, and transversally sectioned into halves. A polyvinyl siloxane with materials of light and heavy consistencies (Express XT, 3M Oral Care) was used to make impressions of the half-restored surfaces after polishing. Epoxy resin (EpoxiCure, Buehler) was then poured into the impressions, and the resulting polymeric replicas were sputter-coated with gold (SCD 050, Bal-Tec; Balzers, Liechtenstein). Then, the presence of internal interfacial gaps was analyzed in a scanning electron microscope (JEOL, JSM-5600LV; Tokyo, Japan). Approximately 40 images were obtained for each specimen at 200X magnification in order to observe the entire length of the bonded interfaces. ImageJ software was used to precisely measure the length of debonded areas along the internal perimeter of the restorations. The scale bar of SEM images was used for distance calibration, and individual measurements of debonded segments were obtained in millimeters. These were converted to percentage of the total length of the bonding interface, resulting in marginal discontinuity measurements for each group.[44]

Statistical Analysis

Data were tested for normal distribution (Shapiro-Wilk and Kolmogorov-Smirnov test) and homoscedasticity (Levene's test). Microtensile bond strength data was analyzed with two-way ANOVA, followed by Bonferroni's post-hoc test, while failure mode data were submitted to Pearson's chi-squared test. Results of integrated density were analyzed with the Kruskal-Wallis test, followed by Mann-Whitney's post-hoc test. CLSM and SEM images were descriptively analyzed through visual comparison between groups by two independent evaluators (MS and RBEL), who consulted with a third researcher (MG) in case of disagreement regarding their conclusions. Statistical analyses were performed using SPSS 21.0 (IBM SPSS, v. 21.0, IBM; Armonk, NY, USA), with a significance level set at 5%.

RESULTS

Microtensile Bond Strength Test

Mean (± SD) dentin bond strengths are presented in Table 3, along with the proportion of pre-test failures for each group (pre-test failures/total number of specimens per group). Two-way ANOVA showed a significant influence of the type of adhesive on bond strength (p = 0.013). On the other hand, no significant influence of the composite (p = 0.764) or significant interaction between type of adhesive and composite (p = 0.901) were found.

The tested bulk-fill composites did not differ between each other when the same adhesive was used (p > 0.05). In regard to the Admira Fusion x-tra results, Adper Scotchbond Multipurpose showed higher bond strength than Clearfil SE Bond (p = 0.016), whereas both OptiBond All-In-One and Futurabond U presented intermediate values that did not differ from the other adhesives (p > 0.05). As for SonicFill 2, no statistically significant differences in bond strength means were found between the adhesives (p > 0.05).

Table 3. Mean (± SD) dentin microtensile bond strength (in MPa), and [pre-test failures/total number of specimens], according to adhesive system and composite.

Adhesive	**Composite**	
	Admira Fusion x-tra	**SonicFill 2**
Adper Scotchbond Multipurpose	33.29 (± 7.4) [0/54] Aa	33.27 (± 3.7) [4/36] Aa
Clearfil SE Bond	24.98 (± 7.0) [2/47] Ab	27.51 (± 9.5) [2/26] Aa
OptiBond All-In-One	30.49 (± 5.2) [2/44] Aab	30.37 (± 6.3) [0/27] Aa
Futurabond U	27.87 (± 2.6) [1/38] Aab	27.39 (± 6.1) [0/22] Aa

Means followed by the same superscript letters are not statistically significantly different (p > 0.05). Uppercase letters compare different composites within the same adhesive (rows), while lowercase letters compare different adhesives within the same composite (columns).

Failure Mode Analysis by SEM

Figure 1 depicts the frequency of occurrence of each failure mode. Pearson’s chi-squared test indicated that the association between adhesive and composite significantly influenced the failure mode distribution [X^2(42 degrees of freedom) = 129.87; p < 0.001]. A lower tendency of occurrence (adjusted residual < -1.96) was observed for the following failure modes within the tested groups: Admira Fusion x-tra + Adper Scotchbond Multipurpose (types II and III); Admira Fusion x-tra + Clearfil SE Bond (types III and VII); Admira Fusion x-tra + OptiBond All-in-One (types I and VI). Conversely, a higher tendency of occurrence (adjusted residual >

1.96) was found for the following fracture patterns within the evaluated groups: Admira Fusion x-tra + Adper Scotchbond Multipurpose (types VI and VII); Admira Fusion x-tra + Clearfil SE Bond (types I and V); Admira Fusion x-tra + OptiBond All-in-One (type IV); Admira Fusion x-tra + Futurabond U (type II); SonicFill 2 + Adper Scotchbond Multipurpose (type VI); SonicFill 2 + Clearfil SE Bond (type II); and SonicFill 2 + OptiBond All-in-One (type VII).

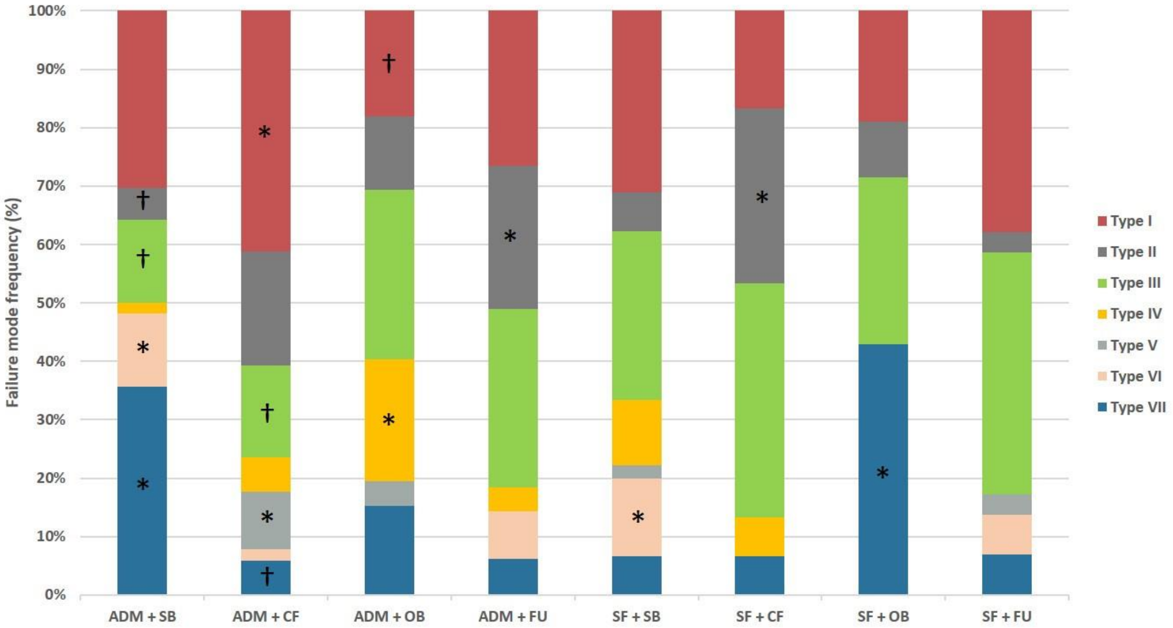

Figure 1. Failure modes (in %) according to each group. Type I: cohesive failure in composite; type II: adhesive failure between bonding agent and composite; type III: adhesive failure between bonding agent and dentin; type IV: mixed failure; type V: cohesive failure at the adhesive layer; type VI: cohesive failure in the hybrid layer; type VII: cohesive failure in dentin. *Represents a statistically significantly higher trend of occurrence of a certain failure mode. †Represents a statistically significantly lower occurrence of a certain failure mode.

Adhesive-Dentin Bonding Interface Morphology Analysis by CLSM

Figure 2 shows representative images of the interface morphology analysis performed by CLSM. For both composites, thick hybridization zones (over 6 μm) and multiple, deep resin tags were observed when used with the adhesive Scotchbond Multipurpose. In the Admira Fusion x-tra + Adper Scotchbond Multipurpose group, a clear differentiation between resin composite and adhesive layer was not possible. The self-etch adhesive Clearfil SE Bond led to hybrid layer formation for both composites, although a thicker hybrid layer and shorter resin tags were observed in the Admira Fusion x-tra + Clearfil SE Bond group compared with its counterpart (SonicFill 2 + Clearfil SE Bond). Hybrid zones and resin tags were also found in the micrographs of the OptiBond All-In-One groups. On the other hand, Futurabond U resulted in thin hybrid layers (less than 1.5 μm), without any resin tags and thick adhesive layers.

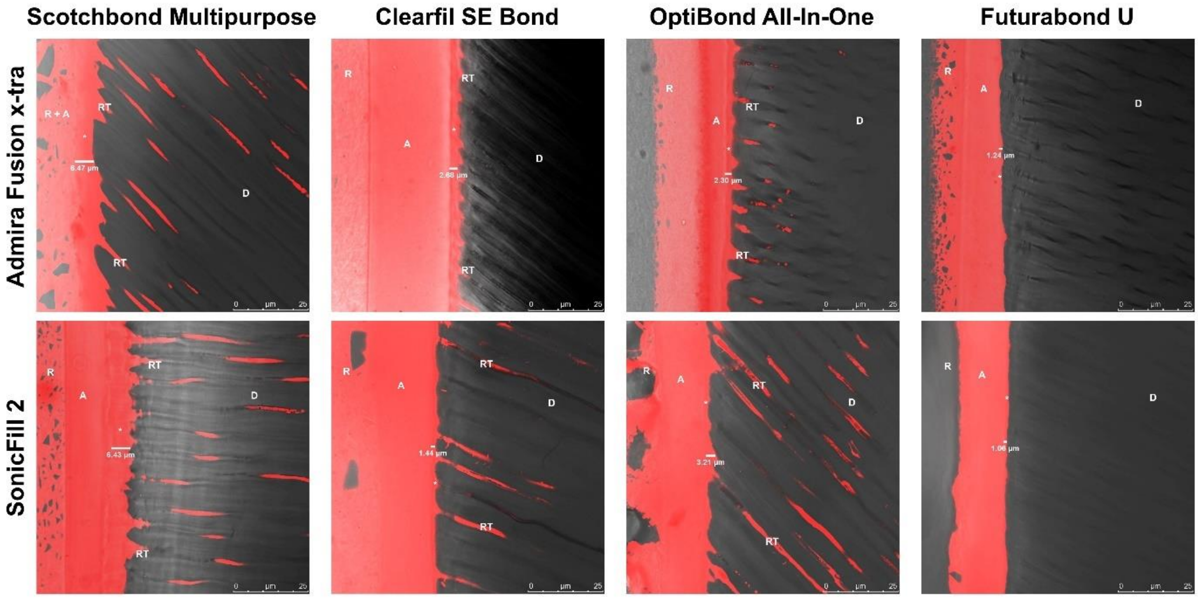

Figure 2. CLSM images (overlap of fluorescent and transmission modes) of the bonding interface for the tested groups. The top row presents micrographs of Admira Fusion x-tra, while the bottom row shows micrographs of SonicFill 2. The columns present micrographs of the tested adhesives, from left to right: Scotchbond Multipurpose, Clearfil SE Bond, OptiBond All-In-One, and Futurabond U. *Indicates hybrid zones. R: resin composite; A: adhesive layer; RT: resin tags; D: dentin.

The integrated density (pixels/μm^2) of the fluorescent signal obtained by confocal microscopy was used to calculate adhesive resin infiltration and is presented in Figure 3. The nonparametric Kruskal-Wallis test showed the type of adhesive significantly influenced the results for both Admira Fusion x-tra (X^2(3 degrees of freedom) = 25.869, p = 0.001), and SonicFill 2 (X^2(3 degrees of freedom) = 8.378, p = 0.039). When the composite Admira Fusion x-tra was used, Scotchbond Multipurpose presented higher adhesive resin infiltration compared to Clearfil SE Bond (adjusted p-value = 0.001) and Futurabond U (adjusted p-value = 0.001), but it did not differ from OptiBond All-In-One (adjusted p-value > 0.05). Futurabond U also showed significantly lower adhesive resin infiltration than OptiBond All-In-One (adjusted p-value = 0.014). For SonicFill 2, OptiBond All-In-One presented higher integrated pixel density than did Futurabond U (adjusted p-value = 0.05), while the remaining adhesives did not differ between each other (adjusted p-value > 0.05).

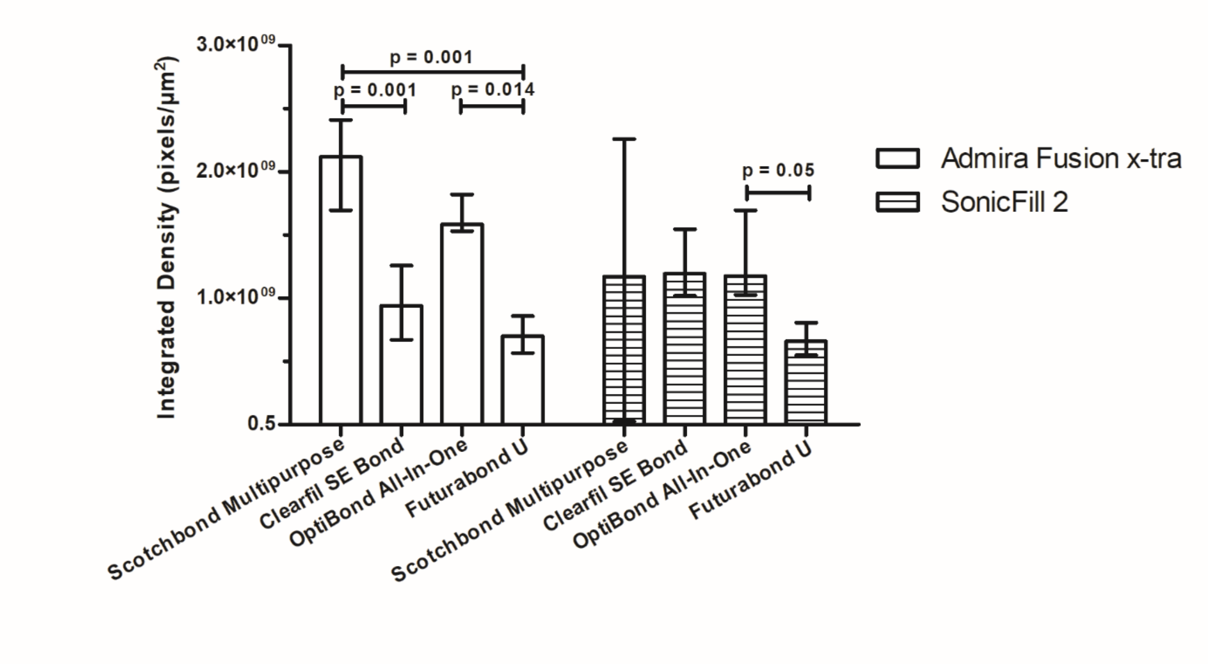

Figure 3. Median and interquartile integrated density (pixels/µm^2) of adhesive resin infiltration into etched dentin for the tested groups. Horizontal bars connecting different groups represent statistically significant differences (adjusted p-value < 0.05; Mann-Whitney test).

Internal Marginal Adaptation Analysis by SEM

Figure 4 shows representative SEM images of the internal adaptation of the restorative material related to the cavity angle and pulpal wall of the tested groups. Overall, Admira Fusion x-tra presented adequate marginal adaptation at both sites (cavity angles and pulpal wall), with low percentages of marginal discontinuity for Scotchbond Multipurpose (5.3%), Clearfil SE Bond (9.4%), and OptiBond All-In-One (8.4%). The group Admira Fusion x-tra + Futurabond U showed higher marginal discontinuity (40.0%). Conversely, SonicFill 2 led to more defects along the adhesive-dentin interfacial perimeter of the restoration, with few occasional small gaps for Scotchbond Multipurpose and OptiBond All-In-One (26.9% and 39.3% marginal discontinuity, respectively), and longer, wider gaps for Clearfil SE Bond and Futurabond U (62.1% and 69.2% marginal discontinuity, respectively).

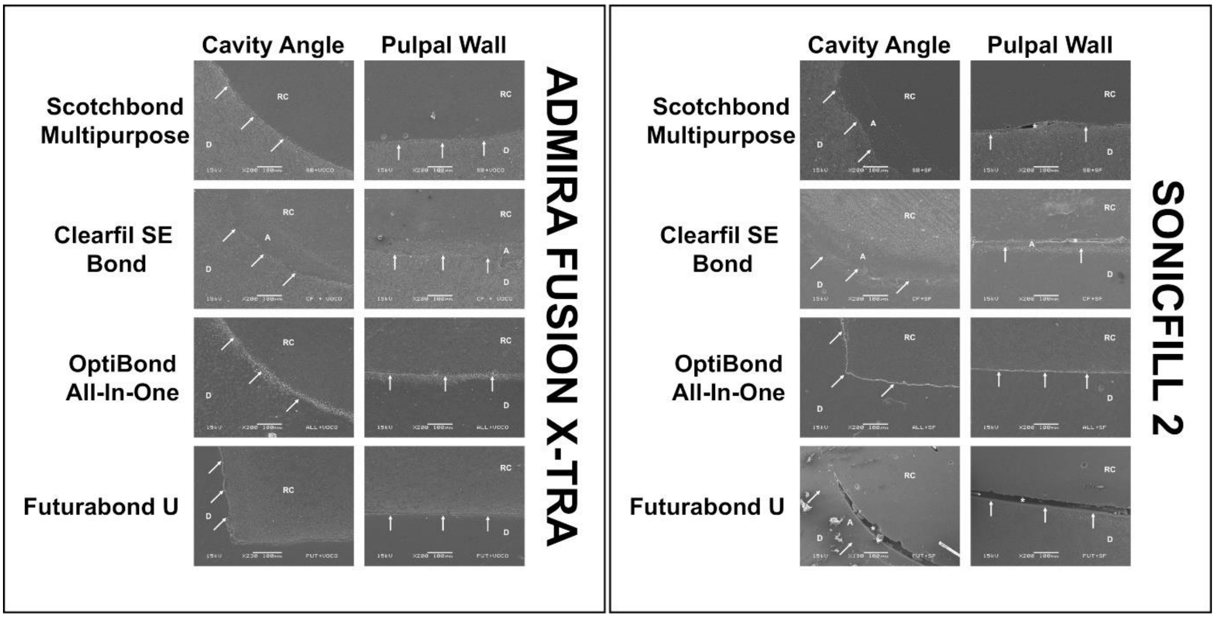

Figure 4. Representative SEM images of internal marginal adaptation for the tested groups at 200X magnification. Arrows indicate the bonding interface, and * indicates gap areas. RC: resin composite; D: dentin; A: adhesive layer.

DISCUSSION

Shrinkage stresses are an unavoidable consequence of the polymerization process that occurs in a confined space (tooth cavity) due to bonding to enamel/dentin walls or other composite layers.[24] These internal stresses are transferred to the bonding interface as tensile forces, which might result in stress-relieving gaps if they exceed the local bond strength.[16] Therefore, the marginal and internal integrity of composite restorations might be compromised by debonding and premature gap formation, which could translate into clinical complications, such as marginal leakage, marginal staining, post-operative sensitivity, and/or recurrent caries.[18,33] Thus, the analysis of the bonding interface quality when using different adhesives in association with low-shrinkage bulk-fill composites is of the utmost importance in the current context of restorative dentistry.

As observed in Table 3, the different composition and application procedures of the tested restorative materials did not affect dentin μTBS, since no statistical differences were found between composites for any of the investigated bonding agents. Hence, the first null hypothesis was accepted. Scarce and conflicting data about the tested composites are available in dental literature. Abbasi et al[1] analyzed the volumetric polymerization shrinkage of six composites, including SonicFill 2, which did not differ from a microhybrid, non-bulk-fill material. Their findings agreed with other publications which stated that the SonicFill restorative system led to similar volumetric polymerization shrinkage compared to non-bulk-fill composites.[6,28] As for Admira Fusion x-tra, a recent study showed its shrinkage stress to be lower than a methacrylate-based, non-bulk-fill composite when larger resin increments of 24 mm^3 were used, which was not the case for 12-mm^3 samples.[48]

Another study demonstrated Admira Fusion x-tra and SonicFill presented lower linear shrinkage and shrinkage force than the non-bulk-fill control group, while Admira Fusion x-tra also showed superior results compared to Sonic-Fill.[14] On the other hand, no differences in cuspal deflection measurements between Admira Fusion x-tra and the SonicFill restorative system have been reported. However, the same study found that both bulk-fill composites performed better than a methacrylate-based, non-bulk-fill material.[55] The bond strength and morphology of the dentin-adhesive interface, as well as the internal adaptation results of the present study, coupled with the volumetric polymerization shrinkage and shrinkage stresses previously reported for Admira Fusion x-tra and Sonic-Fill,[1,6,14,28,48,55] could lead to a better understanding of the behavior of these materials in class I cavities.

However, µTBS results should be cautiously interpreted because there is significant variability between the tested composites regarding composition and application mode. One might argue the only way to achieve a precise, trustworthy comparison between composite-related variables is by using a model, standardized composition. Thus, the target study variable could be modified to isolate its influence on the results by, for example, having the same monomer blend and photoinitiator system for all groups, but with different filler content/ratio,[4,22] or by having different types of monomers between groups, but all with the same filler content and photoinitiator system.[15,19] Consequently, compositional variability is an inherent limitation seen in many in vitro studies evaluating commercially available composites.[21] Another variable with respect to the tested composites would be their mode of application, since SonicFill 2 was applied with an ultrasonic device, while Admira Fusion x-tra was manually inserted into the cavity using a spatula. However, applying Admira Fusion x-tra with the same ultrasonic device would be interesting to evaluate the effect of mode of application in comparison with SonicFill 2. This procedure was not possible because the manufacturer of the SonicFill restorative system has developed its own ultrasonic applicator that is not compatible with the unidose capsules of Admira Fusion x-tra. Additionally, an in-depth evaluation of this variable was not within the scope of the present study, and Admira Fusion x-tra is not recommended for ultrasonic application by its manufacturer.

The creation of a high-quality hybrid layer, the interaction between dentin and adhesive resin, and the presence of a hydrophobic adhesive layer have been suggested as requirements to minimize the main consequence of shrinkage stresses, i. e., the disruption of the contact between resin composite and cavity walls.[6] Even so, the bond strength of SonicFill 2 did not seem to be affected by the different dentin bonding approaches (3E&R with Scotchbond Multipurpose, 2SE with Clearfil SE Bond, 1SE with OptiBond All-In-One or Futurabond U). All the tested adhesives probably established a tight bond between the composite and the dental structure, which could mask the influence of polymerization shrinkage stress, unless the samples are submitted to loading, which allows residual stresses at the restoration margins to weaken the bonding interface.[28]

In contrast, Admira Fusion x-tra presented lower bond strength when used with Clearfil SE Bond compared to Scotchbond Multipurpose, while no other statistical difference was observed between bonding agents for this composite (Table 3), requiring the rejection of the second null hypothesis. Nevertheless, this group had the highest number and a statistically significant higher trend of resin composite cohesive failures (Fig 1). The occurrence of cohesive failures implies that the resin-dentin bond strength could be stronger than the cohesive strength within the composite or the dentin substrate.[11] Hence, this fracture pattern might lead to underestimating the true dentin-adhesive bond strength value.[9] Consequently, the performance of Clearfil SE Bond cannot be questioned solely based on the present results, especially since this bonding agent is regarded as the gold standard for self-etch adhesives, with proven clinical performance.[27,45] Moreover, a comparison between an ormocer-based bonding agent and a non-specific self-etch adhesive, both used with an ormocer composite, showed significantly better interfacial morphology and microleakage results when the ormocer was used with an specific, chemically similar bonding agent.[23] Therefore, the clinical performance of Clearfil SE Bond with Admira Fusion x-tra should be investigated in comparison with non-ormocer composites to determine whether the less favorable results of this study could be composite-dependent due to potential chemical incompatibility between the adhesive resin and the ormocer resin matrix.

Statistical analysis showed higher trends toward adhesive failures (between bonding agent and composite), cohesive failures within the hybrid layer, and mixed failures among the tested groups (Fig 1). Therefore, the third null hypothesis also had to be rejected. Adhesive failures between simplified bonding agents and composites might occur due to chemical reactions between acidic adhesive monomers and tertiary amines from the resin composite, mainly when the uncured acidic adhesive layer and composite remain in contact for a prolonged period.[56] Failures within the adhesive-composite interface might also occur due to regions of poor co-polymerization between the adhesive resin and the lining composite; ideally, the bonding agent should bind to the underlying composite through a process of co-polymerization of residual double bonds (–C═C–) in the oxygen inhibition layer.[60] Cohesive failures within the hybrid layer imply that a defective hybridization zone was formed, and this region fractured because it was the weakest area of the bonding interface.[3] In contrast, mixed failures suggest the presence of a strong bond, with a fracture path probably starting in composite and reaching dentin through the bonding interface.[63] Theoretically, mixed failures are preferable, as they indicate that the structures involved in dentin bonding acted as a single unit rather than separate layers.[31,52]

SEM showed that Clearfil SE Bond + SonicFill 2 and Futurabond U + SonicFill 2 were the groups with the highest percentages of marginal discontinuity (Fig 4), also resulting in high numbers of adhesive failures. Although the dentin bond strength of these two groups was not statistically different than that of Adper Scotchbond Multipurpose + Sonic-Fill 2 or OptiBond All-in-One + SonicFill 2, the latter adhesives showed more cohesive failures, which might suggest their hybridization zones were not as sensitive to tensile stresses generated by composite polymerization shrinkage as were Clearfil SE Bond or Futurabond U. Consequently, the fourth null hypothesis was rejected. Additionally, microtensile bond strength specimens produced from gap areas were more prone to manipulation errors and were not included in the mean bond strengths, which is in keeping with the guidelines of the Academy of Dental Materials.[5] This might have increased the bond strength means for Clearfil SE Bond and Futurabond U, despite the higher percentages of marginal gaps, especially because these adhesives presented reasonably higher standard deviations than their Admira Fusion x-tra counterparts (Table 3).

Low adhesive resin infiltration into dentin and poor interaction between the adhesive and dentin were observed for Futurabond U, as shown in the CLSM images (Fig 2). Based on the information previously discussed, the results for Futurabond U combined with SonicFill 2 might be due to incompatibility between the acidic adhesive resin and the composite,[56] since the use of an ultrasonic applicator would prolong the contact of the adhesive with SonicFill 2, resulting in type-II adhesive failures. However, this does not apply to the other group with a higher proportion of type II failures (almost 30% for the Clearfil SE Bond + SonicFill 2 group), because Clearfil SE Bond is a 2SE adhesive that requires application of a hydrophobic layer of adhesive resin over the acidic dentin primer, preventing its contact with the composite.[58] Thus, we speculate that the higher probability of adhesive-composite failures in this group might have been due to localized regions of insufficient copolymerization between the adhesive resin of Clearfil SE Bond and the ultrasonically applied composite. SonicFill 2 is a reasonably new material and, as far as can be told from the current scientific literature, its compatibility with other polymer-based restorative products has not been studied yet.

Another interesting finding derived from the present results concerns the performance of the two 1SE adhesives. Regardless of the bulk-fill composite used, Optibond All-in-One and Futurabond U did not differ in regard to dentin bond strength or adhesive infiltration into dentin. Still, Futurabond U seemed to generate higher marginal discontinuity than Optibond All-in-One for all of the tested resin-based restorative materials. In the case of Admira Fusion x-tra, although Futurabond U showed higher marginal discontinuity than did Optibond All-in-One, gap formation was observed mostly at the axial walls of the cavities. Consequently, these localized regions of marginal disruption were very unlikely to affect bond strength results, which might explain the similar mean values obtained for the two 1SE adhesives. Conversely, SEM analysis revealed longer and wider gaps at the pulpal wall of the cavities of SonicFill 2 when this composite was coupled with Futurabond U compared to Optibond All-in-One. The difference between the 1SE bonding agents regarding marginal discontinuity, despite their similar bond strength, further supports our hypothesis that dentin bond strengths were not affected by gap formation due to the impossibility of testing specimens obtained from these regions, since they would debond during cutting or manipulation.

Figure 2 depicts the morphological features of the bonding interfaces investigated in the present study. A clear correlation between hybrid layer thickness and bond strength has not been successfully demonstrated,[46] although a hybridization zone of at least 0.5 μm, which would be roughly five collagen fibrils deep, has been suggested as the minimum threshold to create an adequate bonding interface in intertubular dentin.[57] The present CLSM results are in accordance with the bond strengths obtained, since even the thinnest hybrid layer (1.06 μm for Futurabond U + SonicFill 2) was well over 0.5 μm thick. Another interesting finding regarding bonding interface morphology was that some of the groups presented noticeably short or no resin tags (Clearfil SE Bond + Admira Fusion x-tra, Futurabond U + Admira fusion x-tra, and Futurabond U + SonicFill 2). However, the presence of deep resin tags does not contribute much to bond strength, as the surface adhesion and the entanglement between adhesive resin and intertubular collagen fibrils are the two main factors contributing to the overall resin-dentin bond.[51] Also, the cross-sectional area of resin tags decreases along their depth, which reduces their contribution to bonding.[42] Thus, the fifth null hypothesis was not accepted.

Quantitative analysis of integrated density (pixels/μm^2) led to the rejection of the sixth null hypothesis, as it revealed higher adhesive resin infiltration for the etch-and-rinse approach compared to the remaining adhesives for Admira Fusion x-tra (except OptiBond All-In-One), while Futurabond U had lower adhesive resin penetration compared to OptiBond All-In-One for both composites (Fig 3). These results were expected, since the separate etching step used by Scotchbond Multipurpose causes deeper demineralization of around 5-10 μm,[51] leaving a larger area needing to be filled by the bonding agent. Furthermore, CLSM images imply that Futurabond U had a lower ability to demineralize and infiltrate dentin compared to the other self-etch bonding agents, which corroborates previous results in the literature, showing a thin hybrid layer and larger tracer-infused water-rich zones for Futurabond U compared to other self-etch adhesives.[10]

The present results may give a general idea about important aspects to consider when using the tested composites in combination with different dentin bonding approaches in clinical dentistry. The type of bulk-fill restorative material (ormocer-based or ultrasonically applied) does not seem to play a major role in the final quality of dentin-adhesive bonds. Nonetheless, bond strengths and bonding morphology characteristics suggest that the adhesives Adper Scotchbond

Multipurpose and Clearfil SE Bond still remain the more reliable options for bonding ultrasonically applied SonicFill 2 to dentin. However, the chemical compatibility of Clearfil SE Bond with the new composites tested should be further investigated, since the Admira Fusion x-tra specimens bonded with this self-etch adhesive presented lower bond strengths than did Scotchbond Multipurpose, while higher numbers of failures between the adhesive resin and the tested bulk-fill composites were detected when it was combined with SonicFill 2. The adhesive Futurabond U presented lower-quality bonding interfaces and higher percentages of internal gaps, especially when associated with SonicFill 2. Therefore, the long-term performance of this bonding agent is questionable, and it needs to be thoroughly studied in future investigations.

CONCLUSIONS

- The type of bulk-fill composite (Admira Fusion x-tra or SonicFill 2) did not affect dentin microtensile bond strength, regardless of bonding approach.
- The dentin bond strength of Admira Fusion x-tra was affected by the type of adhesive used, although this effect should be interpreted with caution, considering failure mode analysis.
- The etch-and-rinse approach led to thicker hybrid layers with high adhesive infiltration into the dentin substrate.
- SonicFill 2 yielded higher marginal discontinuity compared to Admira Fusion x-tra.

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Clinical relevance: The results provide a general idea about dentin bonding quality when the tested bulk-fill composites are used with different bonding agents. Scotchbond Multipurpose seems a reliable option for bonding bulk-fill composites to dentin, while Futurabond U was not able to prevent gap formation.

3 DISCUSSÃO

A importância da odontologia adesiva passou a ser considerada de fato somente após a famosa publicação de Buonocore demonstrando melhor união da resina acrílica ao esmalte após o tratamento da superfície com ácido fosfórico (Buonocore, 1955). O condicionamento ácido do esmalte aumenta a rugosidade e a energia de superfície do substrato, tornando-o mais receptivo ao adesivo e aumentando consideravelmente a resistência de união entre compósito e esmalte (Zafar e Ahmed, 2015). Assim, quando novos agentes são sugeridos para condicionar o esmalte, o ensaio de resistência de união por cisalhamento pode mostrar se os tratamentos propostos são equiparáveis ao ácido fosfórico tradicionalmente utilizado. No segundo estudo, o condicionamento com ácido fosfórico não apresentou diferença estatisticamente significativa em comparação com os agentes de condicionamento sugeridos.

Ainda na década de 1950, Bowen desenvolveu o monômero bisfenol glicidil metacrilato (bis-GMA) (Bowen, 1956), que viria a ser o principal componente dos materiais restauradores odontológicos, incluindo adesivos, compósitos e cimentos resinosos. No campo dos agentes de união, surgiram monômeros ácidos capazes de condicionar levemente e reagir quimicamente com a dentina (Miyazaki et al., 2014), incluídos nos chamados adesivos autocondicionantes. Isso foi alcançado, principalmente, pela síntese do monômero 10-metacriloiloxidecil dihidrogênio fosfato (10-MDP) (Yoshihara et al., 2015), o qual é capaz de se ligar ionicamente e de maneira hidroliticamente estável ao cálcio da hidroxiapatita (Yoshida et al., 2004), tendo sido incorporado inicialmente ao sistema adesivo de dois passos Clearfil SE Bond (Kuraray Noritake Dental Inc.). O referido adesivo é considerado o padrão-ouro dos sistemas autocondicionantes (Peumans et al., 2015; Pena et al., 2016) e, por isso, foi testado como controle no terceiro artigo desta tese.

Com o intuito de simplificar as etapas de aplicação dos sistemas adesivos durante os procedimentos restauradores, foram criados sistemas adesivos “universais”, que nada mais são do que adesivos autocondicionantes de passo único que também podem, caso assim deseje o operador, ser utilizados como adesivos convencionais de dois passos, por meio da técnica de condicionamento total do esmalte e da dentina com ácido fosfórico (Perdigão e Swift, 2015). A grande diferença desses agentes de união em relação aos autocondicionantes tradicionais é a presença de monômeros funcionais com grupos fosfato e/ou carboxila na sua composição (Perdigão et al., 2021). Ainda se recomenda o condicionamento seletivo do esmalte para esses adesivos, enquanto a aplicação em dentina pode ser realizada ou em substrato seco, sem condicionamento prévio, ou em substrato úmido tratado com ácido fosfórico (Rosa et al., 2015; Ruschel et al., 2018).

Conforme os adesivos universais começaram a substituir gradualmente aqueles mais antigos, se tornou imprescindível a obtenção de conhecimento detalhado a respeito do seu desempenho, sobretudo a longo prazo. Alguns estudos já demonstraram diferentes resultados *in vitro* para adesivos universais quando associados a um tipo específico de tratamento do tecido dental mineralizado (Vermelho et al., 2017; Kaczor et al., 2018). Esses sistemas adesivos apresentam solventes distintos nas suas formulações (diversos tipos de álcool ou acetona), o que também pode influenciar seu desempenho diante de variações da umidade dentinária residual (Irmak et al., 2016; de Siqueira et al., 2020). Além disso, sua compatibilidade química com novos compósitos disponíveis no mercado precisa ser estudada. Por conseguinte, o segundo e o terceiro artigos desta tese focaram no estudo de fatores associados à adesão dos compósitos resinosos à dentina, tendo como ponto comum o teste de adesivos autocondicionantes/universais modernos.

Desde a década de 1980, sabe-se que a união das resinas adesivas à dentina se dá pela difusão de monômeros resinosos ao longo da dentina intra e intertubular, formando uma camada ácido-resistente logo abaixo da interface de união, conhecida como “camada híbrida” (Nakabayashi et al., 1982). A aplicação de agentes ácidos na dentina remove cristais minerais em 5-10 µm de profundidade e expõe fibrilas colágenas, dentre as quais a mistura adesiva penetra, obtendo-se embricamento micromecânico entre o adesivo e o tecido dentinário (Breschi et al., 2018). Independentemente do tipo de adesivo ou compósito utilizado, as imagens de microscopia dos artigos aqui apresentados mostraram claramente a íntima interação entre substrato e resina adesiva, observando-se formação de camada híbrida em todos os grupos testados, embora com espessuras distintas.

O relativo sucesso atrelado ao uso do ácido fosfórico na adesão dentinária, cujo protocolo clínico foi determinado há quase 30 anos (Fusayama, 1992), depende em grande parte da manutenção da umidade residual do substrato condicionado antes da aplicação do adesivo (Gwinnett, 1992; Kanca, 1992). Ao manter a dentina levemente úmida após o condicionamento ácido e antes da aplicação do adesivo, os nanoespaços interfibrilares são preservados, o que melhora a difusão do primer e/ou resina adesiva e favorece o processo de hibridização (Pereira et al., 2001). Entretanto, como discutido na revisão de literatura, o condicionamento da dentina com ácido fosfórico pode prejudicar a longevidade da adesão dentinária por conta da exposição e ativação de MMPs (Mazzoni et al., 2012; DeVito-Moraes et al., 2016; Maravic et al., 2018), o que foi claramente observado nos resultados do segundo artigo, pois ambos os adesivos universais testados mostraram atividade enzimática proeminente e alto grau de nanoinfiltração após armazenamento.

Ao longo desta tese, demonstrou-se que a evolução da odontologia adesiva focou quase exclusivamente no desenvolvimento de novas composições monoméricas. Desde a introdução das técnicas de condicionamento e adesão à dentina úmida, pouca atenção tem sido dada à busca por agentes de condicionamento dentinário alternativos que não apresentem os mesmos efeitos deletérios do ácido fosfórico na longevidade da área de união dente-restauração. Este tipo de pesquisa se mostra bastante relevante no cenário da odontologia brasileira, onde grande parte dos profissionais ainda utiliza, preferencialmente, a técnica tradicional de adesão com passo de condicionamento ácido separado. Por esta razão, o segundo artigo buscou avaliar agentes de condicionamento dentinário experimentais que pudessem permitir adesão à dentina seca e, simultaneamente, inibir a atividade das MMPs (Sebold et al., 2019).

Os resultados mostraram que, do ponto de vista mecânico, não houve prejuízo para a resistência de união em 24 horas dos adesivos Prime&Bond Universal ou Gluma Bond Universal quando a dentina foi condicionada com solução 10:3 ou ácido nítrico. Contudo, ainda que tenham apresentado resultados promissores em 24 horas, estes agentes de condicionamento experimentais não foram capazes de impedir a redução da resistência de união à dentina após armazenamento por longo período, o que está certamente relacionado com a ativação de MMPs vista para ambas as soluções na zimografia *in situ* da área de união do segundo artigo. Teoricamente, compostos contendo íons Fe^{+3} ou NO_3^- teriam a capacidade de interagir com as proteases dentinárias e inibi-las (Wen et al., 2016). Infelizmente, o efeito inibidor de MMPs destas substâncias não foi observado no estudo apresentado, devido às limitações inerentes aos procedimentos de adesão: reduzida área de superfície, lavagem e remoção dos agentes de condicionamento e curto tempo de tratamento da dentina.

Outra observação interessante advinda dos artigos que compõem esta tese se refere a constatação de que o desempenho dos adesivos universais foi material-dependente. No segundo artigo, o adesivo Prime&Bond Universal mostrou menor resistência de união à dentina e nanoinfiltração semelhante ao condicionamento com ácido fosfórico, enquanto o sistema Gluma Bond Universal alcançou resistência de união tão alta quanto a técnica de adesão à dentina úmida com ácido fosfórico, apesar de significativa nanoinfiltração. Já no terceiro artigo, curiosamente, o sistema Futurabond U estabeleceu resistência de união forte entre compósito e estrutura dental, apesar da maior porcentagem de descontinuidade marginal. Isso se deve, muito provavelmente, aos diferentes monômeros e solventes contidos nas formulações adesivas universais atualmente disponíveis, que resultam em pH e mecanismo de união química distintos (Van Landuyt et al., 2007; Giannini et al., 2015; DeVito-Moraes et al., 2016).

Todavia, mesmo que a abordagem de condicionamento da dentina ou o tipo de adesivo universal utilizado sejam fatores determinantes para a qualidade e a estabilidade da área de união, a influência do uso de novos compósitos resinosos também é crítica para o sucesso clínico imediato e para a longevidade das restaurações. Isso porque as tensões de contração são uma consequência inevitável do processo de polimerização dos materiais restauradores, o qual ocorre quase sempre em espaço contido, como a cavidade dental (Hayashi et al., 2019). Por esta razão, tensões internas são transferidas à área de união na forma de forças de tração, podendo resultar na formação de fendas ou *gaps* caso essas tensões excedam a resistência de união local (Ferracane, 2005). Desta forma, torna-se essencial a análise da qualidade da área de união dentina-adesivo ao utilizar agentes de união de abordagens distintas com os atuais compósitos *bulk-fill*.

O terceiro artigo demonstrou, assim como o segundo, que todos os adesivos testados produziram união satisfatória à estrutura dental, sem grande influência do tipo de compósito aplicado. A interação entre dentina e adesivo e a presença de uma camada de resina adesiva são alguns dos principais requerimentos sugeridos para minimizar as consequências das tensões de contração (Benetti et al., 2015). Mesmo assim, potenciais incompatibilidades químicas entre adesivo e compósito devem ser levadas em conta, pois apesar dos valores adequados de resistência de união e da formação de zona de hibridização, altas porcentagens de falhas adesivas entre compósito e adesivo foram observadas para alguns grupos em ambos os estudos laboratoriais desta tese.

Levando-se em conta as informações apresentadas pelos artigos contidos neste trabalho de doutorado, pode-se afirmar que o agente de condicionamento dentinário utilizado e a umidade residual da dentina condicionada podem afetar de maneira significativa a área de união dentina-adesivo, embora modificações nestes fatores relacionados à técnica de adesão não sejam suficientes para reduzir a degradação enzimática da interface adesiva ao longo do tempo. Adicionalmente, a composição e os métodos de aplicação das resinas *bulk-fill* testadas não influenciaram os resultados de resistência de união à dentina por microtração. Em suma, o tipo de material restaurador *bulk-fill* não parece ter um papel importante na qualidade final da união dentina-adesivo, embora os dados de resistência e morfologia da área de união sugiram que os adesivos Scotchbond Multipurpose e Clearfil SE Bond ainda permaneçam como opções mais confiáveis de agentes de união.

4 CONCLUSÃO

Considerando as informações expostas acima, dentro das limitações dos estudos *in vitro* apresentados, pode-se concluir que:

- Dentre os acontecimentos científicos que levaram ao desenvolvimento dos adesivos odontológicos atuais, destacam-se principalmente a descrição do ataque ácido do esmalte por Buonocore, a descoberta da zona de hibridização por Nakabayashi e colaboradores, o uso da técnica de adesão à dentina condicionada e umedecida e a síntese do monômero 10-MDP.
- Grande ênfase tem sido dada à modificação das formulações monoméricas dos sistemas adesivos, de modo a simplificar sua aplicação, oferecendo efeitos terapêuticos como ação antibacteriana, inibição enzimática e remineralização.
- O método de condicionamento dentinário e a umidade residual da dentina condicionada influenciam de maneira significativa a área de união adesivo-dentina.
- O adesivo Prime&Bond Universal teve desempenho superior ao Gluma Bond Universal ao utilizar a técnica de adesão à dentina úmida com ácido fosfórico. Entretanto, os dados obtidos por este estudo sugerem que nenhum destes adesivos deve ser aplicado em dentina condicionada com ácido fosfórico e seca com jato de ar.
- Embora a solução 10:3 e o ácido nítrico diluído tenham apresentado resultados promissores em 24 horas quanto à adesão dentinária, estes agentes não foram capazes de reduzir a atividade enzimática *in situ* da dentina e evitar a degradação da área de união a longo prazo.
- Novas investigações visando incorporar os íons derivados da solução 10:3 e do ácido nítrico em formulações adesivas seriam interessantes, pois estes agentes poderiam apresentar efeito inibidor de MMPs caso fossem mantidos na superfície dentinária por mais tempo.
- A nanoinfiltração mostrou-se dependente do tipo de adesivo utilizado, sendo mais alta nos espécimes fabricados com adesivo “universal” à base de acetona.
- Dentre aqueles testados, o tipo de compósito *bulk-fill* (à base de ormocer ou aplicado por dispositivo ultrassônico) não parece ter influenciado

significativamente a resistência de união à dentina criada por adesivos tanto convencionais, quanto autocondicionantes.

- A abordagem de condicionamento dentinário com ácido fosfórico promoveu maior difusão da resina adesiva e formação de camada híbrida mais espessa, embora não exista correlação destes fatores com a resistência de união à dentina.
- O adesivo Futurabond U levou a formação de áreas de união deficientes, com alta taxa de descontinuidade marginal, principalmente quando associado ao compósito SonicFill 2, o que requer estudos futuros para determinar a estabilidade hidrolítica deste sistema adesivo ao longo do tempo.

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ANEXOS

Anexo 1 - Verificação de originalidade e prevenção de plágio

Estudos in vitro sobre o desempenho de sistemas adesivos utilizando diferentes métodos de condicionamento e compósitos bulk-fill

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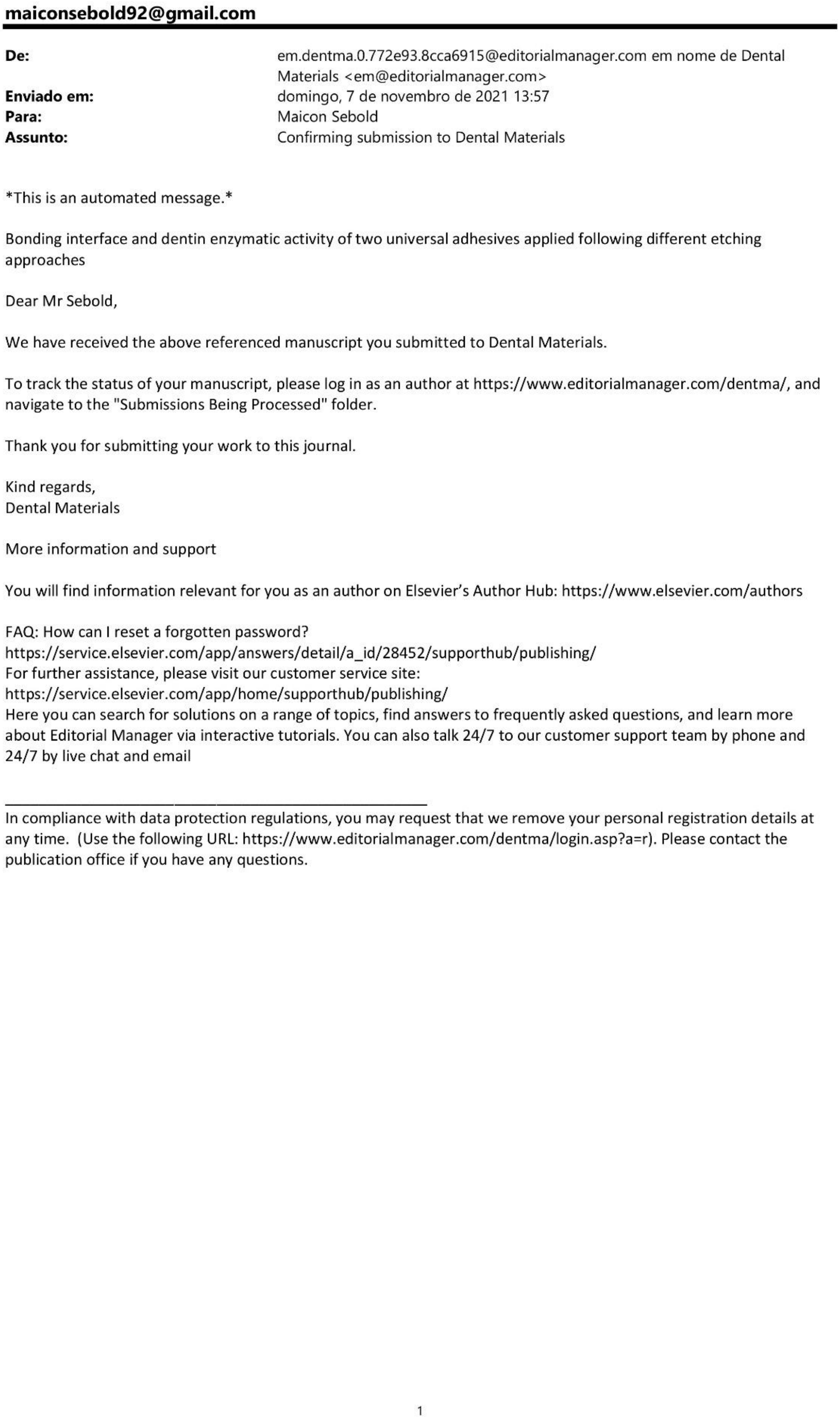

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Anexo 4 - Certificado de aprovação do projeto inicial pelo Comitê de Ética (artigo 2.2)

COMITÊ DE ÉTICA EM PESQUISA
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CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "Avaliação in vitro de agentes de condicionamento dentinário alternativos à base de cloreto férrico e ácido cítrico ou ácido nítrico", CAAE 87550318.0.0000.5418, dos pesquisadores Maicon Sebold e Marcelo Giannini, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 01/06/2018.

The Research Ethics Committee of the Piracicaba Dental School of the University of Campinas (FOP-UNICAMP) certifies that research project "In vitro evaluation of alternative dentin conditioners based on ferric chloride and citric acid or nitric acid", CAAE 87550318.0.0000.5418, of the researcher's Maicon Sebold and Marcelo Giannini, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on June, 01 2018.

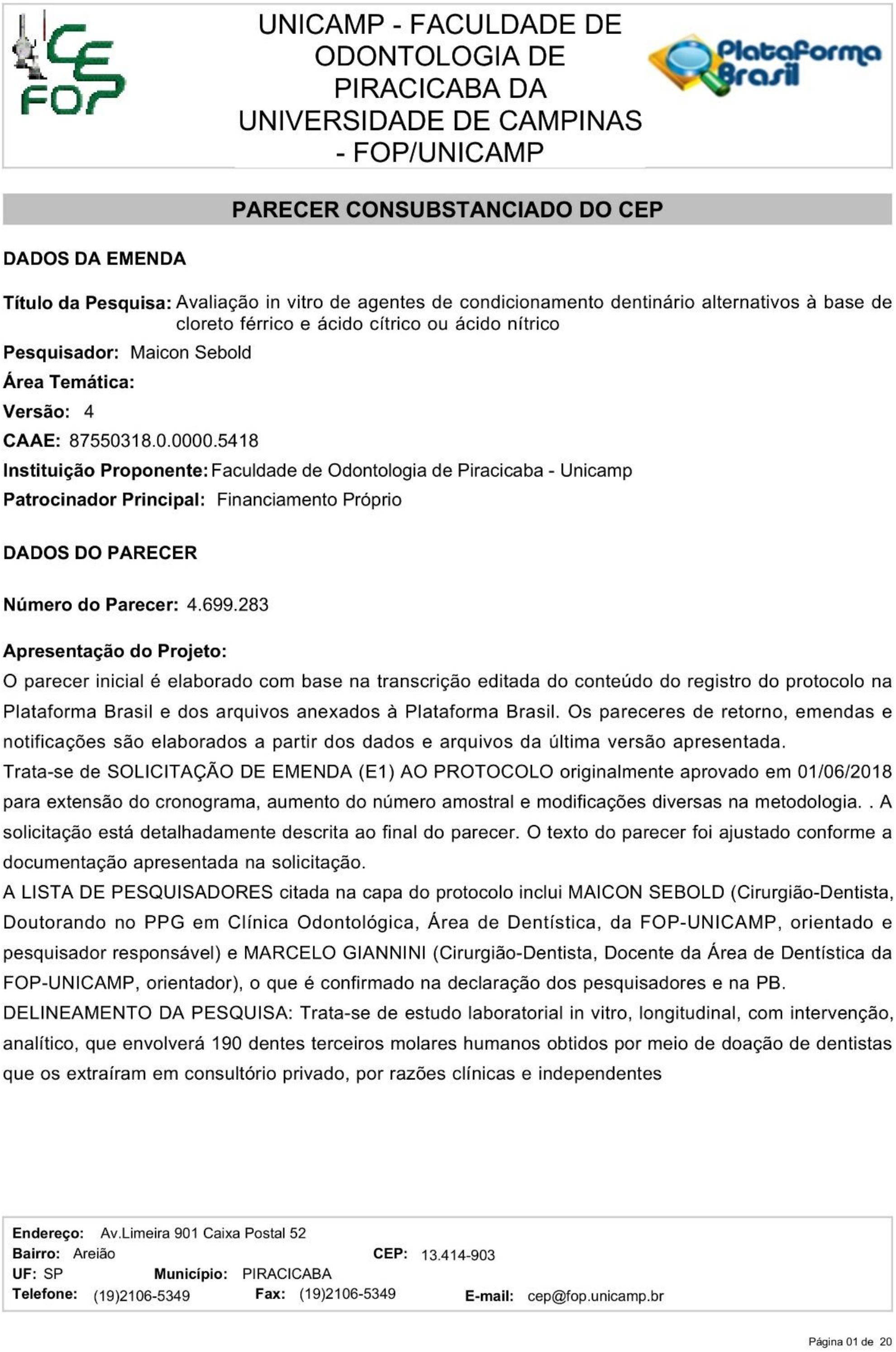
Fernanda Miori Pascon

Profa. Fernanda Miori Pascon
Vice Coordenador
CEP/FOP/UNICAMP

Prof. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
Notice: The title and the list of researchers of the project appears as provided by the authors, without editing.

Anexo 5 - Certificado de aprovação de emenda solicitada ao Comitê de Ética (artigo 2.2)



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DADOS DA EMENDA

Título da Pesquisa: Avaliação in vitro de agentes de condicionamento dentinário alternativos à base de cloreto férrico e ácido cítrico ou ácido nítrico

Pesquisador: Maicon Sebold

Área Temática:

Versão: 4

CAAE: 87550318.0.0000.5418

Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.699.283

Apresentação do Projeto:

O parecer inicial é elaborado com base na transcrição editada do conteúdo do registro do protocolo na Plataforma Brasil e dos arquivos anexados à Plataforma Brasil. Os pareceres de retorno, emendas e notificações são elaborados a partir dos dados e arquivos da última versão apresentada.

Trata-se de SOLICITAÇÃO DE EMENDA (E1) AO PROTOCOLO originalmente aprovado em 01/06/2018 para extensão do cronograma, aumento do número amostral e modificações diversas na metodologia. . A solicitação está detalhadamente descrita ao final do parecer. O texto do parecer foi ajustado conforme a documentação apresentada na solicitação.

A LISTA DE PESQUISADORES citada na capa do protocolo inclui MAICON SEBOLD (Cirurgião-Dentista, Doutorando no PPG em Clínica Odontológica, Área de Dentística, da FOP-UNICAMP, orientado e pesquisador responsável) e MARCELO GIANNINI (Cirurgião-Dentista, Docente da Área de Dentística da FOP-UNICAMP, orientador), o que é confirmado na declaração dos pesquisadores e na PB.

DELINEAMENTO DA PESQUISA: Trata-se de estudo laboratorial in vitro, longitudinal, com intervenção, analítico, que envolverá 190 dentes terceiros molares humanos obtidos por meio de doação de dentistas que os extraíram em consultório privado, por razões clínicas e independentes

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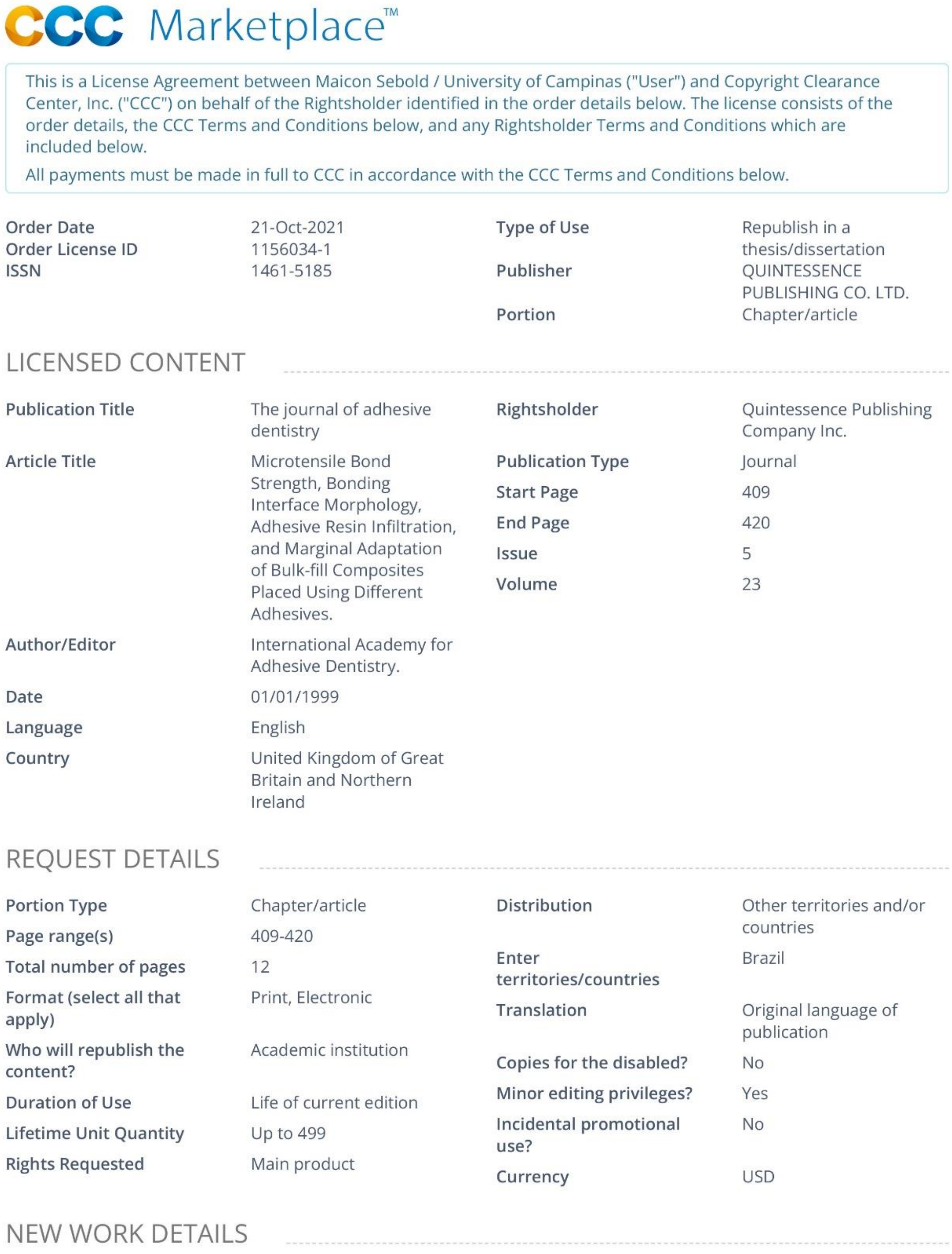
Anexo 6 - Autorização da editora para documento já publicado (artigo 2.3)



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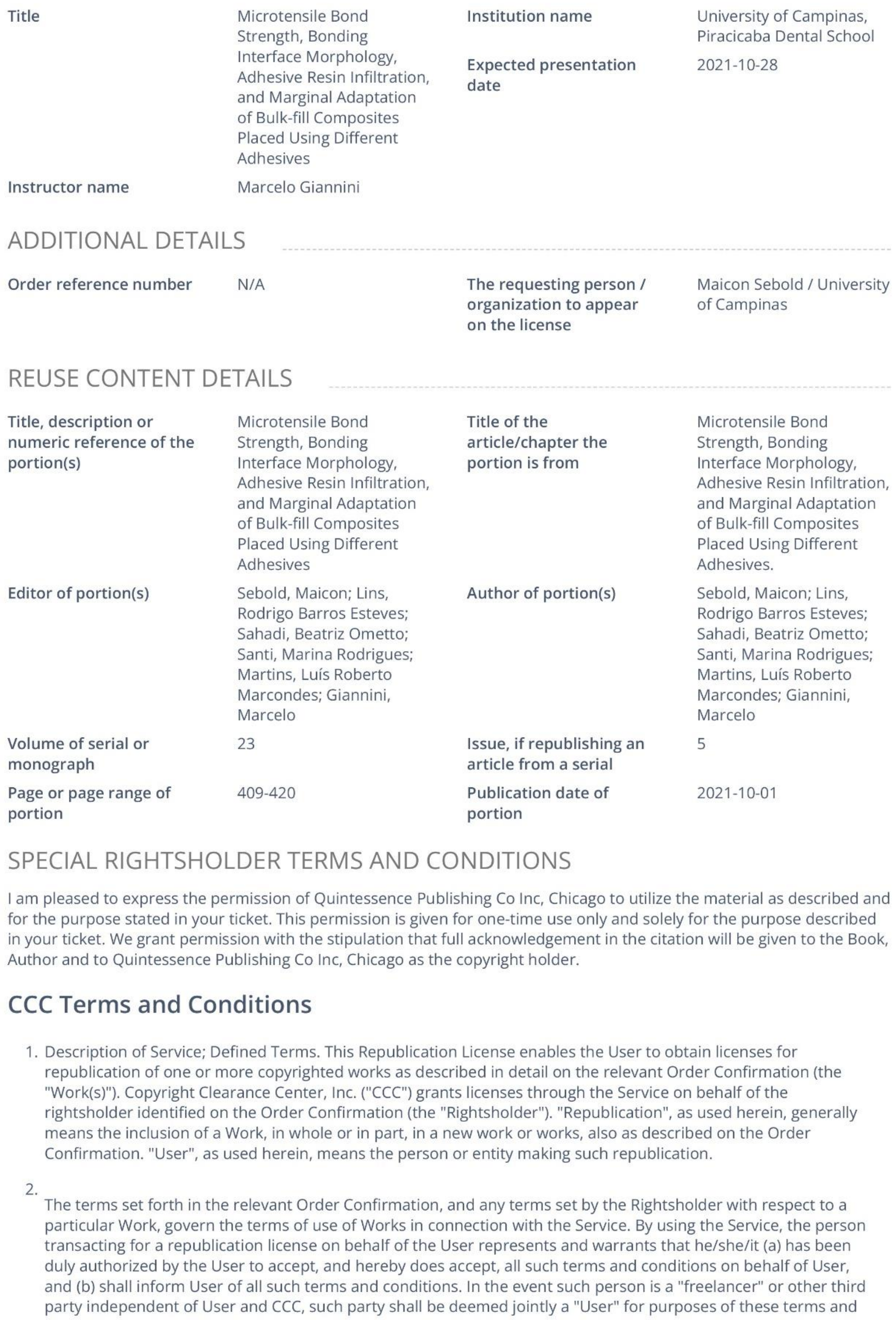

Title	Microtensile Bond Strength, Bonding Interface Morphology, Adhesive Resin Infiltration, and Marginal Adaptation of Bulk-fill Composites Placed Using Different Adhesives	**Institution name** **Expected presentation date**	University of Campinas, Piracicaba Dental School 2021-10-28
Instructor name	Marcelo Giannini		

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Editor of portion(s)	Sebold, Maicon; Lins, Rodrigo Barros Esteves; Sahadi, Beatriz Ometto; Santi, Marina Rodrigues; Martins, Luís Roberto Marcondes; Giannini, Marcelo	**Author of portion(s)**	Sebold, Maicon; Lins, Rodrigo Barros Esteves; Sahadi, Beatriz Ometto; Santi, Marina Rodrigues; Martins, Luís Roberto Marcondes; Giannini, Marcelo
Volume of serial or monograph	23	**Issue, if republishing an article from a serial**	5
Page or page range of portion	409-420	**Publication date of portion**	2021-10-01

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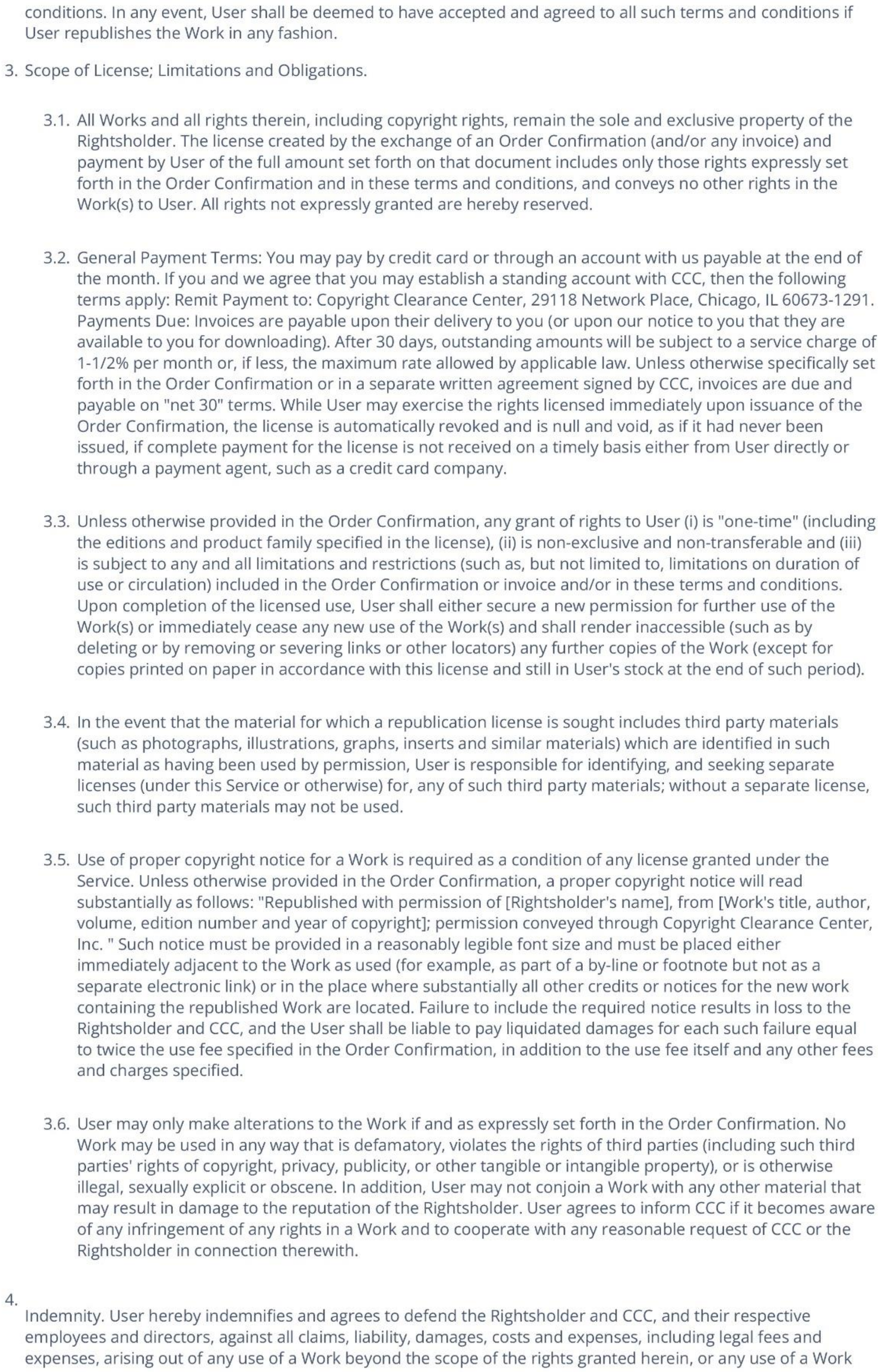

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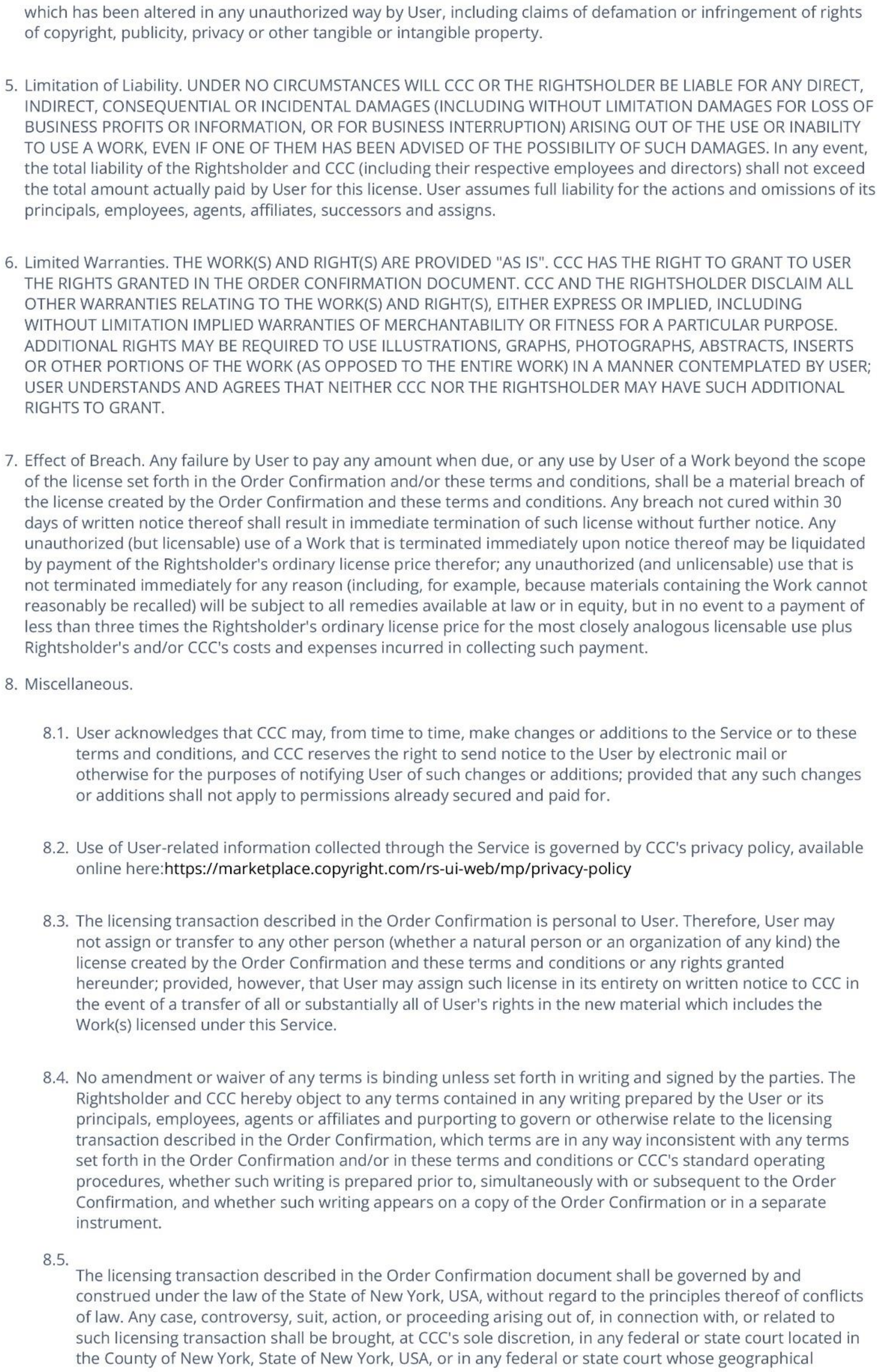

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Anexo 7 - Certificado do Comitê de Ética (artigo 2.3)

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "Avaliação da união resina-dentina de compósitos bulk-fill associados a diferentes abordagens de adesão dentinária", CAAE 86272518.3.0000.5418, dos pesquisadores Vanessa Germano, Maicon Sebold, Rodrigo Barros Esteves Lins e Luís Roberto Marcondes Martins, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 23/05/2018.

The Research Ethics Committee of the Piracicaba Dental School of the University of Campinas (FOP-UNICAMP) certifies that research project "Resin-dentin bond evaluation of bulk-fill composites associated with different dentin bonding strategies", CAAE 86272518.3.0000.5418, of the researcher's Vanessa Germano, Maicon Sebold, Rodrigo Barros Esteves Lins and Luís Roberto Marcondes Martins, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on May, 23 2018.

Fernanda Miori Pascon

Profa. Fernanda Miori Pascon
Vice Coordenador
CEP/FOP/UNICAMP

Prof. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
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