THIAGO HENRIQUE SCARABELLO STAPE

INFLUÊNCIA DO PRÉ-TRATAMENTO DENTINÁRIO COM DIMETILSULFÓXIDO NA RESISTÊNCIA DE UNIÃO, EXPOSIÇÃO DE MATRIZ COLÁGENA E GRAU DE CONVERSÃO DE SISTEMAS ADESIVOS

INFLUENCE OF DIMETHYL SULFOXIDE-WET BONDING TECHNIQUE ON DENTIN BOND STRENGTH, COLLAGEN EXPOSURE AND MONOMER CONVERSION OF ADHESIVE SYSTEMS

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Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba

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

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

Orientador: Prof. Dr. Luis Roberto Marcondes Martins

Este exemplar corresponde à versão final da tese defendida pelo aluno Thiago Henrique Scarabello Stape e orientado pelo Prof. Dr. Luis Roberto Marcondes Martins

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Prof. Dr. LUIS ROBERTO MARCONDES MARTINS

Prof. Dr. PAULO SÉRGIO QUAGLIATTO

Profa. Dra. LÚCIA TRAZZI PRIETO

Prof. Dr. ANDERSON CATELAN

Profa. Dra. THAIS MARQUES SIMEK VEGA GONÇALVES

RESUMO

Essa tese avaliou um novo protocolo adesivo, que consiste no pré-tratamento dentinário com dimetilsulfóxido (DMSO) 50%, com o intuito de otimizar a durabilidade de interfaces adesivas produzidas pelos mecanismos de união convencional e autocondicionante envolvendo o substrato dentinário. Para isso, foram realizados dois estudos independentes para avaliar: (i) o efeito do uso do dimetilsufóxido (DMSO) na resistência de união imediata e na exposição de matriz colágena decorrente da hibridização dentinária na interface adesiva; e (ii) para avaliar o efeito do protocolo proposto na conversão monomérica na camada híbrida e na resistência de união após envelhecimento. Terceiros molares hígidos foram coletados e limpos, a face oclusal foi seccionada, expondo uma superfície dentinária de profundidade média, que foi saturada com uma solução aquosa de DMSO 50% (pH 8.2) após o condicionamento ácido para o sistema convencional de três passos (Adper Scothbond Multi-Purpose: 3M ESPE: SBMP) e previamente a aplicação do sistema autocondicionante de dois passos (Clearfil SE Bond: Kuraray; CF) no substrato mineralizado. Nos grupos controles, as amostras não foram tratadas com DMSO. Os segmentos restaurados com resina composta foram seccionados e submetidos ao ensaio de microtração após 24 h à 0,5 mm/min até a fratura (n=12) e no segundo estudo (n=10), após 1 ano e 2 anos. Foi realizada Two-way ANOVA para análise dos dados imediatos e no segundo estudo foi utilizada ANOVA medidas repetidas (proc mixed), ambos seguidos pelo Teste de Tukey (α=0.05). Foi realizado a análise histomorfométrica (n=12) para avaliação da extensão de matriz colágena exposta na interface adesiva por meio de microscopia ótica e coloração de Tricrômica de Masson após 24 h. Esses dados foram submetidos a Two-way ANOVA, seguido do Teste de Tukey (α=0.05). O grau de conversão monomérica (DC) na camada híbrida foi avaliado por espectroscopia micro-raman após 24 h (n=10), sendo submetidos a One-way ANOVA e Teste de Tukey (α=0.05). A interação entre protocolo adesivo e sistema adesivo influenciou significativamente a extensão de matriz colágena exposta (p<0,0001) e resistência de união imediata

(*p*=0,0091). O protocolo adesivo com DMSO reduziu significativamente o grau de exposição de matriz colágena exposta (SBMP: 85,3% e CF: 61,5%), melhorando a qualidade da hibridização dentinária para ambos sistemas adesivos. Não houve influência negativa do DMSO na conversão monomérica do SBMP (*p*=0.892); já para o CF, houve aumento na conversão monomérica (*p*=0.033). O pré-tratamento com DMSO aumentou a resistência de união imediata do SBMP (*p*<0.0001). Considerando as amostras não tratadas, houve uma redução significativa (SBMP: 45,6% e CF: 36,8%) na resistência de união do SBMP (*p*<0.0001) e CF (*p*<0.0001) após dois anos de envelhecimento. Independentemente do tipo de adesivo, não houve diferença estatística na resistência de união entre valores imediatos e após 2 dois anos quando o DMSO foi empregado (*p*>0,05). A análise dos padrões de falha evidenciou uma tendência na redução no número de falhas adesivas após dois anos para as amostras tratadas com DMSO. Portanto, o protocolo adesivo proposto utilizando o DMSO é uma abordagem promissora para reduzir degradação da interface adesiva para os adesivos convencional e autocondicionante testados.

Palavras-chave: Dimetilsulfóxido, Dentina, Envelhecimento, Adesivos Dentinários, Camada híbrida.

ABSTRACT

This work highlights a new bonding concept using 50% dimethyl sulfoxide (DMSO) as a dentin pre-treatment to optimize resin-dentin bonding for both etch-and rinse and self-etch adhesive systems after long-term aging. Two independent studies were performed: (i) to evaluate the effect of dimethyl sulfoxide wet-bonding technique on resin infiltration depths at the bonded interface and dentin bond strength; and (ii) to examine the effect of dentin pre-treatment with DMSO on the degree of conversion and dentin bond strength after long-term aging. Flat dentin surfaces derived from extracted sound human third molars were saturated with 50% DMSO (pH 8.2) after acid etching for a water-based etch-and-rinse adhesive system (Adper Scothbond Multi-Purpose: 3M ESPE; SBMP), and before acid primer application for a 10-MDP self-etch adhesive (Clearfil SE Bond: Kuraray; CF). In control groups, specimens were not treated with DMSO. The restored tooth segments were sectioned and submitted to microtensile bond strength test at 24 h (n=12), 1 year (n=10) and 2 years (n=10) at 0.5 mm/min until specimen fracture. One slab per tooth (n=12) was stained by Masson Trichrome and the extent of exposed collagen matrix at the bonded interface was evaluated using optical microscopy and a histomorphometric software at 24 h. Statistical analysis of the extension of collagen exposure was performed by two-way ANOVA followed by Tukey Test (α=0.05). The degree of conversion (DC) was measured inside the hybrid layer by micro-raman spectroscopy (n=10) at 24 h. DC statistical analysis was performed by One-way ANOVA and Tukey Test (α=0.05). Immediate microtesnile data was statistically analyzed Two-way ANOVA and aged data was analyzed by repeated measurres ANOVA (proc mixed). Post hoc multiple comparisons were performed by Tukey Test (α=0.05). The interaction between “dentin pre-treatment” and “adhesive system” significantly influence the extent of exposed collagen matrix ($p<0.0001$) and dentin bond strength ($p=0.0091$). The adhesive protocol with DMSO significantly reduced the extent of exposed collagen matrix (SBMP: 85.3% and CF: 61.5%), improving the quality of dentin hybridization for both adhesive systems. There was

no negative influence of DMSO in monomer conversion for SBMP ($p=0.892$); nevertheless, there was an increase in monomer conversion ($p=0.033$) for CF. Dentin pretreatment with DMSO increased the immediate bond strength of SBMP ($p<0.0001$). Considering the untreated samples, there was a significant reduction (SBMP: 45.6% and CF: 36.8%) on the bond strength of SBMP ($p<0.0001$) and CF ($p<0.0001$) after two years of aging. Regardless of adhesive type, no significant reduction in bond strength at two years for DMSO-treated samples was observed. Fracture pattern analysis showed a tendency toward reduction in the number of adhesive failures when the DMSO-wet bonding was performed. Therefore, DMSO-wet bonding is a promising approach to reduce resin-dentin bond degradation of etch-and-rinse and self-etch adhesives.

Key words: Dimethyl sulfoxide, Dentin, Aging, Dentin-Bonding Agents, Hybrid layer.

SUMÁRIO

DEDICATÓRIA

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“*O período de maior ganho em conhecimento e experiência é o período mais difícil da vida de alguém.*”

Dalai Lama

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ANOVA – Análise de variância

BiS-GMA – Bis-Fenol A-Glicidil Metacrilato

CF – Clearfil SE Bond

DC – Grau de conversão

DMSO – Dimetilsulfóxido

Fig – Figura

h – Hora

HEMA – Hidróxietil metacrilato

min – Minuto

fenil-P – 2-metacriloxietil fenil hidrogênio fosfato

s – Segundo

SBMP – Adper Scotchbond Multi-Purpose

vol – Volume

4-MET – 4-metacrilóxietil trimelítico

10-MDP 10-metacriloxidecil di-hidrogênio fosfato

μTBS – Microtração

mm/min – milímetros por minuto

MPa – Megapascal, unidade de medida de pressão no SI.

μm – Micrômetro, unidade de mediada referente a comprimento no SI.

nm – Nanômetro, unidade de mediada referente a comprimento no SI.

μL – Microlitro, unidade de medida referente a volume no SI.

SI – Sistema internacional de medidas

INTRODUÇÃO

O surgimento da odontologia adesiva (Buonocore, 1955) juntamente com a evolução dos sistemas adesivos e técnicas adesivas nas últimas décadas revolucionaram a Odontologia, permitindo a realização de novas alternativas restauradoras de natureza estético-conservadoras até então impraticáveis. No entanto, para que os procedimentos restauradores adesivos apresentem bom desempenho clínico é imprescindível o desenvolvimento de uma união forte e estável aos tecidos dentários. Apesar da união de sistemas adesivos ao esmalte ser consagrada (Van Meerbeek et al., 2003), a união à dentina ainda constitui um desafio e apresenta limitações quando avaliada em longo prazo (Breschi et al., 2008; Liu et al., 2011; Carvalho et al., 2012).

Os primeiros sistemas adesivos desenvolvidos eram compostos por monômeros majoritariamente hidrófobos aplicados diretamente sobre o esmalte e dentina sem nenhum preparo prévio, técnica conhecida como *no-etch bonding*. Baixos valores de resistência de união eram obtidos com a utilização destes sistemas adesivos, especialmente em dentina, em função da permanência da *smear layer*, que é fracamente aderida ao substrato dentário. Na técnica *no-etch,* os valores de resistência de união (5-10 MPa) eram essencialmente resultado da resistência coesiva do *smear layer* e não necessariamente da interação entre o sistema adesivo e a dentina, o que era insuficiente para resistir até mesmo à tensão de contração (15-17 MPa) das resinas compostas da época (Davidson et al., 1984).

Estudos demonstraram que a não remoção da *smear layer* pelos sistemas adesivos utilizados até o final da década de 1980 promovia um desempenho clínico insatisfatório (Van Meerbeek et al., 1994) em função da união micromecânica ineficaz entre os sistemas adesivos e o substrato dentinário. Para que valores de resistência de união satisfatórios fossem alcançados, a remoção da *smear layer* passou a ser considerada, desenvolvendo-se a técnica *total-etch* (Fusayama et al., 1979). A técnica *total-etch* preconiza a desmineralização do substrato dentário removendo completamente a *smear layer*, o que resulta em exposição de fibrilas colágenas presentes no substrato dentinário. No início da década de 1980, obteve-se conhecimento sobre a interação entre sistemas adesivos e dentina criando-se o termo camada híbrida,

hybrid layer, para descrever a estrutura composta por fibrilas colágenas infiltradas por monômeros resinosos formadas na interface adesiva quando a técnica *total-etch* era empregada (Nakabayashi et al., 1982). Inicialmente, o conceito *total-etch* gerou controvérsia na Europa e nos Estados Unidos pois acreditava-se que a aplicação de ácidos diretamente no substrato dentinário poderia aumentar a probabilidade do desenvolvimento de injúrias pulpares (Macko et al., 1978). Após uma vasta revisão de literatura, conclui-se que as injúrias pulpares estavam mais relacionadas à falha do sistema adesivo em selar a interface adesiva em relação à agressividade do condicionamento ácido (Lee et al.; Cox et al., 1987). Assim, os primeiros adesivos comerciais *total-etch* de 3 passos, também conhecidos como adesivos de quarta geração, foram lançados comercialmente no início da década de 1990, dando início à era moderna da Odontologia adesiva.

A camada híbrida é composta por aproximadamente 50% de monômeros resinosos e 50% de fibrilas colágenas (Pashley et al., 2007), abrangendo em torno de 5 µm de profundidade em sistemas adesivos do tipo *total-etch* (Pashley et al., 2007; Spencer et al., 2004), que promove microrretenção mecânica para a camada adesiva e restauração à estrutura dentária. No entanto, sua formação é complexa e dependente de inúmeros fatores inerentes a manutenção do arcabouço da camada de fibrilas colágenas desmineralizadas e o adequado controle de umidade (Pashley et al., 2007). Dentre esses fatores, o colapso da matriz colágena durante o controle da umidade do substrato dentinário (Pashley et al., 2007) e a incompleta difusão dos monômeros resinosos por toda a extensão do substrato desmineralizado previamente produzido (Spencer et al., 2004) invariavelmente resultam na produção de interfaces porosas (Spencer and Wang, 2002; Tay et al., 2002; Tay et al., 2003), susceptíveis a degradação hidrolítica (Hashimoto et al., 2003; Pashley et al., 2004; Mobarak et al., 2010).

Nesse mesmo período, o conceito de "*dry-bonding"*, que consistia na secagem do substrato dentinário desmineralizado com jatos de ar previamente à aplicação do sistema adesivo caiu em desuso sendo substituído pelo conceito "*wet-bonding"* (Gwinnett and Kanca, 1992; Kanca, 1992), que passou a ser o mais indicado para adesivos *total-etch*. A manutenção do substrato dentinário parcialmente úmido, conceito "*wet-bonding",* produz valores de resistência de união significativamente maiores em relação ao

substrato seco (Kanca, 1992), em função da redução do colapso das fibrilas colágenas resultante da desidratação da matriz colágena (Pashley et al., 2007). No entanto, o excesso de umidade também prejudica o desempenho de sistemas adesivos *total-etch* pela ocorrência de separação de fases na interface adesiva (Spencer and Wang, 2002; Ye et al., 2009). Os sistemas adesivos atuais são compostos por monômeros hidrófilos e hidrófobos, dissolvidos em etanol, água, acetona ou em misturas desses solventes (Van Landuyt et al., 2007). Quando o substrato apresenta-se altamente úmido, os solventes (principalmente o etanol e acetona) perdem a capacidade de remover as moléculas de água que se encontram presentes entre as fibrilas colágenas, que tem a função de manter o arcabouço colágeno. O excesso de água residual causa a separação dos monômeros, o que compromete a qualidade da camada híbrida, prejudicando a interface de união.

Com a intenção de reduzir as falhas inerentes à aplicação de sistemas adesivos *total-etch*, no início da década de 1990, iniciou-se o desenvolvimento de sistemas adesivos *self-etch*, que apresentam um mecanismo de união diferente dos adesivos *total-etch*. Os sistemas adesivos *self-etch* apresentam pH inferior (pH 1-2) aos adesivos *total-etch* (pH 2,5-4,5), possibilitando a dissolução do *smear layer* pelos próprios monômeros sem a necessidade do condicionamento ácido em etapa prévia à aplicação do sistema adesivo. Diferentemente dos sistemas *total-etch,* os monômeros acídicos funcionais condicionam e penetram no substrato dentinário simultaneamente, incorporando a *smear layer* na interface adesiva, e promovem ligações químicas primárias entre os monômeros ácidos (fenil-P, 4-MET e 10-MDP) com os íons Ca^{2+} presentes nos cristais de hidroxiapatita (Van Meerbeek et al., 2011). O nível de interação química varia especialmente em função ao tipo de monômero utilizado sendo que o 10-MDP produz maior interação química em relação ao fenil-P e 4-MET. Como resultado, há a formação de uma camada híbrida notavelmente delgada (0,5-1 µm) para os adesivos *self-ecth* do tipo *Mild* (pH~2) onde os monômeros 10-MDP difundem superficialmente na dentina de forma organizada, produzindo camadas nanométricas (4 nm) sequenciais compostas por duas moléculas de 10-MDP em cada camada (Van Meerbeek et al., 2011). Diferentemente dos adesivos *no-etch,* que foram inicialmente utilizados na década de 1960, os adesivos *self-etch* contemporâneos apresentam um maior caráter hidrófilo e pH

inferiores, o que permite que a *smear layer* seja legitimamente incorporada na interface adesiva fazendo com que os monômeros funcionais interajam com os cristais de hidroxiapatita firmemente ancorados na dentina, resultando em desempenho clínico satisfatório (Van Meerbeek et al., 2005; Peumans et al., 2007). Dessa forma, o sistema *self-etch* apresenta vantagens em relação ao sistema *total-etch* quando adequadamente utilizado em dentina: a exposição de fibrilas colágenas na base da camada é reduzida (Carvalho et al., 2005); a redução na sensibilidade técnica operatória, pelo procedimento ser obrigatoriamente realizado em substrato seco, pois a produção de um substrato uniformemente seco é mais fácil em relação a um substrato uniformemente úmido; apresenta duplo mecanismo de união em decorrência da união micromecânica e química (Van Meerbeek et al., 2011).

Mesmo com a grande evolução dos sistemas adesivos nas últimas três décadas, as interfaces adesivas criadas para sistemas *total-etch* e *self-etch* ainda apresentam limitações que contribuem para a sua instabilidade em longo prazo (Breschi et al., 2008), sendo que a interface adesiva ainda pode ser considerada como o tendão de Aquiles de restaurações em resina composta (Peumans et al., 2005; De Munck et al., 2005; Spencer et al., 2011), pois sua relativa baixa durabilidade (Peumans et al., 2005; Carvalho et al., 2012) compromete o desempenho clínico de restaurações adesivas (van Dijken et al., 2007; van Dijken and Hasselrot, 2010). Do ponto de vista clínico, de 60% a 70% dos procedimentos restauradores são constituídos por substituição de restaurações, sendo que os fatores ligados à degradação marginal, como o desenvolvimento de lesões cariosas secundárias, perda da restauração e microinfiltração, são observados corriqueiramente em restaurações adesivas (Murray et al., 2002; Wilson et al., 1997). Diante destas limitações e, sabendo-se que os componentes da camada híbrida são sujeitos a duas formas de degradação: hidrólise das ligações ésteres na porção resinosa e a degradação das fibrilas colágenas principalmente por meio da ação de proteases endógenas (Tjäderhane et al., 2014; Liu et al., 2011) diversos métodos têm sido propostos para melhorar a longevidade dos sistemas adesivos, tais como a técnica alcoólica (Chiba et al., 2006), o uso de inibidores de proteases (Carrilho et al., 2007; Hebling et al., 2005; Tezvergil-Mutluay et al., 2011), aumento no tempo de aplicação dos sistemas adesivos (Reis et al., 2008) e a utilização de substâncias que aumentam as

ligações cruzadas na matriz colágena (Bedran-Russo et al., 2011). No entanto, esses métodos não são eficazes para prevenir a degradação da porção resinosa e da porção colágena presentes na camada híbrida de forma simultânea, o que limita a real capacidade de prevenir a degradação da interface adesiva. A única exceção é a técnica alcoólica, que atua em ambas vertentes de degradação, mas que se torna inviável clinicamente em função do tempo necessário para sua aplicação e do grande número de etapas clínicas.

Na tentativa de melhorar a durabilidade de interfaces adesivas de sistemas adesivos convencionais, o dimetilsulfóxido (DMSO) utilizado em baixa concentrações, 0,004%, foi recentemente proposto como uma alternativa para preservar a resistência de união de adesivos *total-etch* (Tjäderhane et al., 2013). O DMSO é um solvente polar aprótico, classificado como Classe 3 (*FDA Guidance for Industry)* na mesma categoria que solventes como a acetona e o etanol, é completamente miscível em água (MacGregor, 1967), de baixa toxicidade (Marren, 2011), e é capaz de dissolver substâncias polares e apolares, incluindo monômeros resinosos hidrófobos e hidrófilos como o BisGMA, HEMA (Geurtsen et al., 1998). Estudos preliminares têm demonstrado que o DMSO não afeta o metabolismo de células odontoblásticas, apresentando baixa citotoxicidade (Bianchi et al., 2012). O DMSO é uma molécula polifuncional composta por um grupo S=O altamente polar e dois grupos hidrófobos CH_3 (MacGregor, 1967). Em função do seu caráter anfifílico, baixo peso molecular, e natureza dipolar aprótica, o DMSO é possivelmente um dos melhores intensificadores de penetração tecidual para aplicações médicas da atualidade (Marren, 2011). A grande penetração tecidual está associada parcialmente a sua capacidade de substituir as moléculas de água presentes nas fibrilas colágenas e romper as pontes de hidrogênio interfibrilares presentes, aumentando os espaços interfibrilares (Tjäderhane et al., 2013; Bui et al., 2010; Zimmerley et al., 2009). Com isso, a utilização do DMSO na odontologia adesiva poderia contribuir para o aumento da estabilidade da interface adesiva não exclusivamente para sistemas adesivos *total-etch* (Tjäderhane et al., 2013), mas possivelmente para sistemas adesivos *self-tech*. Além do mais, os efeitos positivos do uso do DMSO em sistemas *total-etch* previamente relatados (Tjäderhane et al., 2013) poderiam ser potencilamente exacerbados caso uma maior concentração fosse utilizada.

Devido ao crescente interesse clínico em medidas para melhorar a durabilidade de interfaces adesivas envolvendo o substrato dentinário, essa tese teve como objetivo avaliar, por meio de estudos *in vitro,* o efeito de um protocolo adesivo inédito, que utiliza uma maior concentração de DMSO em relação a estudos prévios, (Tjäderhane et al., 2013) na interface adesiva de sistemas adesivos *total-etch* e *self-etch*. Para isso, foram associadas diferentes metodologias laboratoriais levando em consideração a resistência de união em diferentes tempos de envelhecimento, juntamente à avaliação do grau de conversão monomérica na camada híbrida e à efetividade de envelopamento da matriz colágena exposta produzida pelos diferentes sistemas adesivos submetidos ao protocolo adesivo proposto. Esta tese foi formulada em formato alternativo, sendo dividida em dois capítulos. No primeiro capítulo, os objetivos específicos foram avaliar o efeito do pré-tratamento dentinário com dimetilsulfóxido 50% associado a um sistema adesivo convencional e um sistema autocondicionante: (i) na resistência de união imediata por meio do teste de microtração e análise do padrão de fratura por meio de microscopia eletrônica de varredura; e (ii) na capacidade de envelopamento da matriz colágena exposta na interface adesiva por meio de análise histomorfométrica e microscopia ótica utilizando a coloração Tricrômica de Masson. No segundo capítulo, os objetivos específicos foram avaliar o efeito do protocolo proposto associado aos mesmos sistemas adesivos: (i) na resistência de união após envelhecimento após dois anos utilizando o teste de microtração e análise do padrão de fratura; e (ii) no grau de conversão monomérica na camada híbrida por meio de espectrometria micro-raman.

CAPÍTULO 1

Effect of dimethyl sulfoxide wet-bonding technique on hybrid layer quality and dentin bond strength

Abstract

Objectives: This study examined the effect of a dimethyl sulfoxide (DMSO) wet bonding technique on the resin infiltration depths at the bonded interface and dentin bond strength of different adhesive systems.

Methods: Flat dentin surfaces of 48 human third molars were treated with 50% DMSO (experimental groups) or with distilled water (controls) before bonding using an etch-and-rinse (SBMP: Scotchbond Multi-Purpose, 3M ESPE) or a self-etch (Clearfil: Clearfil SE Bond, Kuraray) adhesive system. The restored crown segments (n=12/group) were stored in distilled water (24 h) and sectioned for interfacial analysis of exposed collagen using Masson's Trichrome staining and for microtensile bond strength testing. The extent of exposed collagen was measured using light microscopy and a histometric analysis software. Failure modes were examined by SEM. Data was analyzed by two-way ANOVA followed by Tukey Test (α=0.05). *Results:* The interaction of bonding protocol and adhesive system had significant effects on the extension of exposed collagen matrix ($p<0.0001$) and bond strength ($p=0.0091$). DMSO-wet bonding significantly reduced the extent of exposed collagen matrix for SBMP and Clearfil ($p<0.05$). Significant increase in dentin bond strength was observed on DMSO-treated specimens bonded with SBMP ($p<0.05$), while no differences were observed for Clearfil ($p>0.05$). *Significance:* DMSO-wet bonding was effective to improve the quality of resin-dentin bonds of the tested etch-and-rinse adhesives by reducing the extent of exposed collagen matrix at the base of the resin-dentin biopolymer. The improved penetration of adhesive monomers is reflected as an increase in the immediate bond strength when the DMSO-wet bonding technique is used with a water-based etch-and-rinse adhesive.

Keywords: Hybrid layer; Collagen; Resin infiltration; Etch-and-rinse adhesive; Self-etch adhesive; Microtensile bond strength, DMSO.

1. Introduction

Modern dental restorations rely on the adhesives providing adequate bonds between the tooth and the restorative composite. Several approaches to enhance the bond strength of adhesive system to dentin have been studied in the past decade [1–4]. Even though they have produced promising *in vitro* results, some concepts defy the principles of user friendliness and technique simplification. Clinically feasible acceptable methods to enhance dentin adhesion improving the collagen-resin biopolymer are still needed.

Current bonding systems to dentin rely on effective adhesive penetration into dentin substrate to form the hybrid layer [3,5] and on chemical interactions between residual hydroxyapatite and specific functional monomers found mainly in self-etch adhesives [6–8]. The resultant micro-mechanical interlocking formed after dentin hybridization is a prerequisite to achieve adequate dentin bonding [3,8]. Ideally, such resin-dentin interfusion zone should form a continuous and stable tooth-restoration interconnection. However, this objective is not achieved by contemporary adhesives [4,9,10].

Dimethyl sulfoxide (DMSO; $(CH_3)_2SO$) is a polar aprotic solvent that dissolves both polar and non-polar compounds. It is a polyfunctional molecule with a highly polar S=O group and two hydrophobic CH_3 groups. DMSO is fully miscible in solvents and most adhesive monomers [11] used in adhesive dentistry. Moreover, DMSO has the ability to dissociate the highly cross-linked collagen into a sparser network of apparent fibrils [12] also in dentin matrix [13], most likely by the suppression of hydrogen bond-mediated attractive forces within the collagen [14]. This allows DMSO to efficiently penetrate biological surfaces, which makes it perhaps the best currently known penetration enhancer for medical purposes [15].

In a recent study, a considerably low concentration of DMSO was shown to reduce dentin bond strength loss after aging [13]. Considering DMSO properties, higher concentrations might have a positive effect on dentin bonding. Since adhesive resins cannot completely infiltrate collagen matrices in demineralized dentin [16], and since monomer diffusion into the dentin substrate is critical to proper dentin bonding [3,4,8,16], the aim of this study was to investigate the effect of high DMSO concentration on dentin

bonding. The hypothesis set were that dentin pretreatment with DMSO would (i) reduce the amount of exposed collagen matrix at the base of the hybrid layer and (ii) increase immediate dentin bond strength values of commercially available adhesive systems.

2. Materials and methods

2.1 Tooth Preparation

Forty-eight recently extracted non-carious human third molars were obtained after patient informed consent under a protocol approved by the Ethical Committee of the Piracicaba Dental School, University of Campinas, Brazil (protocol 017/2013). Teeth were cleaned, ultrasonicated in water for 5 min for cleaning, disinfected for one week in 0.5% chloramine-T solution at 4°C, and stored in distilled water at 4°C for up to one month before use. A flat coronal dentin surface was obtained by sectioning off the occlusal surface (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA). The surface roughness was standardized with 600-grit silicon carbide paper (BuehlerMet, Buehler) for one min under water cooling.

2.2 Dentin Bonding

The teeth were randomly assigned to four groups (n=12) according to the adhesive/bonding technique: (i) three-step etch-and-rinse adhesive (Adper Scotchbond Multi-Purpose, 3M ESPE, St. Paul, MN, USA) (SBMP); (ii) DMSO-wet bonding with SBMP; (iii) two-step self-etch adhesive (Clearfil SE Bond, Kuraray, Osaka, Japan) (Clearfil); and (iv) DMSO-wet bonding with Clearfil. DMSO-wet bonding consisted of light-pressure circular scrubbing movements of a 50 µL of water-based 50% (v/v) DMSO (Sigma-Aldricht, St Louis, MO, USA) (pH 8.2) for 60 seconds, using a disposable cavity brush. For SBMP, DMSO was applied after dentin etching; for Clearfil, DMSO was applied onto smear layer-covered dentin. Control groups consisted of distilled water application for 60 s instead of DMSO. Table 1 displays mode of application, components and manufacturers of the adhesives. Both adhesive systems were applied actively [17]. Adhesive procedures were carried out in a controlled environment with a temperature of 24°C and a relative humidity of 60%. Resin composite build-ups (Z250, shade A2, 3M

ESPE) were built on top of the bonded dentin surfaces in four 1-mm increments that were individually light-cured for 20 s. Light curing of all resin materials was performed using a LED device (Bluephase 20i, Ivoclare Vivadent, Schaan, Liechtenstein). All procedures were carried out by a single operator.

2.3 Specimen Preparation

After storage in distilled water at 37°C for 24 h, the restored segments were sectioned (Isomet 1000 Precision Saw, Buehler) occluso-gingivally into four slabs measuring approximately 0.9 mm. One composite-dentin slab was randomly reserved for morphologic analysis of the adhesive interface with an optical microscope to evaluate the presence of demineralized but unprotected collagen matrix at the base of the hybrid layer. The remaining slabs were further sectioned into composite-dentin sticks, 0.8 mm^2 cross sectional area, in accordance with the “non-trimming” technique [18] for bond strength testing. A minimum of nine sticks were obtained from each tooth.

2.4 Optical Microscopy

One slab from each tooth measuring 0.9 mm in thickness was selected (n=12/group). The slabs were fixed in a glass slide with a cyanoacrylate adhesive adhesive (Super Bonder, Loctite, São Paulo, SP, Brazil) and hand-polished with SiC papers of increasingly fine grits (P1200, P2000, P2500, P4000) under water cooling until its thickness was approximately 10 µm. Specimen were treated with Masson’s trichromic acid staining technique. Briefly, the polished specimen were immersed in Bouin’s solution for 1 h at 56℃, rinsed with distilled water, and incubated in Weigert’s haematoxylin for 10 min. They were then washed with distilled water for 10 min, incubated in acidic scarlet fuchsine for 3 min, rinsed with distilled water, immersed in phosphomolybdic acid for 10 min, and immediately stained with light green for 10 min. Finally, the specimen were rinsed with distilled water and submerged in glacial acetic acid for 3 min.

Masson dye has a high affinity for cationic elements of normally mineralized type I collagen, staining collagen green. Etching with phosphoric acid removes these elements from collagen resulting in different coloration, generally red. Specimens were examined in an optical microscope (Leica DMLP, Leica Microsystems, Heerbrugg, Switzerland) at

400X magnification. In each slab the width of a red band corresponding to demineralized dentin with exposed collagen was analyzed. Minimum of five digital images of the entire bonded interface of each slab were obtained using an open-source image software (ImageJ, National Institute of Health, Bethesda, Maryland USA). From each image, six measurements of the width of the red band were performed. To evenly distribute measurements across the entire bonding interface, each image was divided into three parts and two measurements were performed in each equidistant from each other. All in all, over 1440 measurements of the width of exposed collagen were done in this study. Measurements were performed by one single-blinded examiner. The average width of the red band for all images in each slab was calculated and corresponded for the extent (µm) of exposed collagen layer at the base of the hybrid layer [9,19] for the corresponding tooth.

2.5 Microtensile Bond Strength Test (µTBS)

Sticks were individually attached to a Geraldeli Device (Odeme, Santa Catarina, Brazil) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and submitted to the µTBS test (DL2000, EMIC, São José dos Pinhais, Brazil) at crosshead speed of 0.5 mm/min until failure. The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm to calculate the actual bond strength (MPa). Bond strengths for each tooth were determined by the µTBS average values of a minimum of nine sticks. Sticks with premature failures were recorded as 0 MPa for the statistical analysis.

2.6 Failure Mode Analysis

Both sides of fractured sticks were chemically dehydrated in ascending ethanol concentrations (50% to 90%) for one h in each and for two h in 100% ethanol, and finally by immersion in hexamethyldisilazane for 10 min on filter paper inside a covered glass vial, and air-dried at room temperature. The specimens were mounted on aluminum stubs, gold-sputtered and analyzed under magnification of 100-6000X using a scanning electron microscope (LEO 435 VP; LEO Electron Microscopy Ltd, Cambridge, UK) operating on secondary electron mode at 15 Kv. Failure modes were classified as

adhesive (A), cohesive failure in dentin (CD), cohesive failure in resin composite (CC), and mixed failure (M) [20].

2.7 Statistical Analyses

Data from the μTBS tests and the exposed collagen zone analysis were normally distributed (Kolmogorov-Smirnov Test) and homoscedastic (Levene Test). Data were analyzed separately with a two-way ANOVA design (adhesive system and bonding protocol). *Post hoc* multiple comparisons were performed with Tukey Studentized Range (HSD) Test, with statistical significance set at $\alpha=0.05$ using SAS statistical software (SAS 9.4 Software, SAS Institute, NC, USA). Tooth was considered the statistical unit.

3. Results

3.1 Optical Microscopy

Representative light micrographs of Masson's Trichrome stained sections of the dentin/adhesive interface for SBMP and Clearfil are presented in Figure 1 A-D. In all specimens, exposed demineralized collagen not encapsulated by the adhesive stained red, forming a distinct uniform red layer, while adhesive resin stained beige. Overall values of exposed matrix widths for all groups are shown in Table 2. Two-way ANOVA revealed that the "adhesive system" ($p<0.0001$) and the "interaction of adhesive system and bonding protocol" ($p<0.0001$) had significant effects on the extent of exposed collagen at the base of the hybrid layer. DMSO-wet bonding significantly reduced the extent of exposed collagen for both SBMP and Clearfil ($p<0.05$).

SBMP specimens (Figure 1 A) presented a distinct dark red zone, 4.9 to 8.5 μm wide, at the base of the hybrid layer. For Clearfil (Figure 1 C), the red layer was significantly thinner ($p<0.05$), 1.1 to 1.7 μm wide, showing less exposure of collagen compared to SBMP.

When DMSO-wet bonding was performed, significant reduction ($p<0.05$) of exposed collagen width and color intensity occurred between DMSO-treated and untreated specimens for both SBMP (Figure 1 B) and Clearfil (Figure 1 D). The more intense red color in the control samples may be attributed to the better penetration of stain

into wider zone of exposed collagen, even though the possible effect of slight differences in sample thicknesses cannot be ruled out. DMSO-wet bonding with SBMP still presented significantly (p<0.05) wider zones of exposed collagen (0.8 to 1.3 μm) compared to specimen submitted to DMSO-wet bonding and bonded with Clearfil (0.4 to 0.7 μm).

3.2 Microtensile Bond Strength

Two-way ANOVA revealed that the interaction of adhesive system and bonding protocol had significant effects on the dentin μTBS (p=0.0091). Overall μTBS values are shown in Table 3. No statistical differences in dentin bond strength were observed between SBMP and Clearfil control groups (p>0.05). DMSO-wet bonding with SBMP produced significantly higher dentin bond strength values compared to the control group and DMSO-wet bonding with Clearfil (p<0.05). DMSO-wet bonding did not alter Clearfil bond strength (p>0.05).

3.3 Fracture Mode Analysis

SEM examination of fractured surfaces after μTBS testing and overall numbers of fracture modes are shown in Figure 2 A-H and Table 3, respectively. For SBMP specimens, failures were mostly mixed at the interface involving the adhesive resin or resin composite. Collagen matrix could be frequently observed in areas of the fracture surfaces where the hybrid layer was exposed (Figure 2 B). Adhesive failures frequently occurred at the base and top of the hybrid layer leaving collagen exposed. SBMP specimens treated with DMSO presented mostly mixed failures, and less adhesive failures were observed. Moreover, less collagen was observed when failures occurred at the hybrid layer (Figure 2 F). Adhesive failures occurred in most cases at the top of the hybrid layer, normally associated with higher μTBS values, leaving the underlying dentin partially covered by the adhesive. Small areas of fractures located at the base of the hybrid layer with dentinal tubule entrances filled with resin tags were apparent.

For Clearfil, the most prevalent fracture pattern was mixed, followed by adhesive failures. When adhesive failures occurred at the bottom of the hybrid layer, poorly resin-enveloped collagen matrix and smear plugs left in the dentin tubules were observed (Figure 2 D). When Clearfil was applied to DSMO-treated dentin, the most prevalent

fracture mode remained mixed, followed by adhesive failures. However, fractures leaving exposed collagen rarely occurred, and the underlying dentin remained partially covered by the adhesive resin (Figure 2 H).

4. Discussion

Dentin pretreatment with 50% DMSO significantly decreased the collagen exposure at the bottom of the hybrid layer with both adhesives tested. Therefore, the first hypothesis was accepted. Since application of 50% DMSO to the acid-etched dentin significantly increased dentin bond strength with SBMP, the second hypothesis was partially accepted. The DMSO-wet bonding technique was only effective to increase bond strength when used with the etch-and-rinse adhesive system, but not with the self-etch adhesive.

For the etch-and-rinse dentin bonding approach, the superficial dentin is completely demineralized for subsequent monomers diffusion [3], while for the self-etch approach, acidic monomers demineralize and diffuse into dentin simultaneously [8]. The adhesive monomers capacity to diffuse into the demineralized collagen matrix is invariably impaired [9,19,21]. The main reason for DMSO-related bond strength improvement with SBMP might be attributed to the interaction between DMSO and the dentin substrate/adhesive systems, which may have influenced resin monomer diffusion.

DMSO presents some specific properties that potentially modify collagen matrix interaction with resin monomers. One of such properties is DMSO ability to suppress interpeptide hydrogen bonds within collagen fibrils [12,14], leaving collagen matrix in a condition to be more easily infiltrated by the hydrophilic monomers. By breaking water's self-associative tendency [22] high DMSO concentrations dissociate collagen fibrils into a sparser network by reversibly destabilizing collagen structure in many tissues [12], including dentin [13]. DMSO changes the organized structure of collagen fibers causing dissociation of extracellular matrix collagen [12] which might benefit resin monomer diffusion. Better monomer diffusion was demonstrated by optical microscopy, in which significant 85.3% reduction in the extent of exposed collagen at the base of the hybrid layer was observed with DSMO-wet bonding compared to controls of the etch-and-rinse adhesive group.

DMSO has a lower vapor pressure compared to other commonly used solvents, so evaporation from the dentin substrate after the adhesive system application is less likely to occur compared to higher vapor pressure solvents. Under normal bonding conditions, the infiltration of adhesive system is related, in part to differences in the ratio of the hydrophilic/hydrophobic components, i.e., the ability to dissolve in water. Since dentin is a moist substrate, separation of hydrophobic and hydrophilic monomers hampers the adhesive system capacity to properly diffuse into the dentin substrate [23]. During the acid-etching process, mineral content is solubilized and replaced by rinse-water yielding a new water content of 70 vol% [3]. When three-step etch-and-rinse systems are used, the demineralized wet-dentin is initially flooded with the hydrophilic HEMA monomers dissolved in water, followed by the application of the hydrophobic BisGMA monomers in a separate step. Even though the hydrophilic/hydrophobic components are separately applied in SBMP, the BisGMA monomers have the ability to partially diffuse across the demineralized dentin [24]**.** Nevertheless, BisGMA monomers are contaminated by varying amounts of water present in the demineralized substrate. Phase separation occurs with hydrophobic monomers, such as BisGMA, which resist diffusing into sites where residual water is present [25]. Partitioning of the adhesive components inhibits not only the formation of perfectly integrated collagen/polymer networks [23], but suppresses BisGMA infiltration throughout the width of the demineralized dentin matrix and the subjacent intact dentin [25].

Phase separation [25] of BisGMA/HEMA bonding resins could have hindered the diffusion of relatively large-hydrophobic molecules such as BisGMA (molecular weight 512) in SBMP control group. As a result, hybrid layers in SBMP control specimens may have been infiltrated predominantly by HEMA (molecular weight 130) that was subsequently polymerized into linear poly-HEMA chains, producing a resin-collagen biopolymer with reduced mechanical properties. Phase separation invariably reduces monomer diffusion and produces low quality hybrid layers [23,26]. DMSO has the ability to dissolve a great number of hydrophilic/hydrophobic resin monomers [11] including BisGMA [11,27]. Since collagen matrix present in the demineralized dentin was saturated with DMSO, better monomer diffusion and improved collagen encapsulation occurred with the tested etch-and-rinse adhesive, reducing the formation of poorly hybridized adhesive

interfaces characterized by phase separation of hydrophobic bis-GMA particles distributed in a hydrophilic HEMA matrix [21,23]. As result, DMSO-wet bonding technique increased bond strengths when the etch-and-rinse adhesive system was used.

The paradigm that self-etch adhesives produce simultaneous etching and resin infiltration to the full extent of the bonded interface is not accepted any more [28,10] is also seen in this study. Even though mild self-etch adhesive systems produce low dentin demineralization and narrow hybrid layers [8], exposed collagen was observed along Clearfil adhesive interface using Masson's Trichromic staining, which is in agreement with a previous study [10]. When DMSO was applied to dentin followed by the self-etch adhesive application, a significant 61.5% reduction of exposed collagen was observed. Differently from SBMP, no statistical differences in immediate dentin bond strength were observed between the control group and DMSO-treated dentin bonded with Clearfil. The lack of significant improvement in μTBS may be caused by relatively lower decrease in collagen exposure. Since DMSO was applied onto smear layer-covered dentin and the hydrophilic methacrylate phosphate ester functional monomer 10-MDP simultaneously dissolves and incorporates the smear layer into a narrow hybrid layer, DMSO may not have infiltrated the exposed collagen matrix as effectively as with etch-and-rinse adhesive. This may have limited DMSO capacity to increase micromechanical retention of self-etch systems. Nevertheless, differences in fracture modes were observed. DMSO treated specimen that failed at the bottom of the hybrid layer presented less exposure of collagen, showing a better interaction of the self-etch adhesive system and the dentin substrate.

While mineralized dentin show green in control groups (Fig 1A, C), it is more beige/slightly pinkish color in DMSO treated samples (Fig 1B, D). The reason for that is not exactly known, but is most likely not related to the collagen staining. Since the pH of the DMSO solution was 8.2, exposure of mineralized collagen by DMSO is not possible. The optical clearance effect of completely demineralized dentin collagen required 24 h incubation in 100% DMSO [13]: therefore it is also highly unlikely that DMSO would affect mineralized collagen especially with 50% DMSO concentration and such a short exposure time. The effect of DMSO on water behavior may offer more plausible explanation. DMSO molecule forms two hydrogen bonds with water molecules (1DMSO:2H_2O). DMSO-water

complex replaces cyclic water pentamer, the favorable water form in biological structures, stabilizing water molecules into complexes smaller than cyclic pentamer structure [13]. This may have allowed stain penetration into water-filled dentinal tubules, turning the mineralized dentin into pinkish color in Masson's staining. Since acid etching removes the smear layer and opens the orifices of dentinal tubules, the pinkish color of mineralized dentin under the hybrid layer in DMSO-treated SBMP sample (Fig. 1B) is more intense than in the DMSO-treated Clearfil sample (Fig. 1D), supporting this explanation.

In conclusion, the proposed DMSO-wet bonding technique was effective to increase collagen encapsulation of the tested self-etch/etch-and-rinse adhesive systems improving the quality of the collagen-resin biopolymer at the bonded interface. Moreover, pretreatment of dentin with a high concentration of DMSO increased bond strength of a HEMA/water based etch-and-rinse adhesive system. These findings support previously observed positive effects of low-concentration DMSO on bond strength [13] and suggest that 50% DMSO could be a feasible alternative to improve resin-dentin bond quality especially for water-based etch-and-rinse adhesive systems. More studies should be performed to evaluate the possible interactions between DMSO and self-etch adhesives especially considering long-term aging of the bonded resin-dentin interface.

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5. References

[1] Mazzoni A, Pashley DH, Ruggeri A, Vita F, Falconi M, Di Lenarda R, et al. Adhesion to chondroitinase ABC treated dentin. J Biomed Mater Res B Appl Biomater 2008;86:228–36

[2] Macedo G V, Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res 2009;88:1096–100.

[3] Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1–16.

[4] Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer-A review. Dent Mater 2013;29:999–1011.

[5] Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982;16:265–73.

[6] Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, et al. Evidence of Chemical Bonding at Biomaterial-Hard Tissue Interfaces. J Dent Res 2000;79:709–14.

[7] Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative Study on Adhesive Performance of Functional Monomers. J Dent Res 2004;83:454–8.

[8] Van Meerbeek B, Yoshihara K, Yoshida Y, Mine a, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27:17–28.

[9] Spencer P, Wang Y, Katz JL. Identification of collagen encapsulation at the dentin/adhesive interface. J Adhes Dent 2004;6:91–5.

[10] Erhardt MCG, Toledano M, Osorio R, Pimenta LA. Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure. Dent Mater 2008;24:786–98.

[11] Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. J Biomed Mater Res 1998;41:474–80.

[12] Zimmerley M, McClure RA, Choi B, Potma EO. Following dimethyl sulfoxide skin optical clearing dynamics with quantitative nonlinear multimodal microscopy. Appl Opt 2009;48:D79–87.

[13] Tjäderhane L, Mehtälä P, Scaffa P, Vidal C, Pääkkönen V, Breschi L, et al. The effect of dimethyl sulfoxide (DMSO) on dentin bonding and nanoleakage of etch-and-rinse adhesives. Dent Mater 2013;29:1055–62.

[14] Hirshburg J, Choi B, Nelson JS, Yeh AT. Collagen solubility correlates with skin optical clearing. J Biomed Opt n.d.;11:040501.

[15] Marren K. Dimethyl sulfoxide: an effective penetration enhancer for topical administration of NSAIDs. Phys Sportsmed 2011;39:75–82.

[16] Pashley DH, Tay FR, Carvalho RM, Rueggeberg F a, Agee K a, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007;20:7–20.

[17] Do Amaral RC, Stanislawczuk R, Zander-Grande C, Michel MD, Reis A, Loguercio AD. Active application improves the bonding performance of self-etch adhesives to dentin. J Dent 2009;37:82–90.

[18] Shono Y, Ogawa T, Terashita M, Carvalho RM, Pashley EL, Pashley DH. Regional measurement of resin-dentin bonding as an array. J Dent Res 1999;78:699–705.

[19] Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding. J Dent Res 2003;82:141–5.

[20] Breschi L, Cammelli F, Visintini E, Mazzoni A, Vita F, Carrilho M, et al. Influence of chlorhexidine concentration on the durability of etch-and-rinse dentin bonds: a 12-month in vitro study. J Adhes Dent 2009;11:191–8.

[21] Wang Y, Spencer P. Quantifying adhesive penetration in adhesive/dentin interface using confocal Raman microspectroscopy. J Biomed Mater Res 2002;59:46–55.

[22] Vishnyakov A. Molecular dynamics simulations of dimethyl sulfoxide and dimethyl sulfoxide-water mixture. J Phys. Chem A 2001:1702–10.

[23] Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. J Biomed Mater Res 2002;62:447–56.

[24] Zou Y, Jessop JLP, Armstrong SR. In vitro enzymatic biodegradation of adhesive resin in the hybrid layer. J Biomed Mater Res A 2010;94:187–92.

[25] Ye Q, Wang Y, Spencer P. Nanophase separation of polymers exposed to simulated bonding conditions. J Biomed Mater Res B Appl Biomater 2009;88:339–48.

[26] Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008;24:90–101.

[27] Seiss M, Marquardt W, Hickel R, Reichl FX. Excretion of dental resin monomers and metabolic intermediates via urine in guinea pigs. Dent Mater 2009;25:481–5.

[28] Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, Tay FR. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomaterials 2005;26:1035–42.

Tables

Table 1. Adhesive Systems, main components, and application mode of bonding agents

Adhesive System	Components	Mode of application (Control/DMSO wet-bonding)
Adper Scotchbond Multi-Purpose 3M/ESPE		H_3PO_4 conditioning for 15 s. Rinse with water 30 s. Blot drying leaving dentin slight moist. Active application of either distilled water for 60 s (Control), or 50% DMSO for 60s (DMSO wet-bonding). Blot drying. Active *Primer* application with a fully saturated brush tip 10 s. Gently blow dry 5 s. Active *Bond* application 10s. Light cure for10 s.
Etchant	37% phosphoric acid, fumared silica (pH 0.6)	
Primer	HEMA, polyalkenoic acid methacrylate copolymer, water	
Bond	bis-GMA, HEMA, dimethacrylates photoinitiators,	
Clearfil SE Bond Kuraray		Blot drying until no sign of excess visible moisture was observed. Active application of either distilled water for 60 s (Control), or 50% DMSO for 60s (DMSO wet-bonding). Blot drying. Active *Primer* application with a fully saturated brush tip for 20 s[17].Mild air stream for 5 s. Active *Bond* application. Gentle air stream 5 s. Light cure for 10 s.
Primer	10-MDP; HEMA; CQ; Hydrophilic dimethacrylate; water (pH 2.0)	
Bond	10-MDP; N,N-diethanol-p-toludine; HEMA; Bis-GMA; silanated colloidal silica; hydrophobic dimethacrylate; CQ; MDPB;	

Abbreviations: HEMA=2-hydroxyethyl methacrylate; Bis-GMA=bis-phenolA diglycidylmethacrylate; 10-MDP=10-methacryloxydecyl dihydrogen phosphate; CQ= camphoroquinone; MDPB, 12-methacryloyloxydodecylpyridium bromide; SiO2, silicon oxide.

Table 2. Width of exposed collagen as detected with Massons Tricrome staining. The values are in micrometers (µm; mean and standard deviations (±SD)).

ADHESIVE SYSTEM	DENTIN PRETREATMENT	
	H_2O	DMSO 50%
SBMP	6.8^{aA} (±1.0) [4.9/8.5]	1.0^{aB} (±0.1) [0.8/1.3]
Clearfil	1.3^{bA} (±0.2) [1.1/1.7]	0.5^{bB} (±0.1) [0.4/0.7]

Different lowercase letters indicate significant difference when analyzed per column; different capital letters indicate significant difference when analyzed per row ($p<0.05$; Tukey Studentized Range (HSD) Test). Numbers inside [ ] represent the minimum and maximum values (µm) for each group (n=12). SBMP: Adper Scotchbond Multi-Purpose; Clearfil: Clearfil SE Bond.

Table 3. Dentin microtensile bond strength (MPa: means standard deviations (±SD)), pretest failures, and fracture modes (%) for all groups.

ADHESIVE SYSTEM	DENTIN PRETREATMENT	
	H_2O	DMSO 50%
SBMP	35.58^{aB} *(±6.18)* [7/121] Fracture mode:{35/3/2/60}	53.34^{aA} *(±8.28)* [4/127] Fracture mode:{20/5/3/72}
Clearfil	34.06^{aA} *(±8.47)* [5/119] Fracture mode:{25/5/0/70}	39.46^{bA} *(±4.77)* [2/128] Fracture mode:{18/7/2/73}

Microtensile values followed by different lowercase letters indicate significant difference when analyzed per column; different capital letters indicate significant difference when analyzed per row ($p<0.05$; Tukey Studentized Range (HSD) Test). Numbers inside [ ] represent pretest failures: [sticks with pretest failures/intact sticks]. Numbers inside { } represent fracture modes percentile (%) classified in {A/CD/CC/M}: {A} adhesive; {CD} cohesive failure in dentin; {CC} cohesive failure in resin composite; and {M} mixed failure. SBMP: Adper Scotchbond Multi-Purpose; Clearfil: Clearfil SE Bond.

Figures

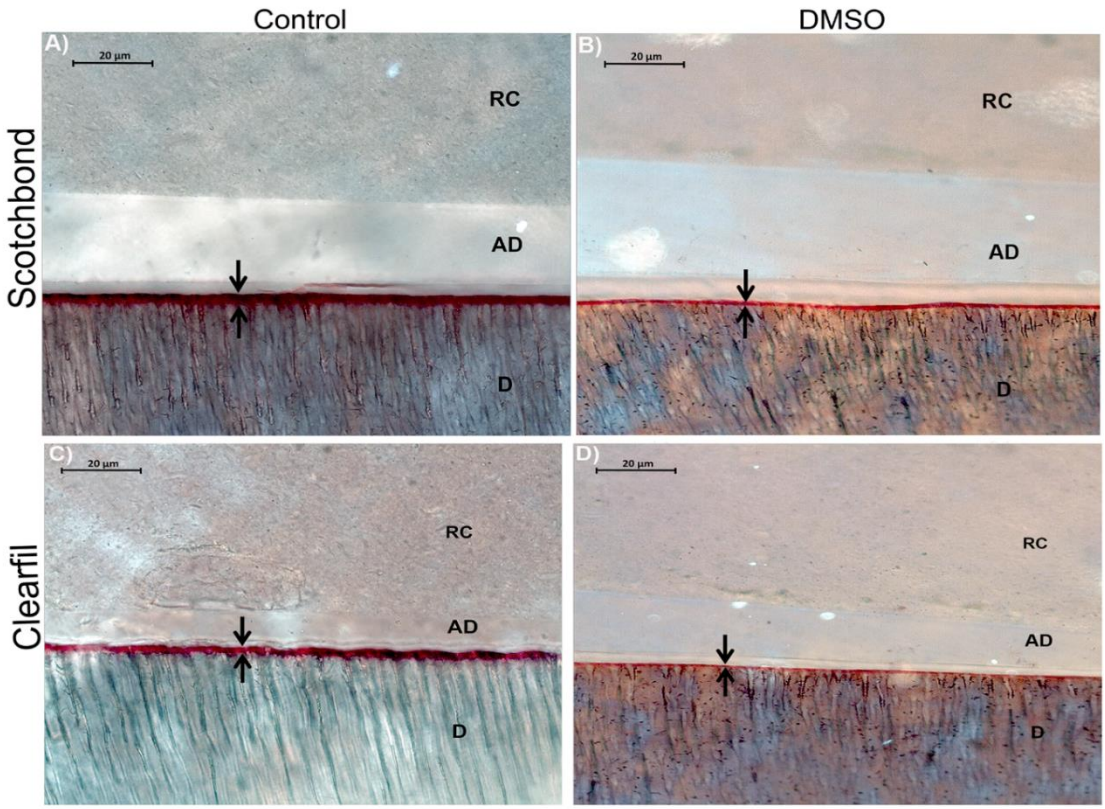

Figure 1. Representative light micrographs of dentin specimens stained with Masson's trichrome (Magnification: 400x): A) SBMP specimen from control group, the wide red band that correspods to the extent of exposed collagen matrix unprotected by the adhesive resin (space between black arrows); B) DMSO-wet bonding with SBMP, the extent of exposed collagen (space between black arrows) is reduced when compared to the specimen from the control group. Histometric analysis showed a significant 85.3% reduction in the width of exposed collagen when DMSO-wet bonding was performed (Table 2); C) Clearfil specimen from control group. Exposed collagen can be observed between the black arrows; D) DMSO-wet bonding with Clearfil, the extent of exposed collagen (space between black arrows) is reduced when compared to the specimen from the control group: histometric analysis showed a significant 61.5% reduction in exposed collagen width when DMSO-wet bonding was performed (Table 2). RC=Resin composite, AD=adhesive layer, D=Mineralized dentin.

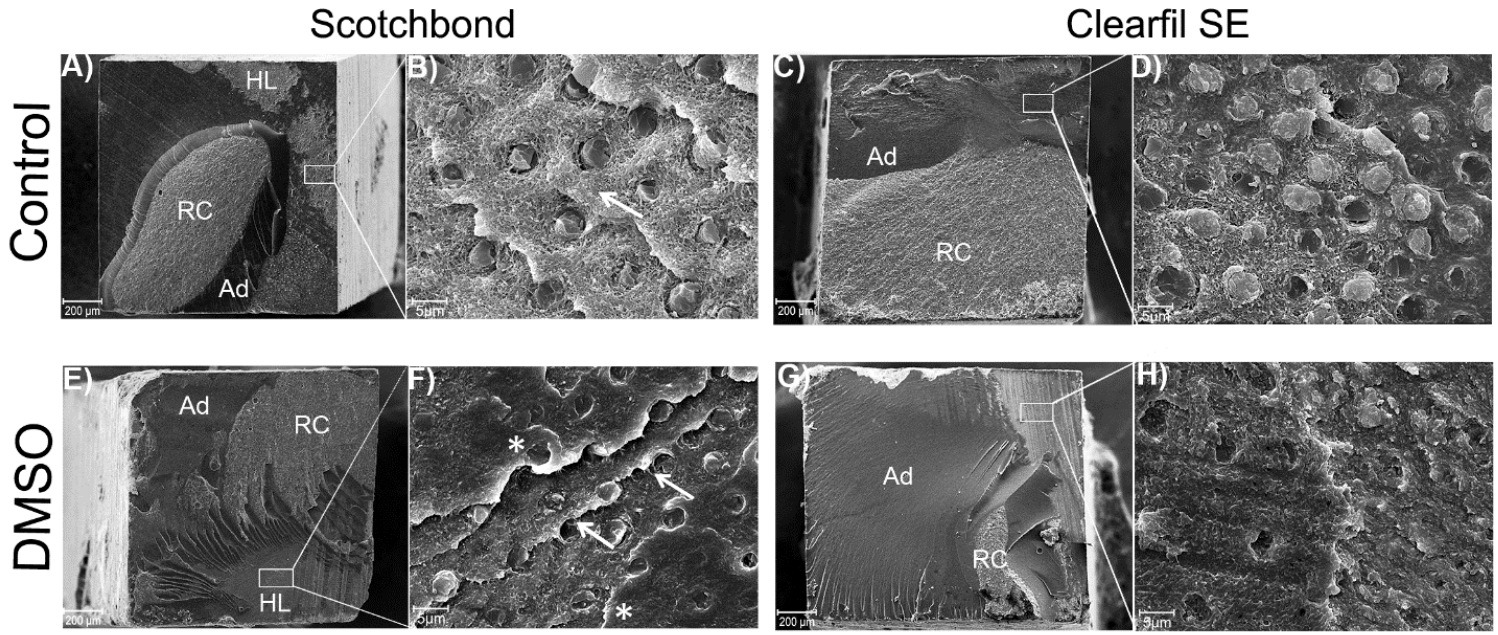

Figure 2. Representative scanning electron micrographs (SEM) of the dentin side of fractured sticks: A) Low-power magnification (100x) of a mixed failure of a SBMP control specimen; B) Higher magnification (3000x) of the area limited by the rectangle in A, showing a region with cohesive failure at the base of the hybrid layer. Note the presence of a substancial amount exposed collagen matrix (white arrow) and resin tags occluding the dentinal tubules; C) Low-power magnification (100x) of a mixed failure of a Clearfil control specimen; D) Higher magnification (3000x) of the area limited by the rectangle in C, showing a region of cohesive failures at the bottom of hybrid layer. Exposed collagen (white arrow) can be seen, but to a lower extent than in SBMP control specimen; E) Low-power magnification (100x) demonstrates a mixed failure for DMSO-wet bonding technique of a SBMP specimen; F) Higher magnification (3000x) of the area limited by a rectangle in E, showing cohesive failure at the hybrid layer; note the presence of resin tags tighly occluding the dentinal tubules, sparse exposed collagen matrix (white arrow) and adhesive remnants on top of dentin (*); G) Low-power magnification (100x) demonstrates a predominantely adhesive failure for DMSO-wet bonding technique with Clearfil; H) Higher magnification (3000x) of the area limited by a rectangle in G, showing a region presenting cohesive failure below the hybrid layer.Note that exposed collagen matrix is not observed. Ad=Adhesive layer, RC=resin composite, and HL=hybrid layer.

CAPÍTULO 2

Dentin bond optimization using the dimethyl sulfoxide-wet bonding strategy

Abstract

There is an imminent need to identify and investigate alternative methods for extending the longevity of resin–dentin bonds. This work evaluated the effect of a new bonding protocol using a high concentration of dimethyl sulfoxide (DMSO) on the dentin bond strength of etch-and rinse and self-etch adhesive systems after long-term aging. The impact of the high concentration of DMSO on the degree of monomer conversion (DC) inside the hybrid layer was evaluated. Dentin flat surfaces (n=10) derived from sound extracted human third molars were saturated with DMSO 50% after acid etching for a water-based etch-and-rinse system (SBMP: Scotchbond Multi-Purpose, 3M ESPE) or a water based self-etch system (Clearfil: Clearfil SE Bond, Kuraray). The restored teeth were sectioned into resin-dentin sticks (cross sectional area 0.8 mm^2) and used for microtensile bond strength test (0.5 mm/min) at 24 h, 1 year and 2 years. Micro-raman spectroscopy for DC analyses inside the hybrid layer was evaluated at 24 h. DMSO-wet bonding had no negative effect on the degree of conversion irrespective of adhesive type: in fact, higher conversion values were observed when Clearfil was associated with DMSO. DMSO-wet bonding increased immediate bond strength of the tested etch-and-rinse adhesive system, and reduced bond strength loss for both tested adhesive systems after long-term aging. A tendency of reduced adhesive failures were observed in DMSO-treated samples after two years. In conclusion, DMSO-wet bonding seems to be a promising approach to reduce resin-dentin bond degradation of etch-and-rinse and self-etch adhesives.

Key words: Dimethyl sulfoxide, Dentin bonding, Durability, Long-term, self-etch, etch-and-rinse

1. Introduction

Adhesion of resin materials to tooth structure has been a challenge in the history of the adhesive dentistry. Currently, the issue of bond durability draws significant attention regarding resin–dentin bonding (van Dijken et al., 2007; Liu et al., 2011; Tjäderhane et al., 2013b). Despite of all improvements in dental adhesive technology and advances in bonding knowledge, resin-dentin bonding still presents significantly limited durability for both etch-and-rinse and self-etch adhesive systems (van Dijken et al., 2007; Hashimoto, 2010).

Resin-dentin bonds created by infiltration of hydrophilic resin monomers into demineralized (Wang and Spencer, 2003) and mineralized dentin (Carvalho et al., 2005) are imperfect and unstable (Hashimoto, 2010). High permeability of the bonded interface, sub-optimal polymerization, and phase separation during adhesive application contributes to hydrolytic degradation of the adhesive resin (Hashimoto, 2010). Another relevant aspect, in face of insufficient resin impregnation of dentin (Wang and Spencer, 2003), is the collagenolysis by endogenous matrix metalloproteinases and cysteine cathepsins of unprotected collagen fibrils (Pashley et al., 2004; Tjäderhane et al., 2013c). Irrespective of adhesive type, hydrolytic degradation of the adhesive resin and collagen matrix degradation occur concurrently, for resin elution from hydrolytically unstable polymeric hydrogels within the hybrid layers increases unprotected collagen matrix over time. Several adjunctive procedures have been suggested to prevent biodegradation of hybrid layers over time (reviewed in Tjäderhane et al., 2013b). Although encouraging results have been produced, they do not effectively address both hydrolytic degradation of the adhesive resin and collagen degradation concurrently, with possibly the exception of ethanol-wet bonding (Tezvergil-Mutluay, Agee, et al., 2011), which is unfortunately clinically unfeasible due to the technique sensitivity and increase in application steps (Tjäderhane et al., 2013b). As a result, such strategies are partially limited in their true potential as methods to optimize the durability of resin restorations.

Dimethyl sulfoxide (DMSO; (CH3)2SO) is a polar aprotic solvent with a highly polar S=O group and two hydrophobic CH3 groups. Its ability to penetrate biological surfaces and tissues makes it perhaps the best penetration enhancer for medical purposes (Marren, 2011) with minor or no effects on the pulp tissue repair-related activity of odontoblast-like cells (Hebling et al., 2015 in press). Recent studies have indicated that a low concentration of DMSO may improve both immediate (Tjäderhane et al., 2013a; Stape et al., in press) and long-term (Tjäderhane et al., 2013a) dentin bond strength. Despite good results after 1-year of water storage, the long-term efficacy has only been demonstrated with two-step etch-and-rinse adhesive (Tjäderhane et al., 2013a). Moreover, several preventive bonding strategies, despite good results after one-year of water storage, show progressive signals of hybrid layer degradation over longer periods of time (Breschi et al., 2010; Sadek et al., 2010). Therefore, this *in vitro* study evaluated the effect of a high concentration aqueous-solution of DMSO on dentin bond durability and degree of conversion at the hybrid layer of two-step etch-and-rinse and three-step self-etch adhesives after two-year long-term storage. The null hypotheses to be tested were that: (i) irrespective of adhesive type, monomer conversion would not be hampered by DMSO; and (ii) application of a high concentration of DMSO on demineralized dentin would not affect immediate or long-term dentin bond strength.

2. Materials and methods

2.1 Teeth selection and preparation

Forty intact non-carious human third molars with complete root formation were extracted for surgical reasons with patients' (age 18–25 years) informed consent and approval by the Ethical Committee of the Piracicaba Dental School, University of Campinas, Brazil (protocol 128/2014). Teeth were cleaned, disinfected for one week in 0.5% chloramine-T solution at 4°C, and stored in distilled water at 4°C for up to one month before use (Perdigão, 2010). A flat coronal dentin surface was obtained by sectioning off the occlusal one-third of the crown (Isomet 1000

Precision Saw, Buehler, Lake Bluff, IL, USA). The surface roughness was standardized with 600-grit silicon carbide paper (BuehlerMet, Buehler, Lake Bluff, Illinois, USA) for 60 s under water cooling and the specimens were randomly assigned to four groups (n=10) according to the bonding protocols used.

2.2 Dentin bonding protocol

Two commercially available unaltered adhesive systems were used: a three-step etch-and-rinse adhesive system (Adper Scotchbond Multi-Purpose, 3M ESPE, St. Paul, MN, USA) (SBMP) and a two-step self-etch adhesive (Clearfil SE Bond, Kuraray, Osaka, Japan) (CF). Dentin bonding in control groups was performed following manufactures' instructions (SBMP and CF; Table 1). In experimental groups (SBMP+DMSO and CF+DMSO), 50 µL of water-based 50% (v/v) DMSO (Dimethyl Sulfoxide, Sigma-Aldricht, St Louis, MO, USA) (pH 8.2) was actively applied for 60 seconds, using a disposable micro brush. For SBMP, DMSO was applied after dentin etching; for Clearfil, DMSO was applied onto smear layer-covered dentin. Table 1 displays the mode of application, components and manufacturers of the adhesive systems. Both adhesive systems were applied actively. Adhesive procedures were carried out in a controlled environment with a temperature of 24°C and a relative humidity of 60%. Resin composite build-ups (Z250, shade A2, 3M ESPE) were built on top of the bonded dentin surfaces in four 1-mm increments that were individually light-cured for 20 s. Light curing of all resin materials was performed using a LED device (Bluephase 20i, Ivoclare Vivadent, Schaan, Liechtenstein). A single operator carried out all bonding procedures.

2.3 Specimen preparation

The restored crown segments were stored in distilled water at 37°C for 24 h and sectioned (Isomet 1000 Precision Saw, Buehler, Lake Bluff, Illinois, USA) occluso-gingivally across the bonded interface into slabs measuring approximately 0.9 mm. The slabs, in turn, were further sectioned into composite-dentin sticks, pursuing a final cross sectional area of approximately 0.8 mm^2 in accordance with

the “non-trimming” technique (Shono et al., 1999) for bond strength testing. A minimum of 20 sticks were obtained from each tooth.

2.4 Specimen aging

Sticks were stored in artificial saliva for up to two years at 37℃. The storage solution was prepared and changed weekly in accordance with a protocol previously described containing (mmoles/L): $CaCl_2$ (0.7), $MgCl_2 \cdot 6H_2O$ (0.2), KH_2PO_4 (4.0), KCl (30), NaN_3 (0.3), and HEPES buffer (20) (Pashley et al., 2004).

2.5 Resin-dentin microtensile testing (μTBS)

Microtensile test was performed at three periods: 24 h, 1 year and 2 years. For each period, six resin-dentin sticks from each restored tooth (n=6) were randomly chosen and individually attached to a Geraldeli’s device (ODEME Biotechnology, Luzerna, SC, Brazil) using cyanoacrylate adhesive (Super Bonder, Loctite, SP, Brazil). Sticks were tested in tensile forces in a universal testing machine (DL2000, EMIC, São José dos Pinhais, SC, Brazil) at a crosshead speed of 0.5 mm/min until failure. The number of premature failures per tooth during specimen preparation was recorded. The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm in order to calculate the actual μTBS. Both surfaces of fractured sticks were observed under a stereomicroscope (Olympus 220670; Tokyo, Japan) with 40x magnification for fracture pattern classification. The fracture modes were classified as follows: cohesive (failure exclusive within dentin or resin composite); adhesive failure (failure at resin/dentin interface); and mixed failure (failure at resin/dentin interface with cohesive failure of the neighboring substrates). For failure modes that could not be accurately established under the stereomicroscope, the surfaces were examined in a scanning electron microscope.

2.6 Degree of conversion (DC) inside the hybrid layer measurements

Two sticks from each tooth (n=10) were randomly evaluated at 24 h. Sticks were wet-polished with P1200, P2000, P2500, and P4000 SiC paper (Buehler Ltd, Lake Bluff, IL, USA), ultrasonically cleaned for 2 min between polishing steps and 20 min after the final step. Raman spectra were collected using a micro-Raman spectrometer (Senterra, BrukerOptik GmbH, Ettlingen, Baden Württemberg, Germany) to investigate the DC inside the hybrid layer of the adhesive interfaces. The micro-Raman spectrometer was first calibrated for zero and then for the coefficient values using a silicon sample. Samples were analyzed using the following micro-Raman parameters: 20 mW Neon laser with 532 nm wavelength, spatial resolution of approximately 3 µm, spectral resolution approximately 5 cm^{-1}, accumulation time of 30 s with 6 co-additions, and 100× magnification (Olympus UK, London, UK) to a ≈1 µm beam diameter. The spectra were taken in the middle of the hybrid layer, in an arbitrary area of the intertubular dentin. Care was taken to select an area between two dentin tubules. One site was examined in each stick (Hass et al., 2013). Spectra of uncured adhesives were taken as reference. Post-processing of spectra was performed using the dedicated Opus Spectroscopy Software version 6.5 (BrukerOptik GmbH, Ettlingen, Baden-Württemberg, Germany). The ratio of double-bond content of monomer to polymer in the hybrid layer was calculated according to the following formula:

$$DC(\%) = \left(1 - \frac{R^{(Cured)}}{R^{(Uncured)}}\right) \quad X\ 100$$

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

2.7 Statistical Analysis

After confirming the normality of the data distribution, Kolmogorov–Smirnov test, and the equality of variances by the Barlett test, µTBS data were subjected to Repeated Measures ANOVA test and DC data were submitted to one-way ANOVA. *Post hoc* multiple comparisons were performed with Tukey Test test, (α=0.05). As

sticks from each tooth were used for different evaluation times, sticks from each group and from each aging period were averaged for statistical purposes.

3 Results

3.1 Degree of conversion (DC)

Raman images based on R1639/1609 were generated to show the distribution of unconverted C=C bond content in the hybrid layer. Means and standard deviations of degree of conversion inside the hybrid layer obtained from Raman spectra and statistical differences for all groups are reported in Figure 1. One-way ANOVA showed significant differences between groups. For SBMP, the degree of conversion between SBMP+DMSO and SBMP control group were not statistically different from each other *(*p=0.892). For CF, the monomer conversion value of CF+DMSO was statistically higher (p=0.033) than CF control group.

3.2 Microtensile bond strength evaluation

The mean cross-sectional area of tested resin-dentin sticks ranged from 0.74 to 0.83 mm^2 and no statistical differences among groups was detected (p=0.43). Microtensile overall means and the respective standard deviations are reported in Figure 2. Repeated measure ANOVA detected that “adhesive type” (p=.0038), “dentin treatment” (p<.0001), and “aging” (p<.0001) significantly affected dentin bond strength. The interaction between “dentin treatment” and “adhesive type” significantly affected microtensile values irrespective of “aging” (p=.0003): bond strength of DMSO treated SBMP specimens were significantly higher (37.4%) than untreated specimens at 24 h (p<.0001), However, no statistical differences were observed for CF between DSMO treated and untreated specimens at 24 h (p=.6817). The interaction between “aging” and “adhesive type” was not statistically significant (p=.1196), showing that aging affected bond strength irrespective of adhesive type. Moreover, the interaction of “aging” and “dentin treatment” significantly affected dentin bond strength (p<.0001), showing a different effect between DMSO treatment

and no-treatment on bond strength over time. Bond strengths of untreated specimens at one year were not statistically different compared to specimens tested at 24 h. However, bond strength of untreated specimens reduced significantly at two years compared to 24 h microtensile values. It was observed a significant reduction in bond strength of 45.7% for untreated SBMP specimens ($p<.0001$), and 36.8 % for untreated CF specimens ($p<.0001$). Differently, no statistical differences were observed for DMSO-treated specimens for both SBMP and CF at the three testing periods. Fracture mode distribution are shown in Figure 3.

4. Discussion

Since the proposed DMSO-wet bonding technique did not negatively influence monomer conversion inside the hybrid layer for SBMP and increased monomer conversion for CF, the first null hypothesis was rejected. One concern that may arise considering dentin saturation with low vapor pressure solvents (i.e. DMSO), prior to dentin bonding, is that residual solvents at the hybrid layer may compromise monomer conversion and the rate of conversion of adhesive systems (Cadenaro, 2012). Residual solvents may dilute monomer concentration and separate growing polymer chains (Cho and Dickens, 2004; Cadenaro et al., 2008), which may in turn compromise dentin bond strengths (Hashimoto et al., 2006). However, such effect on polymerization kinetics of dental adhesives is concentration dependent (Cadenaro, 2012). Even though residual DMSO stays within the dentin substrate in face of DMSO low vapor pressure, no negative effects in monomer conversion were observed for both tested adhesive systems. During the bonding protocol, dentin wetness after DMSO application was controlled according to the adhesive system used: dentin was maintained slightly wet for SBMP and with no signs of visible moisture for CF in order to optimize the bonding performance of both systems (Pashley et al., 2011; Van Meerbeek et al., 2011). During the total-etch approach, demineralized dentin is composed by approximately 70% water (Pashley et al., 2011; 2007). Dentin pre-treatment with a water-based 50% DMSO solution after etching, likely replaced water content in dentin. Since the control of dentin wetness

was performed in the same manner for both treated and untreated specimens according to the adhesive system used, the overall amount of solvent within the demineralized dentin (i.e. water for control groups; and water/DMSO for experimental groups) remained the same. Moreover, the composition of the remaining solvents at the bonded interface differed between DMSO-treated and untreated samples, not necessarily the overall solvent amount. The proposed technique merely replace dentin solvent content. Therefore, residual DMSO did not affect SBMP degree of conversion when a wet-bonding technique was used, for SMPB is originally applied in a substrate highly saturated with water.

For the self-etch approach, dentin was kept dry so residual DMSO might have influenced monomer conversion. Depending on the concentration and solvent type, solvents might improve the degree of conversion of hydrophilic adhesive systems (Cadenaro et al., 2008). The increased monomer conversion observed in CF might be related to a possible increase in monomer mobility along the bonding interface caused by the low amount of residual DMSO. In addition, previous results showed that low pH could considerably affect the initiating efficiency of amines thus resulting in poor polymerization of self-etch adhesives (Guo et al., 2009; Moszner et al., 2005). Since the pH of the DMSO solution used was 8.2, we speculate that a slight increase in dentin pH caused by DMSO during the 60 s active application, without over-wetting the bonding substrate, might have also have improved CF monomer conversion.

Considering dentin bonding performance, the measurement of DC inside the hybrid layer can provide useful information for reduction in conversion values may be correlated with reduced bond strengths (Hass et al., 2013) compromising the adhesive interface. Since higher immediate dentin bond strengths were produced and preserved after long-term aging when the proposed DMSO-wet bonding technique was employed with the tested *etch-and-rinse* adhesive system, the second null hypothesis was rejected. Improvement in dentin bond strength may be attributed to several factors related to the biomodification of the bonding substrate brought up by DMSO, which most likely enhanced the interaction between adhesive

and dentin improving bond strength. DMSO easily penetrates into biological surfaces, which makes it perhaps the best currently known penetration enhancer for medical purposes (Marren, 2011). Differently than high vapor pressure solvents (e.g. ethanol/acetone), DMSO has a low vapor pressure, so dentin remained saturated by DMSO during adhesive application. The fact that DMSO remained trapped within the bonded interface certainly contributed for immediate bond strength increase for SBMP and for the preservation of bond strengths of SBMP and Clearfil after aging.

Enhanced collagen matrix infiltration by hydrophilic monomers in SBMP/DMSO occurs (Stape et al. in press) partially due to suppression of interpeptide hydrogen bonds within demineralized collagen fibrils produced by DMSO (Zimmerley et al., 2009; Hirshburg et al., 2006). By breaking water's self-associative tendency (Vishnyakov, 2001), high DMSO concentrations dissociate collagen fibrils into a sparser network by reversibly destabilizing collagen structure (Zimmerley et al., 2009) including dentin (Tjäderhane et al., 2013). Collagen biomodification caused by a high DMSO concentration played an important role in SBMP immediate bond strength increase. Moreover, DMSO ability to be fully miscible in water and to dissolve most currently known adhesive hydrophilic/hydrophobic monomers, including HEMA and BisGMA (Geurtsen et al., 1998), certainly reduced the occurrence of phase separation during dentin hybridization improving resin monomer diffusion within demineralized dentin. One important aspect regarding resin dentin bonding is that bond strengths in total-etch adhesive systems vary directly with the width of interfibrillar spaces within the hybrid layer (Pashley et al., 2007; Carvalho et al., 2003). Since DMSO remains within the collagen matrix during dentin hybridization, most likely maintaining the interfibrilar spaces by breaking interpeptide H-bonds (Zimmerley et al., 2009; Hirshburg et al., 2006) higher immediate bond strengths were produced.

Differently from SBMP, DMSO-wet bonding had no influence on immediate dentin bond strength of the tested self-etch adhesive system, which might be explained by differences in the bonding mechanisms. For the etch-and-rinse bonding approach, superficial dentin is completely demineralized for subsequent monomers

diffusion (Pashley et al., 2011), while for the self-etch approach, acidic monomers demineralize and diffuse into dentin simultaneously promoting chemical interaction with hydroxyapatite (Van Meerbeek et al., 2011). For the self-etch approach, DMSO wet-bonding had no influence on immediate bond strength. One reasonable explanation for such finding is that self-etch systems, unlike total-etch systems, do not solely rely on collagen encapsulation to produce micromechanical retention. Improved collagen encapsulation is possibly the main factor involved in bond strength involved in the immediate increase of dentin bond strength in total-systems (Stape et al., in press). Interaction of self-etch monomers with dentin is more superficial compared to total-etch systems, and the chemical interaction with the mineralized tissue plays an important role on the adhesive capacity to properly bond to the dentin substrate (Van Meerbeek et al., 2011). Even though higher monomer conversion values occurred in DMSO-tretaed CF specimens, the increase in DC was not reflected as immediate bond strength increase.

Although high bond strengths are crucial to the longevity of resin restorations, they are only meaningful if they are stable over time. Irrespective of adhesive type, bond strengths were not statistically reduced at 1 year. However, aging significantly affects dentin bond strength (De Munck et al., 2003), which was observed in the present study with a significant reduction in microtensile values of untreated specimens at 2 years. This is the first study to evaluate the long-term effect of aging on bond strength of highly DMSO-saturated hybrid layers. After adhesive light-curing, residual water or solvents may become pathways for water movement within hybrid layers (Tay et al., 2002) which increases permeability (Chersoni et al., 2004) and subsequent susceptibility to degradation via resin hydrolysis (Hashimoto, 2010; De Munck et al., 2003) and collagen degrading enzymes (Liu et al., 2011; Pashley et al., 2004). Nonetheless, DMSO-wet bonding produced bond strengths that were not statistically reduced at 2 years, while control groups presented a 57.8% reduction compared to DMSO-treated specimens. The ability of DMSO to prevent dentin bond degradation of *etch-and-rinse* adhesives might be partly attributed to DMSO capacity to reduce the gelatinolytic activitiy produced by endogenous proteases as it was

demonstrated in a previous study that used a significantly lower 0.004% DMSO concentration (Tjäderhane et al., 2013). Apart from DMSO endogenous protease inhibition, improvements within the hybrid layer by high DMSO concentration regarding monomer diffusion (Stape et al., in press) possibly contributed for the stability of the SBMP bonded interface over time. Direct measurements of the mechanical/physical properties of the hybrid layer were not performed in the present study. However, considering the immediate bond strength increase in DMSO treated specimens for SBMP, we speculate that improvements in hybrid layer quality may have contributed to reduce the deleterious effects of aging at the adhesive interface. Analysis of fracture patterns at 2 years comparing DMSO-treated and untreated specimen showed a tendency of reduction in adhesive failures in DMSO-treated dentin, which suggests a possible improvement of the bonded interface. Moreover, BisGMA (molecular weight 512) has a limited ability to diffuse across the hybrid layer (Zou et al., 2010). As a result, the bulk of the hybrid layers is infiltrated predominantly by HEMA (molecular weight 130) (Zou et al., 2010; Spencer and Wang, 2002) subsequently polymerizing mostly into linear poly(HEMA) chains. Since DMSO dissolves both HEMA and BisGMA (Geurtsen et al., 1998), BisGMA/HEMA rates inside the hybrid layer possibly increased, favoring polymer crosslinking. Therefore, a hypothetical reduction of polymer crosslinks at the hybrid layer in untreated specimens could have resulted in a higher degree of water sorption (Spencer and Wang, 2002). As endogenous proteases require water to function, this could also have expedited the collagenolytic activities within the hybrid layer of untreated specimens. More studies are required to assess differences in etch-and-rinse dentin hybridization produced by DMSO-wet bonding.

To date, it is still unclear the most predominant cause for resin-dentin bond instability. Even though factors involved in bond degradation occur simultaneously, the degradation caused by endogenous enzymes seems to be more pronounced in etch-and-rinse adhesives, while hydrolysis of polymeric matrix caused by water sorption is most likely the principal mechanism of bond degradation of self-etch adhesives (De Munck et al., 2009). Although CF presents a two-fold micro-

mechanical and chemical bonding mechanism (Van Meerbeek et al., 2011), untreated CF specimens suffered a significant 36.8% reduction in bond strength at 2 years. However, when DMSO-wet bonding was employed, dentin bond strength was preserved as in SBMP. Differently form SBMP, DMSO was applied on smear layer covered dentin for CF, which hindered DMSO contact with the demineralized collagen matrix at the bonded interface certainly producing a different mechanism of bond strength preservation compared to total-etch adhesive tested. Since DMSO increased CF monomer conversion at the hybrid layer, we speculate that hydrolysis of polymeric matrix over time caused by water sorption certainly occurred to a lesser degree, preventing bond strength loss when DMSO was used.

In conclusion, this study presents compelling evidence that dentin pre-treatment with 50% DMSO improves the bonding performance of both self-etch and etch-and-rinse tested adhesives after long-term aging. The proposed DMSO wet-bonding technique not only prevented bond strength loss, but also produced significantly higher dentin bond strengths over time when a water-based total-etch adhesive was used. Dentin pre-treatment with a high DMSO concentration did not negatively affect the degree of monomer conversion of the tested total-etch system, in fact, higher monomer conversion occurred for the self-etch system.

References

1. Bedran-Russo AKB, Castellan CS, Shinohara MS, Hassan L, Antunes A (2011). Characterization of biomodified dentin matrices for potential preventive and reparative therapies. Acta Biomater. 7:1735–41.

2. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E (2008). Dental adhesion review: aging and stability of the bonded interface. Dent. Mater. 24:90–101.

3. Cadenaro M (2012). Effects of residual ethanol on the rate and degree of conversion of five experimental resins. Dent. Mater. 29:997–1003.

4. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, et al. (2010) Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. Dent Mater. 26:320–5.

5. Cadenaro M, Breschi L, Antoniolli F, Navarra CO, Mazzoni A, Tay FR, et al. (2008). Degree of conversion of resin blends in relation to ethanol content and hydrophilicity. Dent. Mater. 24:1194–200.

6. Carrilho MRO, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, et al. (2007). In vivo preservation of the hybrid layer by chlorhexidine. J. Dent. Res. 86:529–33.

7. Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, Tay FR (2005). A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomaterials 26:1035–42.

8. Carvalho RM, Mendonça JS, Santiago SL, Silveira RR, Garcia FCP, Tay FR, et al. (2003). Effects of HEMA/solvent combinations on bond strength to dentin. J. Dent. Res. 82:597–601.

9. Chersoni S, Suppa P, Breschi L, Ferrari M, Tay FR, Pashley DH, et al. (2004). Water movement in the hybrid layer after different dentin treatments. Dent. Mater. 20:796–803.

10. Cho B-H, Dickens SH (2004). Effects of the acetone content of single solution dentin bonding agents on the adhesive layer thickness and the microtensile bond strength. Dent. Mater. 20:107–15.

11. Van Dijken JW V, Sunnegårdh-Grönberg K, Lindberg A (2007). Clinical long-term retention of etch-and-rinse and self-etch adhesive systems in non-carious cervical lesions. A 13 years evaluation. Dent. Mater. 23:1101–7.

12. Geurtsen W, Lehmann F, Spahl W, Leyhausen G (1998). Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. J. Biomed. Mater. Res. 41:474–80. Hashimoto M (2010).

13. Guo X, Peng Z, Spencer P, Wang Y. (2009). Effect of initiator on photopolymerization of acidic, aqueous dental model adhesives. Journal of Biomedical Materials Research - Part A.; 90(4):1120–27.

14. Hashimoto M (2010). A review--micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions. J. Biomed. Mater. Res. B. Appl. Biomater. 92:268–80.

15. Hashimoto M, Tay FR, Svizero NR, de Gee AJ, Feilzer AJ, Sano H, et al. (2006). The effects of common errors on sealing ability of total-etch adhesives. Dent. Mater. 22:560–8.

16. Hass V, Dobrovolski M, Zander-Grande C, Martins GC, Gordillo LAA, Rodrigues Accorinte MDL, et al. (2013). Correlation between degree of conversion, resin-dentin bond strength and nanoleakage of simplified etch-and-rinse adhesives. Dent. Mater. 29:921–8.

17. Hebling J, Pashley DH, Tjäderhane L, Tay FR (2005). Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J. Dent. Res. 84:741–6.

18. Hirshburg J, Choi B, Nelson JS, Yeh AT (2006). Collagen solubility correlates with skin optical clearing. J. Biomed. Opt. 11:040501.

19. Liu Y, Tjäderhane L, Breschi L, Mazzoni a, Li N, Mao J, et al. (2011). Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J. Dent. Res. 90:953–68.

20. Marren K (2011). Dimethyl sulfoxide: an effective penetration enhancer for topical administration of NSAIDs. Phys. Sportsmed. 39:75–82.

21. Moszner N, Salz U, Zimmermann J. (2005) Chemical aspects of self-etching enamel-dentin adhesives: A systematic review. Dental Materials.; 21(10):895–910.

22. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. (2010). Relationship between bond-strength tests and clinical outcomes. Dent. Mater. 26:e100–21.

23. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine a, De Munck J, Van Landuyt KL (2011). State of the art of self-etch adhesives. Dent. Mater. 27:17–28.

24. De Munck J, Van Landuyt K, Peumans M, Poitevin a., Lambrechts P, Braem M, et al. (2005). A Critical Review of the Durability of Adhesion to Tooth Tissue: Methods and Results. J. Dent. Res. 84:118–132.

25. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. (2003). Four-year water degradation of total-etch adhesives bonded to dentin. J. Dent. Res. 82:136–40.

26. De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G, et al. (2009). Inhibition of enzymatic degradation of adhesive-dentin interfaces. J. Dent. Res. 88:1101–6.

27. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. (2011). State of the art etch-and-rinse adhesives. Dent. Mater. 27:1–16.

28. Pashley DH, Tay FR, Carvalho RM, Rueggeberg F a, Agee K a, Carrilho M, et al. (2007). From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am. J. Dent. 20:7–20.

29. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. (2004). Collagen Degradation by Host-derived Enzymes during Aging. J. Dent. Res. 83:216–221.

30. Perdigão J (2010). Dentin bonding-variables related to the clinical situation and the substrate treatment. Dent. Mater. 26:e24–37.

31. Hebling J, Bianchi L, Basso FG, Scheffel DL, Soares DG, Carrilho MR, Pashley DH, Tjäderhane L, de Souza Costa CA (2015). Cytotoxicity of dimethyl sulfoxide (DMSO) in direct contact with odontoblast-like cells. Dent. Mater *in press.*

32. Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR. (2010) Ethanol wet bonding challenges current anti-degradation strategy. J Dent Res;89:1499–504.

33. Shono Y, Ogawa T, Terashita M, Carvalho RM, Pashley EL, Pashley DH (1999). Regional measurement of resin-dentin bonding as an array. J. Dent. Res. 78:699–705.

34. Spencer P, Wang Y (2002). Adhesive phase separation at the dentin interface under wet bonding conditions. J. Biomed. Mater. Res. 62:447–56.

35. Stape THS, Tjäderhane L, Marques MR, Aguiar FHB, Martins LRM. Effect of dimethyl sulfoxide wet-bonding technique on hybrid layer quality and dentin bond strength Dental Materials (in press).

36. Tay FR, Pashley DH, Yoshiyama M (2002). Two modes of nanoleakage expression in single-step adhesives. J. Dent. Res. 81:472–476.

37. Tezvergil-Mutluay A, Agee KA, Hoshika T, Uchiyama T, Tjäderhane L, Breschi L, et al. (2011). Inhibition of MMPs by alcohols. Dent. Mater. 27:926–33.

38. Tezvergil-Mutluay A, Mutluay MM, Gu L-S, Zhang K, Agee KA, Carvalho RM, et al. (2011). The anti-MMP activity of benzalkonium chloride. J. Dent. 39:57–64.

39. Tjäderhane L, Mehtälä P, Scaffa P, Vidal C, Pääkkönen V, Breschi L, et al. (2013). The effect of dimethyl sulfoxide (DMSO) on dentin bonding and nanoleakage of etch-and-rinse adhesives. Dent. Mater. 29:1055–62.

40. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. (2013). Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater. 29:116-35.

41. Vishnyakov A (2001). Molecular dynamics simulations of dimethyl sulfoxide and dimethyl sulfoxide-water mixture. J. Phys. 1702–1710.

42. Wang Y, Spencer P (2003). Hybridization efficiency of the adhesive/dentin interface with wet bonding. J. Dent. Res. 82:141–5.

43. Zimmerley M, McClure RA, Choi B, Potma EO (2009). Following dimethyl sulfoxide skin optical clearing dynamics with quantitative nonlinear multimodal microscopy. Appl. Opt. 48:D79–87.

44. Zou Y, Jessop JLP, Armstrong SR (2010). In vitro enzymatic biodegradation of adhesive resin in the hybrid layer. J. Biomed. Mater. Res. A 94:187–92.

Tables

Table 1. Adhesive Systems, main components, and application mode of bonding agents

Adhesive System	Components	Mode of application	
		Control	***DMSO wet-bonding***
Adper Scotchbond Multi-Purpose (SBMP) 3M/ESPE		(1) H_3PO_4 conditioning for 15 s; (2) rinse with water for 30 s; (3) blot drying leaving dentin slight moist; (4) *Primer* application with a fully saturated brush tip*; (5) gently blow dry 5 s; (6) *Bond* application*; and (7) Light cure for10 s.	1) H_3PO_4 conditioning for 15 s; (2) rinse with water for 30 s; (3) blot drying leaving dentin slight moist; (4) active application of 50% DMSO for 60 s; (5) blot drying leaving dentin slight moist; (6) *Primer* application with a fully saturated brush tip*; (7) gently blow dry 5 s; (8) *Bond* application*; and (9) Light cure for 10 s.
Etchant	37% phosphoric acid, fumared silica		
Primer	HEMA, polyalkenoic acid methacrylate copolymer, water		
Bond	bis-GMA, HEMA, dimethacrylates photoinitiators,		
Clearfil SE Bond (CF) Kuraray		(1) Blot drying until no sign of visible moisture was observed; (2) active *Primer* application with a fully saturated brush tip for 20 s; (3) mild air stream for 5 s; (4) *Bond* application*; (5) gentle air stream 5 s; and (6) light cure for 10 s.	(1) active application of 50% DMSO for 60s; (2) Blot drying until no sign of visible moisture was observed; (3) active *Primer* application with a fully saturated brush tip for 20 s; (4) mild air stream for 5 s; (5) active *Bond* application*; (6) gentle air stream 5 s; and (7) light cure for 10 s.
Primer	10-MDP; HEMA; CQ; Hydrophilic dimethacrylate; water (pH 2.0)		
Bond	10-MDP; N,N-diethanol-p-toludine; HEMA; Bis-GMA; silanated colloidal silica; hydrophobic dimethacrylate; CQ; MDPB;		

Abbreviations: HEMA=2-hydroxyethyl methacrylate; Bis-GMA=bis-phenolA diglycidylmethacrylate; 10-MDP=10-methacryloxydecyl dihydrogen phosphate; CQ= camphoroquinone; MDPB, 12-methacryloyloxydodecylpyridium bromide; SiO2, silicon oxide; * application time was standardized (10 s).

Figures

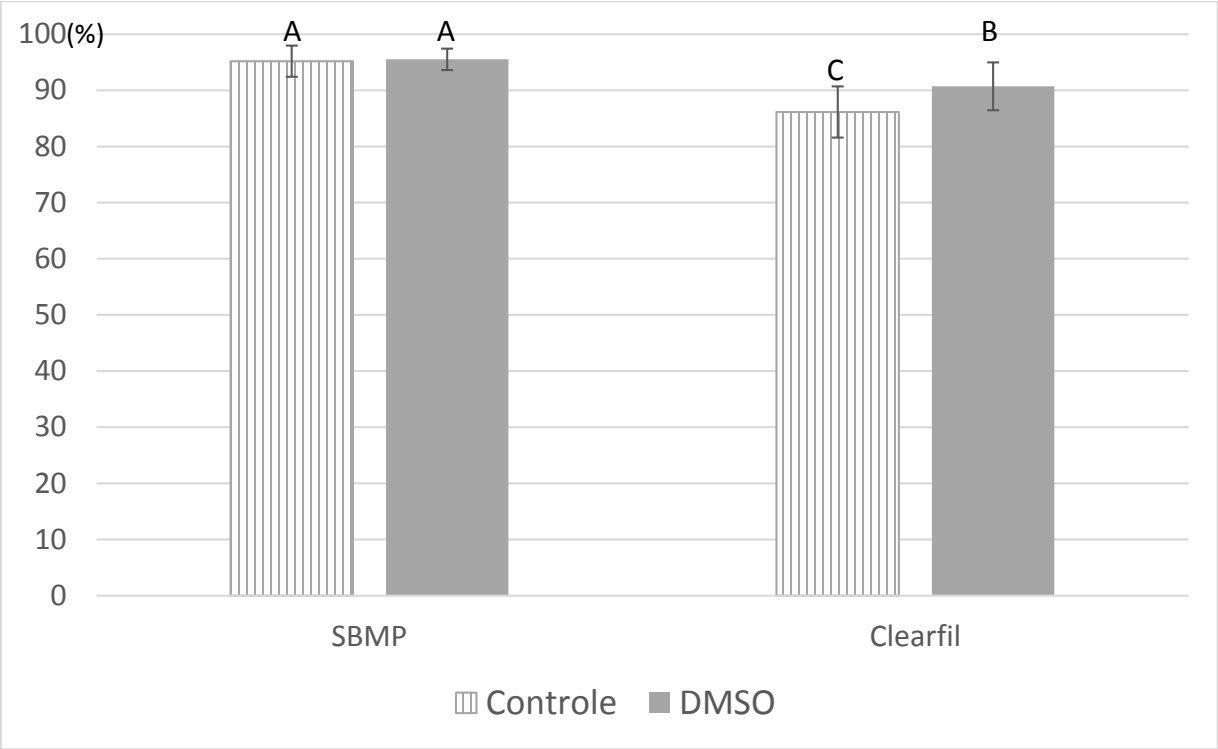

Figure 1. Degree of conversion (%) inside the hybrid layer for all groups. Different letters indicate significant difference according to Tukey Test ($p<0.05$).

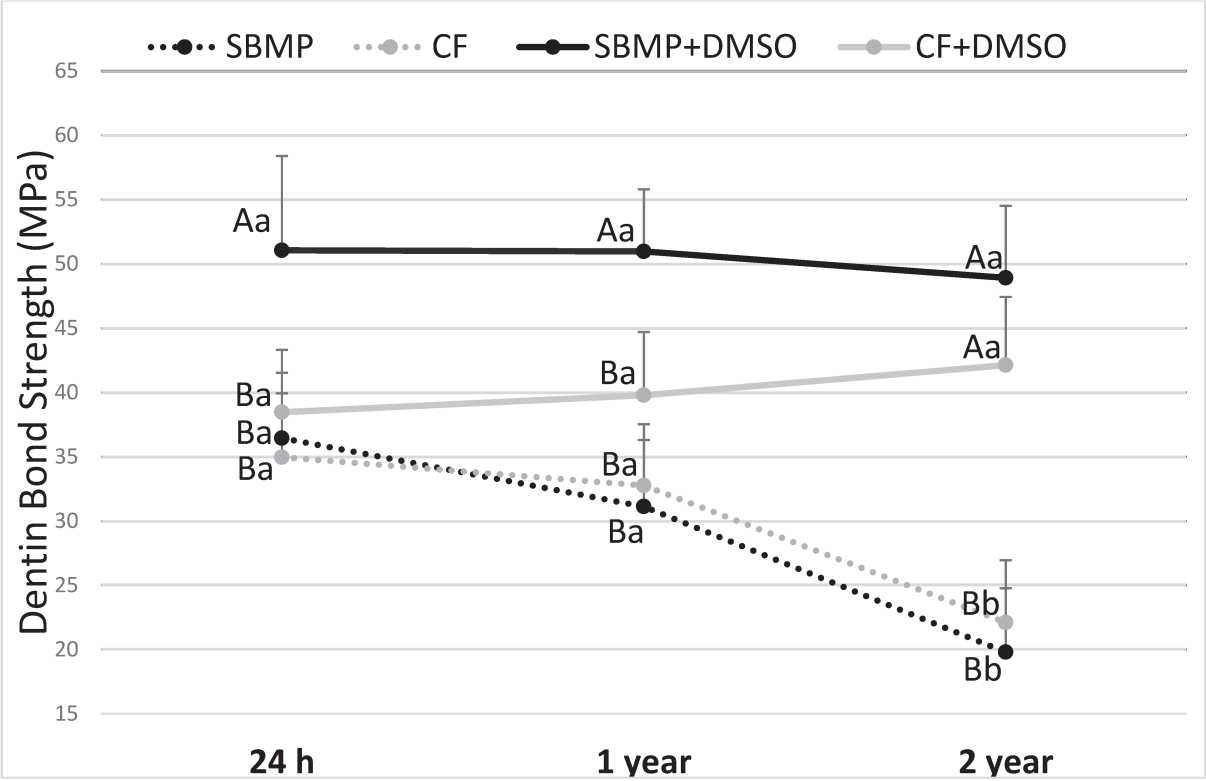

Figure 2. Dentin bond strength values for all groups. Different capital letters indicate significant difference according to Tukey Test ($p<0.05$) for each aging period; different lowercase letters indicate significant difference according to Tukey Test ($p<0.05$) comparing different aging periods.

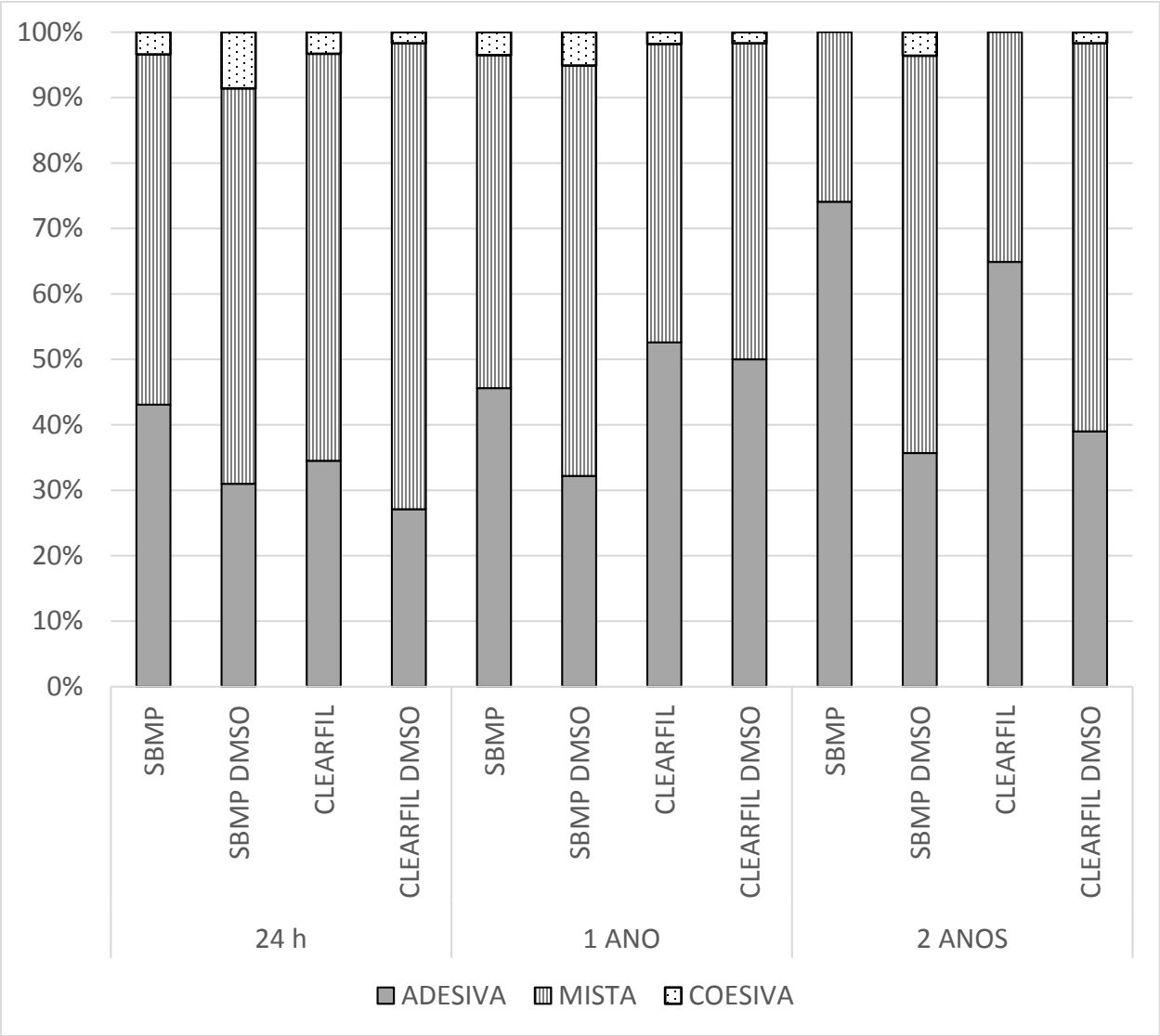

Figure 3. Fracture mode distribution (%) for all groups.

CONCLUSÃO

De acordo com os resultados obtidos e considerando as limitações das metodologias utilizadas, pôde-se concluir que:

1. O mecanismo de ação do protocolo adesivo com 50% DMSO depende do sistema adesivo utilizado. O protocolo adesivo proposto não influenciou a resistência de união imediata do adesivo autocondicionante de 2 passos testado, no entanto, promoveu aumento na resistência de união do adesivo convencional de 3 passos;

2. Ambos adesivos testados produziram menores extensões de matriz colágenas não encapsuladas na base da camada híbrida quando o pré-tratamento dentinário com 50% DMSO foi realizado, melhorando a hibridização da dentina;

3. A saturação do substrato dentinário com 50% DMSO, não afetou a conversão monomérica na camada híbrida do sistema adesivo convencional testado; no entanto houve aumento no grau de conversão do sistema adesivo autocondicionante;

4. A técnica adesiva com DMSO proposta contribuiu para a preservação dos valores de resistência de união de ambos adesivos testados por dois anos, aumentando a durabilidade de interfaces adesivas envolvendo o substrato dentinário.

REFERÊNCIAS

Bedran-Russo AKB, Castellan CS, Shinohara MS, Hassan L, Antunes A. Characterization of biomodified dentin matrices for potential preventive and reparative therapies. *Acta Biomater.* 2011; 7: 1735–41.

Bianchi L, Basso FG, Carrilho MRO, Tjäderhane L, De Souza Costa CA, Hebling J. Effects of dimethyl sulfoxide (DMSO) on odontoblast-like cells activity. *Dent. Mater.* 2012; 28(Suppl. 1): e4 [Abstr. No. 7].

Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. *Dent. Mater.* 2008; 24: 90–101.

Bui AK, Mcclure RA, Chang J, Stoianovici C, Yeh AT, Choi B. Revisiting optical clearing with dimethyl sulfoxide (DMSO). *Lasers Surg Med.* 2010; 41:142–148.

Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J. Dent. Res.*1955; 34: 849–53

Carrilho MRO, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, et al. In vivo preservation of the hybrid layer by chlorhexidine. *J. Dent. Res.* 2007; 86: 529–33.

Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, Tay FR. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. 2005; *Biomaterials* 26: 1035–42.

Carvalho RM, Manso AP, Geraldeli S, Tay FR, Pashley DH. Durability of bonds and clinical success of adhesive restorations. *Dent. Mater.* 2012; 28: 72–86.

Chiba Y, Yamaguchi K, Miyazaki M, Tsubota K, Takamizawa T, Moore BK. Effect of air-drying time of single-application self-etch adhesives on dentin bond strength. *Oper. Dent.* 2006; 31: 233–9.

Cox CF, Keall CL, Keall HJ, Ostro E, Bergenholtz G. Biocompatibility of surface-sealed dental materials against exposed pulps. *J. Prosthet. Dent.* 57:1–8. Davidson CL, de Gee AJ, Feilzer A (1984). The competition between the composite-dentin bond strength and the polymerization contraction stress. *J. Dent. Res.* 1987; 63:1396–9.

Van Dijken JW V, Hasselrot L. A prospective 15-year evaluation of extensive dentin-enamel-bonded pressed ceramic coverages. *Dent. Mater.* 2010; 26: 929–39.

Van Dijken JW V, Sunnegårdh-Grönberg K, Lindberg A. Clinical long-term retention of etch-and-rinse and self-etch adhesive systems in non-carious cervical lesions. A 13 years evaluation. *Dent. Mater.* 2007; 23: 1101–7.

Fusayama T, Nakamura M, Kurosaki N, Iwaku M. Non-pressure adhesion of a new adhesive restorative resin. *J. Dent. Res.* 1979; 58: 1364–70.

Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. *J. Biomed. Mater. Res.* 1998; 41: 474–80.

Gwinnett AJ, Kanca JA. Micromorphology of the bonded dentin interface and its relationship to bond strength. *Am. J. Dent.* 1992; 5: 73–7.

Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin–dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. *Biomaterials* 2003; 24: 3795–3803.

Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. *J. Dent. Res.* 2005; 84: 741–6.

Kanca J. Resin bonding to wet substrate. 1. Bonding to dentin. *Quintessence Int.* 1992; 23: 39–41.

Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials* 2007; 28: 3757–85.

Lee HL, Orlowski JA, Scheidt GC, Lee JR Effects of acid etchants on dentin. *J. Dent. Res.* 1973 52: 1228–33.

Liu Y, Tjäderhane L, Breschi L, Mazzoni a, Li N, Mao J, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *J. Dent. Res.* 2011; 90: 953–68.

MacGregor WS. The chemical and physical properties of DMSO. *Ann. N. Y. Acad. Sci.* 1967; 141: 3–12.

Marren K. Dimethyl sulfoxide: an effective penetration enhancer for topical administration of NSAIDs. *Phys. Sportsmed.* 2001; 39: 75–82.

Van Meerbeek B, Peumans M, Verschueren M, Gladys S, Braem M, Lambrechts P, et al. Clinical status of ten dentin adhesive systems. *J. Dent. Res.* 1994; 73: 1690–702.

Van Meerbeek B, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Peumans M. A randomized controlled study evaluating the effectiveness of a two-step self-etch adhesive with and without selective phosphoric-acid etching of enamel. *Dent. Mater.* 2005; 21: 375–83.

Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper. Dent.* 2003; 28: 215–35.

Van Meerbeek B, Yoshihara K, Yoshida Y, Mine a, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dent. Mater.* 2011; 27: 17–28.

Mobarak EH, El-Badrawy W, Pashley DH, Jamjoom H. Effect of pretest storage conditions of extracted teeth on their dentin bond strengths. *J. Prosthet. Dent.* 2010; 104: 92–7.

De Munck J, Van Landuyt K, Peumans M, Poitevin a., Lambrechts P, Braem M, et al. A Critical Review of the Durability of Adhesion to Tooth Tissue: Methods and Results. *J. Dent. Res.* 2005; 84:118–132.

Murray PE, Windsor LJ, Smyth TW, Hafez AA, Cox C. Analysis of pulpal reactions to restorative procedures, materials, pulp capping, and future therapies. *Crit. Rev. Oral Biol. Med.* 2002; 13:509–20.

Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. *J. Biomed. Mater. Res.* 1982; 16: 265–73.

Pashley DH, Tay FR, Carvalho RM, Rueggeberg F a, Agee K a, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am. J. Dent.* 2007; 20: 7–20

Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen Degradation by Host-derived Enzymes during Aging. *J. Dent. Res.* 2004; 83: 216–221.

Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. *Dent. Mater.* 2005; 21: 864–81

Peumans M, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Five-year clinical effectiveness of a two-step self-etching adhesive. *J. Adhes. Dent.* 2007; 9: 7–10.

Reis A, de Carvalho Cardoso P, Vieira LCC, Baratieri LN, Grande RHM, Loguercio AD. Effect of prolonged application times on the durability of resin-dentin bonds. *Dent. Mater.* 2008; 24: 639–44.

Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. *J. Biomed. Mater. Res.* 2002; 62: 447–56.

Spencer P, Wang Y, Katz JL. Identification of collagen encapsulation at the dentin/adhesive interface. *J. Adhes. Dent.* 2004; 6: 91–5.

Spencer P, Ye Q, Park J, Topp EM, Misra A, Wang Y, et al. Adhesive/Dentin Interface: The Weak Link in the Composite Restoration. *Ann Biomed Eng.* 2011; 38: 1989–2003.

Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SCN, Yiu CKY, et al. Aging Affects Two Modes of Nanoleakage Expression in Bonded Dentin. *J. Dent. Res.* 2003; 82: 537–541.

Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. *J. Dent. Res.* 2002; 81: 472–476.

Tezvergil-Mutluay A, Mutluay MM, Gu L-S, Zhang K, Agee KA, Carvalho RM, et al. The anti-MMP activity of benzalkonium chloride. *J. Dent.* 2011; 39: 57–64

Tjäderhane L, Mehtälä P, Scaffa P, Vidal C, Pääkkönen V, Breschi L, et al. The effect of dimethyl sulfoxide (DMSO) on dentin bonding and nanoleakage of etch-and-rinse adhesives. *Dent. Mater.* 2013; 29: 1055–62.

Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. *J. Dent. Res.* 2014; 29: 116–135.

Wilson NHF, Burke FJT, Mjör IA. Reasons for placement and replacement of restorations of direct restorative materials by a selected group of practitioners in the United Kingdom. *Quintessence Int. (Berl).* 1997; 28: 245–248.

Ye Q, Wang Y, Spencer P. Nanophase separation of polymers exposed to simulated bonding conditions. *J. Biomed. Mater. 2009;* 88: 339–348.

Zimmerley M, McClure RA, Choi B, Potma EO. Following dimethyl sulfoxide skin optical clearing dynamics with quantitative nonlinear multimodal microscopy. *Appl. Opt.* 2009; 48: 79–87.

APÊNDICE 1

METODOLOGIA ILUSTRADA

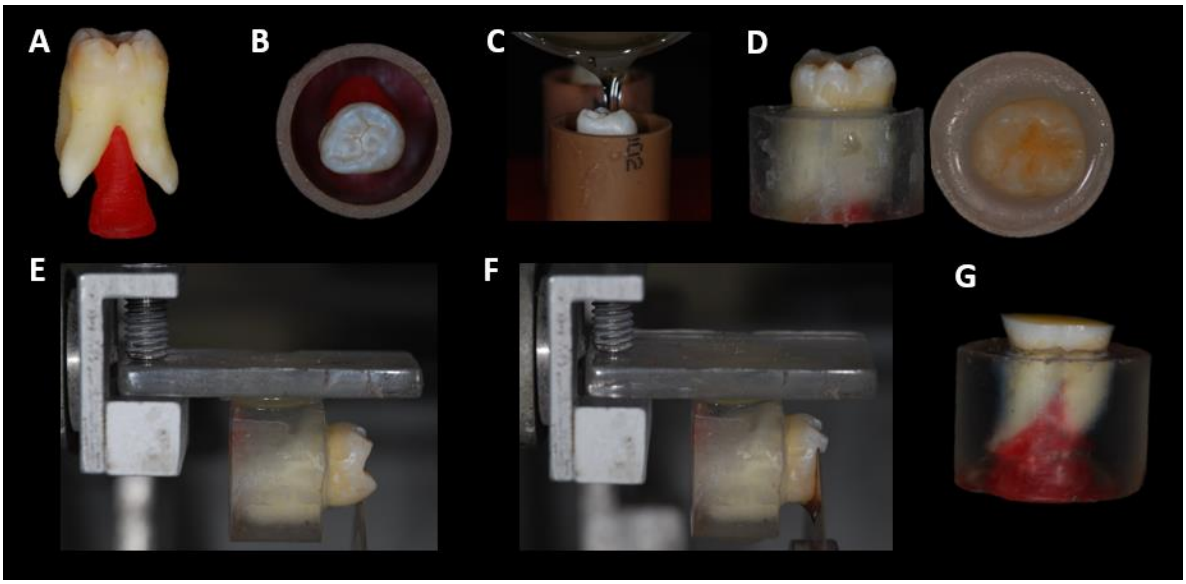

Figura 1. Preparo das amostras. A): confecção de suporte em cera utilidade e fixação do terceiro molar hígido mantendo as cúspides na mesma altura e perpendiculares ao longo eixo do dente; B): posicionamento e fixação do tubo de PVC vaselinado ao redor do elemento dentário, sobre uma lâmina de cera; C): inclusão da porção radicular em resina de poliestireno, 1 mm aquém do limite amelo-cementário; D): vista vestibular e oclusal após a remoção do tubo de PVC; E): posicionamento do dente incluído em resina de poliestireno em placa acrílica com cola quente e fixação da placa na cortadeira de precisão; F): secção da face oclusal para exposição de dentina de profundidade média; G): vista vestibular após secção da face oclusal e remoção da placa acrílica.

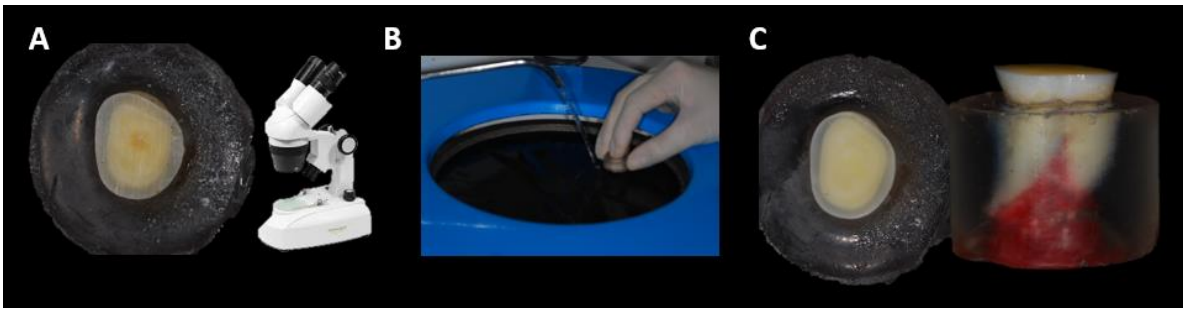

Figura 2. Remoção de ilhas remanescentes de esmalte e padronização da *smear layer*. A): verificação da presença de esmalte remanescente em lupa estereoscópica (aumento 40×); B): remoção de esmalte remanescente utilizando lixas de granulação 400, seguido da padronização da *smear layer* utilizando lixas de granulação 600 por 60 s em politriz sob irrigação constante de água; C): superfície de dentina média plana pronta para realização dos protocolos adesivos e restauração.

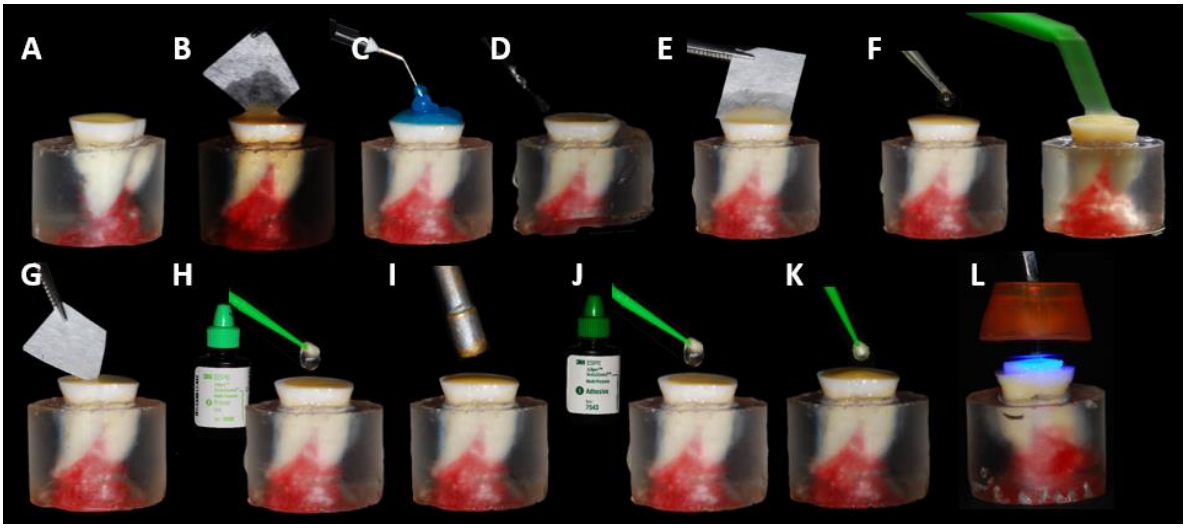

Figura 3. Protocolo de aplicação do sistema adesivo Scotchbond Multi-purpose. A): manutenção da superfície dentinária úmida previamente à hibridização; B): controle de umidade com tiras de papel absorvente; C): condicionamento com ácido fosfórico por 15 s; D): lavagem com água por 30 s; E): controle de umidade mantendo a dentina levemente úmida; F): aplicação ativa de 50 µL de água destilada por 60 s;

G): controle de umidade mantendo a dentina levemente úmida; H): aplicação do *Primer*; I): volatilização do solvente por 5 s; J): aplicação do *Bond*; K): remoção de excesso do sistema adesivo; L): fotoativação por 10 s utilizando um fotopolimerizador LED (Bluephase 20i, Ivoclare Vivadent).

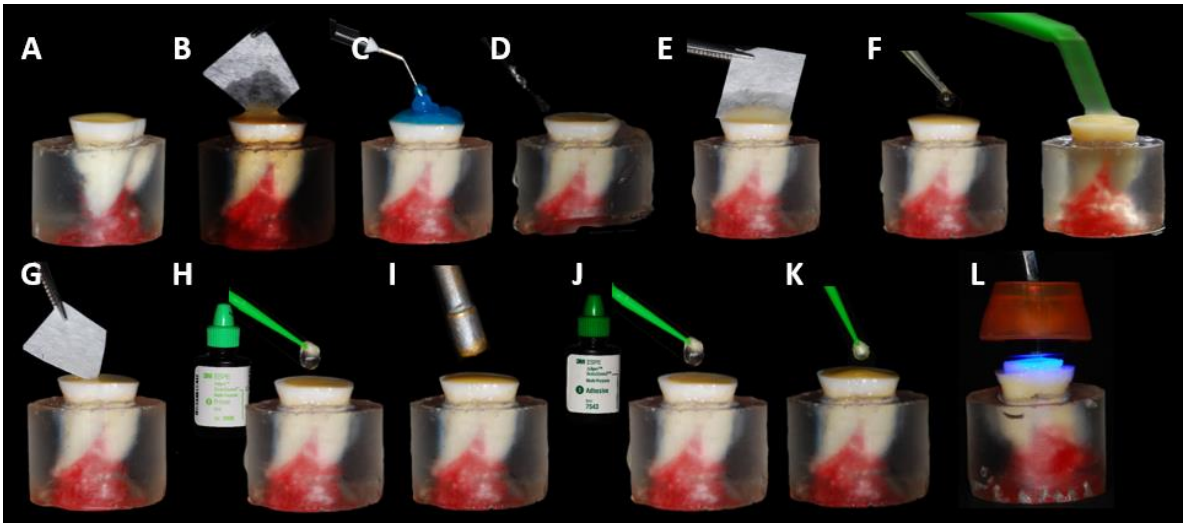

Figura 4. Protocolo de aplicação do sistema adesivo Scotchbond Multi-purpose segundo a técnica *DMSO-wet bonding*. A): manutenção da superfície dentinária úmida previamente à hibridização; B): controle de umidade com tiras de papel absorvente; C): condicionamento com ácido fosfórico por 15 s; D): lavagem com água por 30 s; E): controle de umidade mantendo a dentina levemente úmida; F): aplicação ativa de 50 µL de DMSO 50% por 60 s; G): controle de umidade mantendo a dentina levemente úmida; H): aplicação do *Primer*; I): Volatilização do solvente por 5 s; J): aplicação do *Bond*; K): remoção de excessos do sistema adesivo; L): fotoativação por 10 s, utilizando um fotopolimerizador LED (Bluephase 20i, Ivoclare Vivadent).

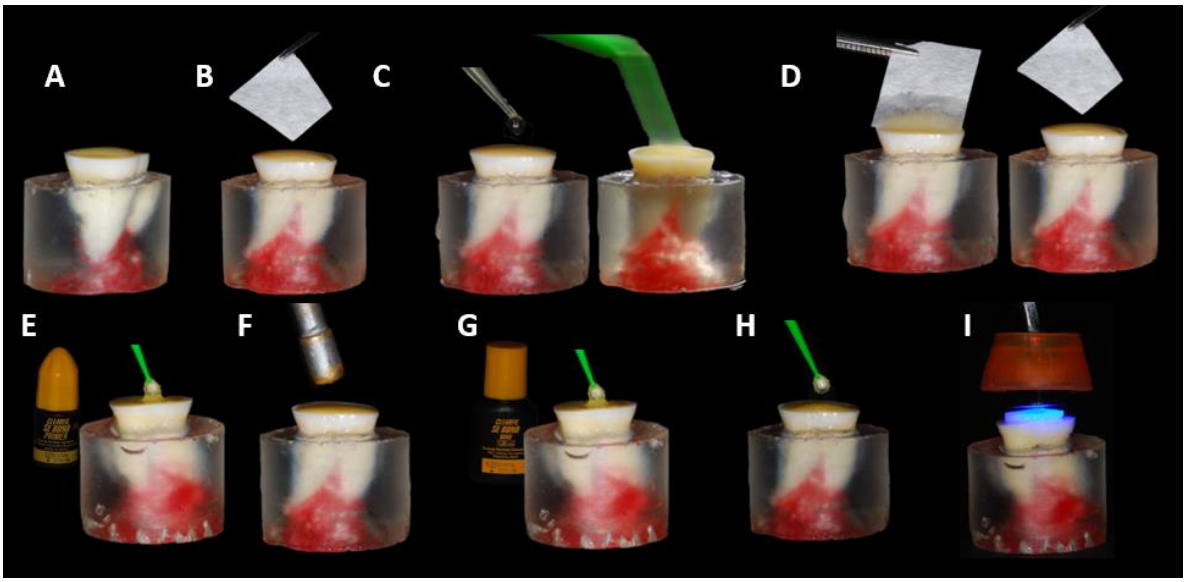

Figura 5. Protocolo de aplicação do sistema adesivo Clearfil SE Bond. A): manutenção da superfície dentinária úmida previamente à hibridização; B): controle de umidade com tiras de papel absorvente criando um substrato seco; C): aplicação ativa de 50 µL de água destilada por 60 s; D): controle de umidade com tiras de papel absorvente criando um substrato seco; E): aplicação ativa do *Primer* acídico por 20 s; F): volatilização do solvente por 5 s; G): aplicação do *Bond*; H): remoção de excesso do sistema adesivo; L): fotoativação por 10 s, utilizando um fotopolimerizador LED (Bluephase 20i, Ivoclare Vivadent).

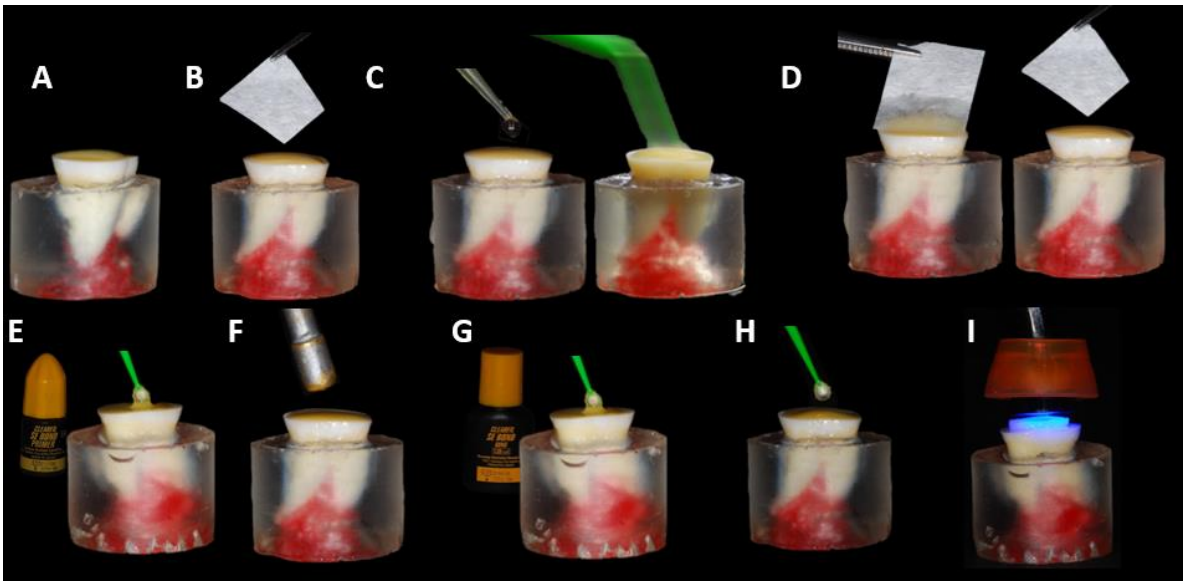

Figura 6. Protocolo de aplicação do sistema adesivo Clearfil SE Bond segundo a técnica *DMSO-wet bonding*. A): manutenção da superfície dentinária úmida previamente à hibridização; B): controle de umidade com tiras de papel absorvente criando um substrato seco; C): aplicação ativa de 50 µL de DMSO 50% por 60 s; D): controle de umidade com tiras de papel absorvente criando um substrato seco; E): aplicação ativa do *Primer* acídico por 20 s; F): volatilização do solvente por 5 s; G): aplicação do *Bond*; H): remoção de excesso do sistema adesivo; L): fotoativação por 10 s, utilizando um fotopolimerizador LED (Bluephase 20i, Ivoclare Vivadent).

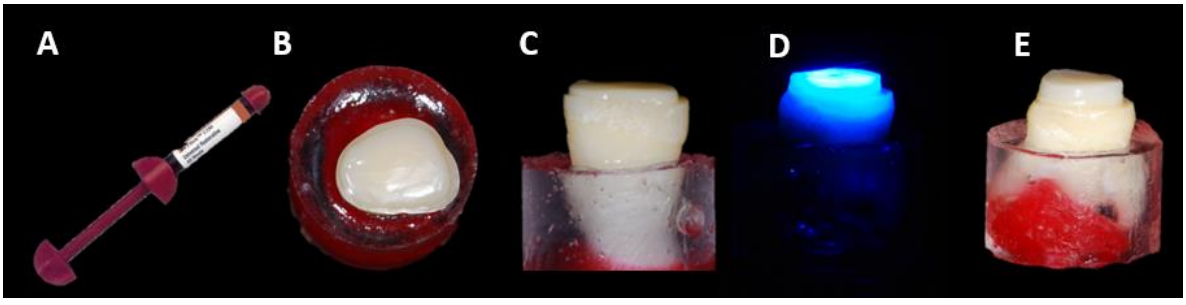

Figura 7. Confecção das Restaurações. A): resina microhíbrida (Z250 3M ESPE); B) estratificação do primeiro incremento de 1mm exclusivamente sobre dentina; C)

vista lateral do primeiro incremento; D) fotoativação por 20 s; E): restauração finalizada.

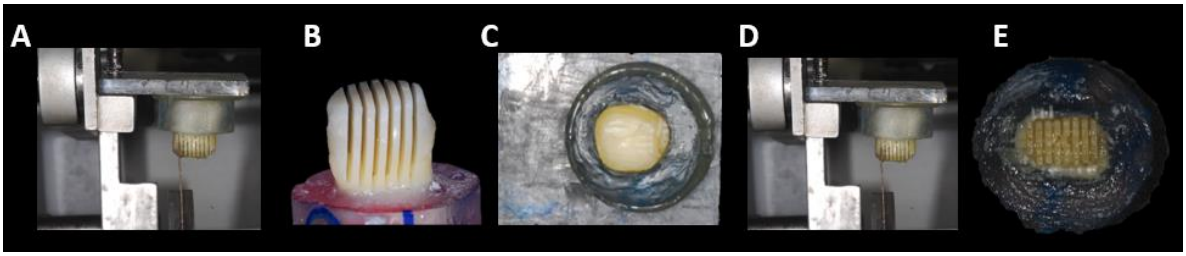

Figura 8. Confecção das fatias e palitos. A): fixação dos dentes restaurados em placa acrílica utilizando cola quente e montagem da placa em cortadeira de precisão para realização de secções longitudinais de 0,9 mm; B) fatias obtidas; C) remoção do dente restaurado, estabilização das fatias com cera e fixação em placa acrílica com cola quente girando o dente restaurado em 90º. D) confecção de secções longitudinais; E): vista oclusal dos palitos obtidos.

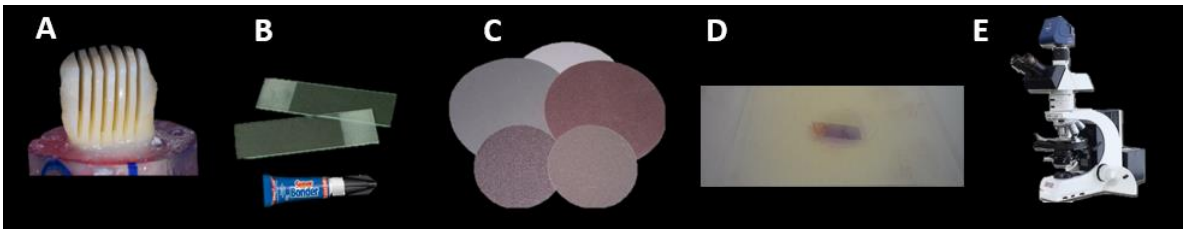

Figura 9. Avaliação da exposição de fibrilas colágenas não encapsuladas na base da camada híbrida. A) seleção de uma fatia por dente; B) colagem da fatia em lâmina histológica com adesivo à base de cianoacrilato; C) polimento manual da fatia utilizando lixas de granulação P1200, P2000, P2500 e P4000; D) fatia após a coloração de Masson; E): análise da interface adesiva em microscópio ótico (Leica DMLP, Leica Microsystems).

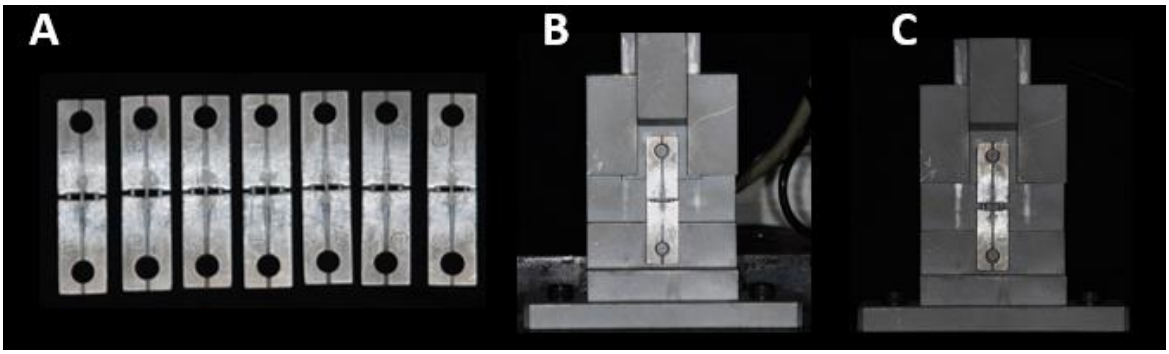

Figura 10. Teste de microtração. A): colagem dos palitos com adesivo à base de cianoacrilato em garras com canaleta central; B): fixação individual das garras no dispositivo de Geraldeli montado em máquina de ensaio mecânico (DL2000, EMIC); C) carregamento de tração, velocidade de 0,5 mm/min, até a fratura.

Figura 11. Análise do grau de conversão na camada híbrida. A): polimento dos palitos utilizando lixas de granulação P1200, P2000, P2500 e P4000; B) limpeza em cuba ultrassônica; (C) espectrômetro (Senterra, BrukerOptik GmbH); (D) obtenção dos espectros-raman na região de camada híbrida.

ANEXO 1

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 **"Influência da aplicação de DMSO na resistência de união dentinária e na morfologia da camada híbrida de sistemas adesivos"**, protocolo nº 017/2013, dos pesquisadores Luis Roberto Marcondes Martins, Beatriz Oliveira Silva Capelli e Thiago Henrique Scarabello Stape, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 24/06/2013.

The Ethics Committee in Research of the Piracicaba Dental School - University of Campinas, certify that the project **"Influence of DMSO on dentin bond strength and hybrid layer morphology of adhesive systems"**, register number 017/2013, of Luis Roberto Marcondes Martins, Beatriz Oliveira Silva Capelli and Thiago Henrique Scarabello Stape, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee on Jun 24, 2013.

Prof. Dr. Felippe Bevilacqua Prado
Secretário
CEP/FOP/UNICAMP

Profa. Dra. Lívia Maria Andaló Tenuta
Coordenadora
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2

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 das técnicas adesivas DMSO-wet bonding e Etanol-wet bonding na resistência de união dentinária de sistemas adesivos convencionais"**, protocolo nº 128/2014, dos pesquisadores Luis Roberto Marcondes Martins e Thiago Henrique Scarabello Stape, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 05/11/2014.

The Ethics Committee in Research of the Piracicaba Dental School - University of Campinas, certify that the project **"Influence of DMSO-wet bonding and ethanol wet-bonig on dentin bond strength of conventional adhesive systems"**, register number 128/2014, of Luis Roberto Marcondes Martins and Thiago Henrique Scarabello Stape, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee on Nov 05, 2014.

Prof. Dr. Felippe Bevilacqua Prado
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Felippe Bevilacqua Prado
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 3

Manuscript Number: DEMA-D-14-00240R1

Title: Effect of dimethyl sulfoxide wet-bonding technique on hybrid layer quality and dentin bond strength

Article Type: Full Length Article

Keywords: Resin-dentin biopolymer; Collagen; Resin infiltration; Etch-and-rinse adhesive; Self-etch adhesive; Microtensile bond strength, DMSO

Corresponding Author: Prof. Thiago Henrique Scarabello Stape, DDS, MS

Corresponding Author's Institution: Piracicaba Dental School, University of Campinas

First Author: Thiago Henrique Scarabello Stape, DDS, MS

Order of Authors: Thiago Henrique Scarabello Stape, DDS, MS; Leo Tjäderhane, DDS, MS, PhD; Marcelo R Marques, DDS, MS, PhD; Flávio H Aguiar, DDS, MS, PhD; Luís R Martins, DDS, MS, PhD

Abstract: Objectives: This study examined the effect of a dimethyl sulfoxide (DMSO) wet bonding technique on the resin infiltration depths at the bonded interface and dentin bond strength of different adhesive systems.Methods: Flat dentin surfaces of 48 human third molars were treated with 50% DMSO (experimental groups) or with distilled water (controls) before bonding using an etch-and-rinse (SBMP: Scotchbond Multi-Purpose, 3M ESPE) or a self-etch (Clearfil: Clearfil SE Bond, Kuraray) adhesive system. The restored crown segments (n=12/group) were stored in distilled water (24 h) and sectioned for interfacial analysis of exposed collagen using Masson's Trichrome staining and for microtensile bond strength testing. The extent of exposed collagen was measured using light microscopy and a histometric analysis software. Failure modes were examined by SEM. Data was analyzed by two-way ANOVA followed by Tukey Test (α=0.05). Results: The interaction of bonding protocol and adhesive system had significant effects on the extension of exposed collagen matrix ($p<0.0001$) and bond strength ($p=0.0091$). DMSO-wet bonding significantly reduced the extent of exposed collagen matrix for SBMP and Clearfil ($p<0.05$). Significant increase in dentin bond strength was observed on DMSO-treated specimens bonded with SBMP ($p<0.05$), while no differences were observed for Clearfil ($p>0.05$). Significance: DMSO-wet bonding was effective to improve the quality of resin-dentin bonds of the tested etch-and-rinse adhesives by reducing the extent of exposed collagen matrix at the base of the resin-dentin biopolymer. The improved penetration of adhesive monomers is reflected as an increase in the immediate bond strength when the DMSO-wet bonding technique is used with a water-based etch-and-rinse adhesive.