UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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DURABILIDADE DA UNIÃO RESINA-DENTINA: EFEITO DA UMIDADE DENTINÁRIA NA RESISTÊNCIA DA UNIÃO MEDIADA POR *PRIMERS* EXPERIMENTAIS

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de Doutor em Materiais Dentários.
Orientador: Prof. Dr. Mario Fernando de Goes.
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“If you can dream it, you can do it”

(Walt Disney)

RESUMO

O objetivo geral deste estudo foi avaliar *in vitro* os efeitos de variações na umidade dentinária no estabelecimento e durabilidade da união resina-dentina através da associação de materiais e técnicas que apresentam potencial para uso em restaurações adesivas. Para atender ao objetivo geral, dois estudos com objetivos específicos foram propostos: 1) avaliar os efeitos da condição de umidade da dentina na durabilidade da resistência da união (RU) à dentina obtida por meio da associação de oxalato de potássio com *primers* a base de água ou etanol. 2) avaliar os efeitos da técnica úmida utilizando etanol na durabilidade da RU à dentina obtida através do uso de *primers* com características hidrofílicas e hidrofóbicas. Os *primers* experimentais foram preparados utilizando-se a proporção 35% monômeros / 65% solventes (v/v), como se segue: HEMA/água, HEMA/etanol, Bis-GMA/etanol e um agente de união com características hidrofóbicas (Adper Scothbond Multi-Purpose adhesive, 3M/ESPE) foi usado nos dois estudos. A RU foi avaliada pelo teste de resistência à microtração 24 h após a realização do procedimento adesivo ou após 6 meses de armazenamento em água, utilizando-se dentes humanos hígidos recém extraídos para confecção das amostras de acordo com os objetivos específicos de cada estudo. Os resultados do estudo 1 mostraram que de uma maneira geral a associação de oxalato de potássio com *primers* a base de água e etanol reduziu significativamente a RU imediata e a longo prazo para os *primers* a base de água e etanol, independente da condição de umidade da dentina. Os resultados do estudo 2 indicaram que a técnica úmida com etanol resultou em maior RU imediata para os *primers* com características hidrofílicas e hidrofóbicas enquanto o armazenamento em água por 6 meses diminuiu a RU para o *primer* hidrofílico, independente da técnica utilizada.

Palavras Chave: longevidade, adesivos dentinários, resistência à tração e materiais dentários.

ABSTRACT

The overall goal of this investigation was to evaluate *in vitro* the effects of dentin moisture variations in the establishment and durability of the resin-dentin bonds by the association of materials and techniques with potential for use in adhesive restorative procedures. In order to meet the overall objective, two studies with specific goals were proposed. 1) to evaluate the effects of dentin moisture condition on the durability of the bond strength (BS) to dentin produced by the association of potassium oxalate with experimental water-and ethanol-based primers; 2) to evaluate the effects of the ethanol wet-bonding technique on the durability of the BS produced with the use of experimental primers with hydrophilic and hydrophobic characteristics. The experimental primers were prepared using the proportion 35% monomers / 65% solvents (v/v) as follows: HEMA/water, HEMA/ethanol, Bis-GMA/ethanol and the same bonding agent with hydrophobic characteristics was used in both studies (Adper Scothbond Multi- Purpose adhesive, 3M/ESPE). A microtensile bond strength test was performed 24 h after bonding procedures and 6 months after water storage, using freshly extracted noncarious human teeth for specimen preparation according to the specific goals of each study. In general, the results of study 1 showed that the association of potassium oxalate with water-and ethanol-based primers reduced significantly the immediate and long-term BS, regardless of the dentin moisture condition. The results of study 2 showed that the ethanol wet-bonding technique resulted in higher immediate BS for the hydrophilic and hydrophobic primers, while 6-month water storage reduced the BS for the hydrophilic primer, regardless of the employed bonding technique.

Keywords: longevity, dentin bonding agents, tensile strength and dental materials.

SUMÁRIO

INTRODUÇÃO

Nos sistemas adesivos convencionais, o condicionamento ácido remove a *smear layer,* desmineraliza a zona mais superficial, reduzindo o conteúdo de hidroxiapatita das camadas subjacentes, amplia o diâmetro dos túbulos, aumentando a permeabilidade da dentina e expõe a trama de fibrilas de colágeno (Nakabayashi *et al*.,1992). Como conseqüência desse procedimento, ocorre modificação das características morfológicas da dentina (Montes *et al*., 2005), implicando no aumento da permeabilidade e da umidade intrínseca do substrato (Pashley *et al*., 1989; Itthagarun *et al*., 2000). Estas variações na permeabilidade se refletem diretamente na união dos sistemas adesivos à dentina (Giannini *et al*., 2001). É fundamental que após a desmineralização com ácido as fibrilas de colágeno se mantenham expandidas, preservando os espaços interfibrilares necessários para a permeação dos monômeros resinosos presentes nos sistemas adesivos. A água desempenha papel fundamental na manutenção das fibrilas de colágeno em uma condição expandida, pois quando está presente nos espaços interfibrilares forma pontes de hidrogênio (P-H) com qualquer molécula das fibrilas de colágeno que seja capaz de formar P-H entre os peptídeos, prevenindo assim o estabelecimento de ligações interpeptídicas, o que resulta em uma condição de total expansão da matriz de dentina desmineralizada (Pashley *et al*., 1993, Carvalho *et al*., 1996, Pashley *et al*., 2001a;). Portanto, a infiltração dos monômeros resinosos na matriz de dentina desmineralizada é realizada através de uma técnica úmida de adesão (Gwinett 1992; Kanca 1992a; Kanca *et al*., 1992b; Kanca, Sandrik, 1998), o que implica na permanência de água no substrato previamente à aplicação do sistema adesivo.

Prévios estudos relatam que não há um consenso com relação à umidade dentinária ideal para cada tipo de sistema adesivo (Perdigão, Frankeberger, 2001; Reis *et* *al*., 2003). Embora a presença de água seja fundamental para o estabelecimento da união, a sua permanência em excesso (Tay *et al*., 1996a; Tay *et al*., 1996b; Tay *et al*., 1997; Jacobsen, Söderholm, 1998; Perdigão *et al*., 2002), durante a aplicação dos sistemas adesivos dificulta a infiltração dos monômeros nos espaços interfibrilares. Adicionalmente, a presença de água promove a diluição de alguns monômeros resinosos, provocando a emulsificação e separação dos seus componentes, o que resulta em um selamento incompleto da dentina (Tay *et al*. 1996b; Spencer, Wang, 2002), afetando a durabilidade da união (Paul *et al*., 1999; Carrilho *et al*., 2004; Yiu *et al*. 2005a; Carrilho *et al*., 2007). Dessa forma, a habilidade que os componentes dos sistemas adesivos apresentam em suportar a presença de água em uma técnica úmida é afetada por fatores como a natureza hidrofílica ou hidrofóbica dos componentes resinosos e tipo de solvente (Wang, Spencer, 2003). Assim, a umidade da dentina é fundamental no procedimento adesivo e de difícil padronização, o que demonstra a dificuldade na execução de uma técnica capaz de manter a umidade ideal e uniforme em toda a superfície condicionada (Pereira *et al*., 2001; Perdigão *et al*., 2002). A despeito dos altos valores iniciais de resistência da união obtidos com o uso dos sistemas adesivos convencionais associados à técnica úmida, a camada híbrida formada em tais condições apresenta-se porosa e susceptível à degradação ao longo do tempo (Tay *et al*., 2002, Wang, Spencer 2002; Breschi *et al*., 2008). Além disso, quando se estabelece uma técnica de união à dentina vital profunda, a maior umidade do substrato representa um fator complicador no alcance e manutenção dessa união (Shono *et al*., 1999).

Ao mesmo tempo em que apresenta um papel primordial para obtenção da união nos sistemas adesivos convencionais, a água estabelece condições que podem deflagrar o processo de degradação. Por outro lado a secagem excessiva da superfície dentinária após o condicionamento ácido dificulta a infiltração dos monômeros resinosos nos espaços interfibrilares por ocasionar o colapso das fibrilas de colágeno, diminuindo os valores de resistência da união à interface resina-dentina (Gwinett, 1994; Carvalho *et al*., 1996; Pashley & Carvalho, 1997; Eick *et al*., 1997). Quando a secagem do substrato é realizada com um breve jato de ar existe o colapso das fibrilas de colágeno no topo da zona desmineralizada, preservando os espaços interfibrilares abaixo dessa zona (Nakajima *et al*., , 1999). Porém, se o processo de secagem continuar poderá ocorrer uma contração adicional da fibrila de colágeno (Nakaoki *et al*., 2000). Com a evaporação da água da superfície de dentina há maior interação molecular entre os peptídeos das fibrilas de colágeno, por meio de P-H, o que estabiliza a matriz em uma condição colapsada e contraída. Entretanto, o colapso das fibrilas é um processo reversível pelo simples re-umedecimento da superfície dentinária com água (Gwinnett, 1994; Perdigão, Frankerberger, 2001) ou com solventes anidros com valor de parâmetro de solubilidade maior que o das fibrilas de colágeno (Pashley *et al*., 2001a). Dessa forma, não somente a água é capaz de re-expandir as fibrilas de colágeno colapsadas após a secagem com ar, mas também alguns solventes anidros têm se mostrado eficazes para re-expandir a matriz de dentina desmineralizada a uma condição semelhante à obtida com a água (Pashley *et al*., 2001a; Pashley *et al*., 2002; Carvalho *et al*., 2003; Eddleston *et al*., 2003). Os solventes adicionados aos sistemas adesivos, quando aplicados sobre a matriz desidratada, induzem diferentes graus de re-expansão de acordo com o seu parâmetro de solubilidade (Pashley *et al*., 2001a). Idealmente, os solventes utilizados para carrear os monômeros na dentina desmineralizada deveriam manter os espaços interpeptídeos expandidos. Para que isso ocorra é necessário que eles previnam o desenvolvimento de ligações do tipo P-H interpeptídeos. Essa capacidade parece estar relacionada também com a mistura de co-monômeros presentes nos sistemas adesivos. Asmussen *et al*. (1991); Asmussen, Uno (1993), foram os primeiros a sugerir que o mecanismo de ação dos *primers* é baseado na sua capacidade de molhamento das fibrilas de colágeno. A habilidade dos *primers* em estabelecer maiores valores de resistência da união está relacionada ao valor do seu parâmetro de solubilidade. Uma vez que monômeros resinosos possam facilmente penetrar nos espaços interfibrilares preservados até uma zona desmineralizada mais profunda, isto acarretaria em uma quantidade e volume suficiente de monômero para preencher os espaços interfibrilares, evitando a contração e colapso da matriz de colágeno, com adequada formação da camada híbrida (Pashley, Carvalho, 1997).

Com o objetivo de reduzir a permeabilidade dentinária sob restaurações adesivas, Pashley *et al.* (2001b) propuseram uma técnica associando o uso de agentes dessensibilizantes à base de oxalato de potássio, aplicados na dentina previamente condicionada, com sistemas adesivos convencionais. Esta técnica promove a oclusão dos túbulos dentinários com cristais de oxalato de cálcio, os quais são formados na sub-superfície dos túbulos sem comprometimento da resistência da união (Pashley *et al.*, 2001b; Tay *et al.*, 2003). Entretanto, adesivos ácidos ou com presença de grande quantidade de flúor em sua composição têm a resistência da união à dentina comprometida quando associados ao oxalato de potássio por meio desta técnica (Yiu *et al.*, 2005b).

Assim, ao bloquear o fluido proveniente dos túbulos dentinários, associado à secagem da cavidade, uma maior homogeneidade da umidade superficial durante as etapas subseqüentes de aplicação do sistema adesivo é esperada. Um dos principais fatores envolvidos na degradação da união resina-dentina ao longo do tempo está correlacionado à hidrólise dos monômeros resinosos hidrofílicos que compõem a camada híbrida (Shono *et al.*, 1999a; Sano *et al.*,1999; Carvalho *et al.*, 2000; Hashimoto *et al.*, 2000; Hashimoto *et al.*, 2001; Santerre *et al.*, 2001; Hashimoto *et al.*, 2003; De Munck *et al*, 2003, De Munck et al., 2005; Carrilho *et al.*, 2005; Yiu *et al.*, 2005b). Dessa forma, a possibilidade de se conjugar uma técnica de união à dentina utilizando solventes anidros associados à redução do fluido dentinário através da oclusão tubular poderia resultar em uma maior durabilidade da união resina-dentina. Por meio dessa técnica, monômeros resinosos com características mais hidrofóbicas poderiam ser empregados para a formação da camada híbrida, conferindo a esta menor susceptibilidade à absorção de água e, consequentemente, maior probabilidade de resistir à degradação hidrolítica. Por outro lado, se a superfície dentinária é seca após a obliteração dos túbulos dentinários, a capacidade de re-expansão das fibrilas colapsadas depende diretamente do parâmetro de solubilidade dos solventes utilizados e da sua velocidade em induzir essa expansão. Uma vez que essa técnica tem potencial para uso clínico, a viabilidade de associarmos a oclusão tubular e secagem da dentina com o uso de *primers* experimentais com parâmetro de solubilidade que sejam capazes de re-expandir a matriz dentinária desmineralizada para posterior infiltração de um agente de união com características hidrofóbicas merece ser investigada, tanto com relação à formação quanto à durabilidade da união à dentina.

Recentemente, Becker *et al*. (2006) corroborado por outros estudos (Nishitani *et al*., 2006; Pashley *et al*., 2007; Sadek *et al*., 2007; Tay *et. al*, 2007; Sadek *et al*., 2008) sugeriram a possibilidade de utilizar o etanol, ao invés da água, em uma técnica úmida de adesão à dentina. Conhecido como "técnica úmida com etanol", esse protocolo é baseado na substituição da água da superfície da dentina pela desidratação com concentrações ascendentes de etanol. O objetivo primordial dessa técnica é possibilitar a permeação de monômeros com características hidrofóbicas entre as fibrilas de colágeno desmineralizadas, o que poderia resultar em camada híbrida menos susceptível à ação da água. (Nishitani *et al*., 2007 ; Sadek *et al*., 2007 ; Tay *et al*, 2007; Sadek *et al*., 2008). Os resultados imediatos dos trabalhos empregando essa técnica alternativa utilizando etanol têm se mostrado promissores no estabelecimento de uma união eficaz e também melhor selamento da dentina, quando comparados à técnica úmida convencional (Carrilho *et al.*, 2009; Sauro et al., 2009). Entretanto, não existem estudos acerca da eficiência desse protocolo na manutenção da durabilidade da união resina-dentina (Nishitani *et al.*, 2007; Pashley et al., 2007; Sadek *et al.*, 2007; Tay *et.al*, 2007; Sadek *et al.*, 2008; Sauro *et al.,* 2009).

As diferentes pesquisas que abordam o tema durabilidade da união à dentina deixam claro que a manutenção da união resina-dentina estável ao longo do tempo é ainda um problema que demanda investigação (Sano *et al.*, 1999; Shono *et al.*, 1999; Hashimoto *et al.*, 2000; De Munck *et al.*, 2005; Breschi *et al.*, 2008; Ikeda *et al.*, 2008). O presente estudo desenvolveu uma linha de investigação visando à obtenção da união resina-dentina com um maior controle sobre a quantidade de água envolvida no processo adesivo, bem como depositou expectativas no sentido de contribuir para o desenvolvimento de materiais e técnicas que resultassem na obtenção de restaurações adesivas mais duradouras.

Diante do exposto, os objetivos deste trabalho foram: 1) avaliar os efeitos da condição de umidade da dentina na durabilidade da resistência da união à dentina obtida através da associação de oxalato de potássio com *primers* experimentais a base de água ou etanol. 2) avaliar os efeitos da técnica úmida utilizando etanol na durabilidade da resistência da união à dentina obtida através do uso de *primers* experimentais com características hidrofílicas e hidrofóbicas.

CAPÍTULO 1

Title: Effect of wet versus dry bonding technique on the durability of water and ethanol-based primers bonded to oxalate-treated acid-etched dentin

Short Title: Durability of wet vs. dry bonding to oxalate-treated acid-etched dentin

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Summary

This study evaluated the effect of potassium oxalate on the bond strength (μTBS) of resin-dentin bonds comprised of water- or ethanol-based HEMA primers that were applied to wet *versus* dry acid-etched dentin. Forty extracted human third molars had their deep-coronal dentin exposed, acid-etched and rinsed with distilled water. Half of the teeth were treated with potassium oxalate (Bis-Block/Bisco) for 30 s, while the remained teeth were kept untreated (control). Both control and oxalate-treated dentin surfaces were washed with water and gently blot-dried or strongly air-dried for 10 s. Two experimental primers containing 35/65% (v/v) HEMA/Water (HEMA/H_2O) or HEMA/Ethanol (HEMA/EtOH) were applied to both untreated and oxalate-treated dentin surfaces. After a dwell time of 30 s, the primed-dentin surfaces were bonded with a bonding agent (Adper Scotchbond Multi-Purpose adhesive, 3M/ESPE). Resin composite (Z100, 3M/ESPE) build-ups were constructed and 0.9 mm^2-specimens were prepared for μTBS. Specimens were either immediately pulled to failure or stored in distilled water for 6 months prior to testing. Data were evaluated by multiple ANOVA and Tukey's test ($\alpha=0.05$). In general, results showed that potassium oxalate treatment reduced significantly both the immediate and 6-month μTBS, regardless of the primer and dentin moisture condition ($p<0.05$). The only exception was HEMA/H_2O control group for which the μTBS did not differed significantly from that obtained for HEMA/H_2O oxalate-treated group, when this primer was applied to wet dentin and tested after 6 months of water storage ($p>0.05$). In general, the 6-month storage reduced significantly the μTBS of both experimental primers ($p<0.05$), with the exception of HEMA/H_2O control group that after 6 months of water storage exhibited mean μTBS similar to that observed at the baseline period (immediate test) ($p>0.05$).

Introduction

The durability of resin-dentin bonding is related to the capacity of resin monomers to infiltrate and optimally to copolymerize into inter and intrafibrillar nanospaces of dentin matrix created after removal of apatite minerals by acid-etching [1]. The etch-and-rinse bonding approach requires that the demineralized dentin matrix is, then, suspended in water so that resin monomers diluted in polar solvents can penetrate the expanded dentin matrix without or with minimum resistance. However, it has been demonstrated that resin monomers cannot fully embed the demineralized dentin matrix [2,3], resulting in porous resin-bonded interfaces [4]. If on the one hand the wet bonding technique ensures that the dentin matrix is in full expansion during monomers infiltration [5], on the other hand the presence of residual water before the photo-activation of adhesives/formation of hybrid layers is responsible for producing localized areas of incomplete monomer polymerization [6,7], generating porosities within bonded interfaces that, in turn, may permit inward diffusion of oral fluids [8-10].

Previous studies have pointed out a lack of consensus regarding the optimal moisture required for demineralized dentin to be perfectly sealed with dimethacrylates (i.e HEMA, TEGDMA, Bis-GMA) [11,12]. Under over-wet conditions the residual water entrapped within the collagen fibrils may undermine the diffusion and polymerization of the resin monomers [13], while under dry conditions the collapse of dentin matrix may also prevent proper resin infiltration into dentin [14-16]. Since the moist of dentin is a subjective criterion that varies among operators, under a clinical scenario the water-wet bonding technique has been shown to be critically sensitive.

Although the so-called “dry bonding technique” has been shown to cause a marked reduction in the immediate bond strength of the etch-and-rinse adhesive systems to dentin [14,17], some studies has demonstrated that high-quality adhesion can be achieved when employing bonding agents with solvents that are able to re-expand the shrunken dried dentin matrix [18-20]. The ability of polar solvents, such as the alcohols (i.e methanol, ethanol), to form hydrogen bonds with the peptides of the collagen fibrils it is thought to be important to guarantee their capacity to re-expand and maintain the demineralized dentin in an expanded state during resin infiltration [21,22]. This could likely produce resin-bonded interfaces that are more durable over time.

An attempt to reduce the negative consequences caused by the permeability of resin-bonded dentin interfaces is to take advantage of the use of oxalate desensitizers prior to adhesives’ application [23,24]. Due to its ability to occlude the dentinal tubules, oxalate-based desensitizers are considered as being potent agents in the treatment of dentin sensitivity [24,25]. The calcium oxalate crystals formed into the dentinal tubules were shown to reduce the fluid conductance of dentin [26,27] reducing the pain sensation [28]. As a secondary effect, the obstruction of dentinal tubules with oxalate crystals may help clinicians to have a better control on the moisture that is present on the surface of acid-etched dentin during bonding procedures. In theory, the presence of oxalate crystals reducing the free fluid conductance of dentin creates an environment wherein the infiltrated resin monomers may polymerize more accurately, with any or limited presence of water.

The purpose of this study was to determine whether the treatment of demineralized dentin with potassium oxalate may alter the bond strength of water- or ethanol-solvated HEMA-based primers to wet *versus* dry demineralized dentin. The null hypotheses tested were that there is no difference in the bond strength (μTBS) of water- and ethanol-solvated

HEMA-based primers when: 1) either applied to oxalate-treated dentin or 2) when applied to moist or air-dried acid-etched dentin.

Materials and Methods

Teeth Preparation

Forty freshly extracted noncarious human third molars were collected, stored in saline containing 0.1% thymol at 4°C and used within 6 months after extraction. This study protocol was approved by the Human Assurance Committee of the Piracicaba School of Dentistry, University of Campinas, Brazil. Crown-segments were prepared by removing the occlusal enamel and roots of these teeth, using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water-cooling. The crown segments were ground wet with #240-grit silicon carbide paper until a remaining thickness of 0.5 -1 mm as measured from the highest pulp horn, indicating that the bonding interfaces were located in deep dentin. Deep dentin was chosen due to its high wetness [5], representing one of the most challenging substrate for bonding procedures. Dentin surfaces were further polished with #600-grit silicon carbide paper under running water for 20 s to create a standard smear layer.

Primers Preparation

Two experimental HEMA/solvent solutions were prepared in order to simulate a primer similarly to that of three-step etch-and-rinse adhesive systems. HEMA was purchased from Sigma-Aldrich (St. Louis, MO, USA). The experimental primers consisted of (1) (v/v%) 35% HEMA/65% Water (HEMA/H_2O) and (2) 35% HEMA/65% Ethanol (Merck, Darmstadt, Germany) (HEMA/EtOH). These primers were prepared based on their reported ability to re-expand demineralized dry dentin matrix. Primers composition and Hoy's solubility parameters values are listed in Table 1. A fixed amount of each neat component - HEMA (7 μL)/solvents (13 μL) - was measured with a calibrated micropipette (Microman M 25, Gilson Medical Eletronics, S.A., Villiers le Bel, France) and mixed in a disposable vial. Freshly prepared HEMA/solvent mixtures (20 μL) were all ultra-sonicated for 5 minutes to ensure homogeneity.

Bonding Procedures

The exposed dentin surfaces were etched with 35% phosphoric acid (3M/ESPE, St. Paul, MN, USA) for 15 s and rinsed with water for 20 s. After etching, half of the teeth had their dentin surfaces blot-dried and treated with oxalic acid (Bisblock, Bisco Inc, Chicago, USA) that was applied under constant agitation for 30 s and left undisturbed for additional 30 s. The remained teeth were not treated with oxalate and consisted of the control group. The dentin surfaces from both groups were thoroughly rinsed with water for 20 s and they were either strongly dried (80 psi) with an oil/water-free air-stream for 10 seconds at a distance of 10 cm (dry bonding technique/DB) or gently blot-dried with an absorbent paper leaving the surface visibly moist (wet bonding technique/WB). The HEMA/solvent experimental primers were then applied to both untreated and oxalate-treated dentin surfaces under constant agitation for 30 s. In the event that matte dentin was observed, an additional coat of primer was applied to ensure that a shiny surface was obtained prior to light-activation of the adhesive. The excess of solvent (water or ethanol) was evaporated with a gentle air stream for 20 s at distance of 20 cm from the dentin surface. Then, a layer of a hydrophobic bonding agent (Adper Scotchbond Multi-Purpose adhesive, 3M/ESPE, St. Paul, MN, USA) was applied and photo-activated for 20 s with a halogen light curing unit (XL 3000, 3M/ESPE, St. Paul, MN, USA) with a power output of 600 mw/cm^2. Resin composite (Z100, 3M/ESPE, St. Paul, MN, USA) build-ups of 5 mm in height were incrementally constructed on hybridized dentin surfaces. The 1-mm thick increments of resin composite were individually light-activated for 40 s. The bonding procedures were performed by a single operator under climate-controlled conditions (23 ± 2 ^{o}C and 50 ± 5 % relative humidity) [29]. The specimens were randomly assigned to 8 groups of five teeth for each combination of experimental primers, oxalate treatment and dentin moisture condition.

Microtensile bond strength testing

After storage in distilled water at 37 ^{o}C for 24 h, each bonded tooth was longitudinally sectioned by means of diamond impregnated saw (Buehler Ltd., Lake Bluff, IL, USA) under water cooling according to the "non-trimming" technique for microtensile test [30]. The sticks-shaped specimens from each tooth were either tested immediately or after storage in distilled water at 37 ^{o}C for 6 months. Each stick was individually fixed to a custom-made testing jig (Geraldeli's device) [31] with a cyanoacrylate glue (Super Bonder Gel, Loctite Brazil Ltda, Itapevi, SP, Brazil) and pulled until failure in a universal testing machine (Instron Corporation, Canton, MA, USA) operating at a cross-head speed of 0.5 mm/min [32]. Immediately after testing, the fractured specimens were removed from the jig and the cross-sectional area at the site of fracture measured with a digital caliper (Starret 727, Starret Ltda, Itu, SP, Brazil) to allow for calculating the μTBS values in MPa.

Statistical Analysis

The experimental unit in this study was the tooth. A multiple ANOVA and post hoc Tukey tests were used to analyze the effects of the oxalate treatment, types of primers, dentin moisture condition and time of specimens test on the μTBS to dentin. Statistical significance level was preset at $\alpha = 0.05$.

Results

Results showed that mean µTBS was affected by the experimental primers ($p<0.001$), oxalate treatment ($p<0.001$) and period of storage ($p<0.001$). Interactions among all factors were significant ($p<0.001$). Multiple comparisons of µTBS data are shown in Table 2.

For HEMA/H_2O primer, the treatment of dentin with potassium oxalate resulted in µTBS that in comparison to their control group at the immediate period was significantly lower when was employed the wet bonding technique ($p<0.05$), but statistically similar when was employed the dry bonding technique ($p>0.05$). After 6-month water storage, no significant differences in µTBS were found between control and oxalate-treated groups when the wet bonding was used ($p>0.05$). In contrast, when dry bonding was employed, µTBS decreased significantly ($p<0.05$).

For HEMA/EtOH primer, the treatment of dentin with potassium oxalate resulted in reduction of µTBS regardless of the storage time and dentin moisture condition ($p<0.05$).

In general, the 6-month storage reduced significantly the µTBS of both experimental primers ($p<0.05$), with the exception of HEMA/H2O control group that after 6 months of water storage exhibited mean µTBS similar to that observed at the baseline period (immediate test) ($p>0.05$).

Discussion

The present results showed that for most of experimental conditions the oxalate-treated dentin exhibited bond strength values that in comparison to untreated dentin (controls) are significantly lower. These findings, therefore, lead us to reject the first anticipated hypothesis that there is no difference in the bond strength of water- and ethanol-solvated HEMA-based primers when applied to oxalate-treated dentin. It was also shown that for control specimens primed with HEMA/EtOH and for oxalated-treated specimens primed with HEMA/H_2O the baseline μTBS (immediate results) to wet *versus* dry dentin did not varied significantly. However, for the other experimental conditions the μTBS to dry dentin was in general significantly lower if compared with its respective wet dentin counterpart. These combined results, thus, requires only the partial acceptance of the second anticipated hypothesis that there is no difference in the bond strength of water- and ethanol-solvated HEMA-based primers when applied to air-dried acid-etched dentin.

The HEMA/EtOH primer provided immediate bond strength that under wet bonding conditions were significantly higher ($p<0.05$) than that provided by HEMA/H_20 primer both control and oxalate-treated specimens. Under dry bonding conditions, however, there was no significant difference ($p>0.05$) in the immediate bond strength provided by both primers, regardless of dentin had treated or not with potassium oxalate. Specimens bonded with both primers exhibited significant reduction (i.e. from 35 to 67%) of their bond strength after 6-month of storage in water. The only exception was the control group that was bonded with the HEMA/H_20 primer in dry conditions. Curiously, after 6 months of storage in water, the most dramatic reduction of mean bond strengths was also observed for the control group bonded with the HEMA/H_20 primer but now under wet conditions (i.e. 67% of reduction when compared to its baseline results), as recommended for the etch-and-rinse bonding approach. These results confirm the anticipated concern on the critical sensitivity of the wet bonding technique to provide durable resin-dentin bonds.

The protocol for dry bonding varies among different studies. Some authors chose to dry dentin for only 1 s [33], while others applied the air-stream for 5 s [34,35], 10 s [36], 15 s [33,34] or 30 s [12,19]. In the present study, the acid-etched dentin was strongly air-dried to render a challenging degree of dehydration. It is likely that when the acid-etched, oxalate-treated dentin was strongly dried with an air-stream for 10 s, much of the water located in the matrix was removed, which would have caused the matrix to collapse, approximating the collagen fibrils [13,15,37]. Because water is removed and collagen fibrils are brought close together, they form new interpeptide H-bonds that stabilize and stiffen the matrix in a shrunken state [18,37]. The decrease in width of the interfibrillar channels compromises the diffusion of resin monomers and may result in compromised bonds [38,39].

One way to reverse such an undesirable situation it would be to use a priming agent that contains water or other solvents that are capable to break the interpeptide H- bonds formed in dried dentin [19]. In theory, monomer/solvents mixtures that can re-expand the collapsed dentin matrix must have solubility parameters for hydrogen bonding (δ_h) greater than 16.9 $(J/cm^3)^{1/2}$, that is, a δ_h value for dried dentin [20]. If not, little infiltration of solvated resin monomers can occur within interfibrillar spaces compromising bond strengths. The ability of water-based primers to re-expand air-dried, shrunken demineralized dentin matrix based due to their relatively high solubility parameter for hydrogen bonding [40-42] may, in fact, explain why the HEMA/$H_2$0 (δ_h=32.1) primer provided relatively high and more durable bond strength to over-dried acid-etched dentin that was not treated with potassium oxalate. We believe that the primer application mode (i.e. 30 s under constant agitation) may explain this outcome. Previous studies have shown that rubbing motion during primer application was able to achieve high immediate and long-term µTBS at dry bonding conditions [6, 43]. It is likely that this approach enhanced water evaporation from dentin surface increasing the degree of conversion and polymer cross-linking, thereby improving the mechanical properties of the resin within the hybrid layer [6, 43-45].

There was an expectation that the adjunctive use of potassium oxalate and dry bonding could minimize the problems regard with standardizing surface moisture, thereby contributing to provide durability for resin-dentin bonds [12,26]. The present results, however, do not support this assumption, since the μTBS of oxalate-treated, air-dried dentin has shown to be significantly affected at the immediate testing period and, then, it was reduced after 6 months of water storage, no matter this substrate had been hybridized with water- or ethanol-based HEMA primers. It has been shown that oxalate treatment of acid-etched dentin reduces the hydraulic conductance through dentinal tubules [23,25]. Tubules occlusion, in turn, prevents dentin surface from being naturally re-wetted by the outward fluid movement. This might not to be a problem if a wet bonding technique was used. However, if dentin is air-dried and not re-wetted, the tubule occlusion by oxalate crystals might compromise the immediate bond strength of etch-and-rinse adhesive systems, thereby rendering them weak from the baseline on.

The bond strength exhibited for HEMA/EtOH primer was significantly affected when applied to oxalate treated dentin both at immediate testing period and after 6 months of storage in water. Ethanol concentration in HEMA/EtOH primers was consistent (i.e. 65% in volume) and it may explain in part such results. It has been shown that the solubility of calcium oxalate, a by-product of the reaction between potassium oxalate and dentinal $Ca2^{+}$, is significantly increased in ethanol solutions [46]. In this case, calcium (Ca^{2+}) oxalate crystals that occlude the dentinal tubules could have been dissolved and adversely affected the bond strength to dentin [47]. Yiu *et al* [48], reported a 15 %-decrease in μTBS after oxalate desensitizer application followed by bonding with a two-step ethanol-based etch-and-rinse adhesive. Similarly, but more dramatic decreases in μTBS were observed in the present study. Since μTBS decreased by 64 % and 44 % respectively for dry and wet bonding techniques, differences in materials and methodology should be considered when comparing these results. It was expected that due to its solubility parameter value (δ_h =18.9 $(J/cm^3)^{1/2}$) HEMA/EtOH primer was able to re-expand the dried acid-etched dentin matrix irrespectively of dentin has been treated or not with oxalate. While ethanol is capable of re-expanding collapsed collagen matrix, the dentin re-expansion occurs in a very slow rate [42,49] and probably not sufficient within the priming time allowed in this study. It is likely that the collagen matrix remained collapsed after HEMA/EtOH application, which resulted in low μTBS bond strengths for the oxalate-treated group. On the other hand, the immediate μTBS of HEMA/EtOH was not compromised when applied to dry dentin in the control group. In this case, it can be speculated that, in the absence of dentinal tubule occlusion by calcium oxalate crystals, the water flow from subsurface dentin was capable to re-expand the collagen network, preventing a severe collapse of the demineralized dentin matrix and permitting HEMA/EtOH infiltration within the collagen fibrils [33], which resulted in the highest immediate μTBS values of all tested groups.

The fact that this study was performed *in vitro* should be underscored because the possibility of water transudation from dentin is rather limited when compared to the clinical condition. Since the occluding ability of potassium oxalate solutions may be markedly reduced with increasing diameter of the dentinal tubules and by demineralization of dentin surface [50], it is possible that these results would be different if applied to acid-etched deep dentin in vital teeth. Nevertheless, it has been reported that the benefits derived from the use of oxalate may not be long-lasting since the calcium oxalate crystals can be affected by the acidic nature of simplified adhesives [48], presence of ethanol as a solvent [46] and also undergo slow dissolution over time [26,51]. Spaces occupied by dissolved crystals may act as pathways for water permeation, which increase the probability of hydrolytic degradation on exposed collagen fibrils and the bonding agent [52].

In a recent study Vachiramon et al [53] showed that the application of the acidic oxalate after 35% phosphoric acid etching may produce an additional etching of dentin. The authors suggested that hydrolytic degradation of resin-dentin bond may be further enhanced if resin infiltration is compromised by any additional etching or by the presence of residual oxalic acid after rinsing. Moreover, data from that study also showed that oxalate-treated resin-bonded dentin undergo significant degradation over a period of 3 months.

Conclusions

Under the limited conditions of this study, it is concluded that the use of potassium oxalate affected both the immediate and 6-month μTBS of experimental HEMA/based primers solvated in water or ethanol-based and applied to moist or dry acid-etched dentin. Thus, clinicians who regularly apply this technique should be aware that clinical usage of oxalate to reduce dentin fluid conductance and, consequently, to enlarge the durability of resin-dentin bonds remains unclear and needs to be further investigated.

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Table 1. Chemical composition and Hoy's solubility parameters of the experimental primers used in this study

Primers	Composition			Hoy's solubility parameters $(J/cm^3)^{1/2}$			
	HEMA	Ethanol	Water	δ_d	δ_p	δ_h	δ_t
HEMA/H_2O	35%	_	65%	12.6	19.2	32.1	39.9
HEMA/EtOH	35%	65%	_	12.9	11.7	18.9	25.7

Percentages are all (v) percentage. Hoy's solubility parameters: $\delta_{d,}$ dispersives forces; $\delta_{p,}$ polar forces; $\delta_{h,}$ hydrogen bonding forces; $\delta_{t,}$ total cohesive forces; HEMA: 2-hydroxyethyl methacrylate.

Table 2. Bond strength values (MPa) for the different primers to dentin according oxalate treatment, bonding technique and storage periods.

		Control (No oxalate)		**After Oxalate treatment**	
		HEMA/H_2O	*HEMA/EtOH*	*HEMA/H2O*	*HEMA/EtOH*
Wet bonding	**24 hours**	31.0 ± 4.9 A b	48.9 ± 9.7 A a	13.7 ± 3.3 A c	31.4 ± 1.9 A b
	6 months	10.2 ± 2.1 B c	31.4 ± 5.6 B a	8.7 ± 1.6 B c	19.8 ± 5.4 B b
Dry bonding	**24 hours**	35.2 ± 5.5 A ab	43.2 ± 5.0 A a	24.2 ± 9.0 A bc	19.0 ± 6.8 B c
	6 months	37.8 ± 5.4 A a	23.1 ± 8.4 B b	9.7 ± 1.7 B c	9.3 ± 1.4 C c

Values are Mean ± SD (n=5 teeth/experimental condition).

Different upper-case letters indicate significant differences between values in each column ($p<0.05$).

Different lower- cases letters indicate significant differences between values in each row ($p<0.05$).

CAPÍTULO 2

Durability of bonded-dentin treated with hydrophilic versus hydrophobic primers*

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Abstract

Objectives**:** This study evaluated the immediate and long-term resin-dentin bond strength of different hydrophilicity primers bonded to acid-etched dentin saturated with water or ethanol.

Methods: Flat dentin surfaces from extracted human third molars were obtained on deep-coronal dentin. The surfaces were acid-etched, left moist with water (water wet-bonding) or saturated with ethanol (ethanol wet-bonding). Two consecutives coats of each experimental primer 35/65% (v/v) HEMA/EtOH or Bis-GMA/EtOH, were then applied to both water- and ethanol-saturated dentin surfaces for 30 sec and left undisturbed for additional 30 sec. Excess solvent was evaporated for 20 sec and a layer of bonding agent was then applied. Resin composite buildups were constructed incrementally. After storage in water for 24 h at 37^{o}C, the specimens were prepared for microtensile bond strength (μTBS) testing. Data were analyzed statistically by three-way ANOVA and Holm- Sidak method for multiple comparisons (α=0.05%).

Results: The highest immediate and long-term μTBS values were observed when both primers, HEMA/EtOH and Bis-GMA/EtOH, were applied to ethanol-wet dentin. Theses values were significantly higher than those observed to water-wet dentin ($p<0.05$). HEMA/EtOH exhibited higher immediate μTBS than Bis-GMA/EtOH when applied to water-wet dentin ($p<0.05$). Significant reduction in μTBS using HEMA/EtOH was observed after 6 months of water storage.

Conclusion: The ethanol wet-bonding promoted more reliable immediate and long-term bond strengths for the Bis-GMA/EtOH. Water storage resulted in reduced bond strength for HEMA/EtOH, regardless of the bonding technique employed.

Introduction

The durability of adhesive-resin bonding is currently an area of great interest in adhesive dentistry. Several studies have been conducted to evaluate the integrity of resin-dentin bond interfaces over time.[1,2,3,4] Reduced bond strength values after only a few months of storage have been demonstrated, indicating that the bonded interface degrades over time.[5,6]

In etch-and- rinse bonding, the mineral is removed from around the collagen fibrils of the dentin surface by acid etching[7], followed by application of adhesive resin monomers that infiltrate to the depth of the demineralization. However, several studies have shown that this infiltration is incomplete.[8,9,10] The reasons for incomplete infiltration are numerous, but the main concern relies on the fact that hydrophilic resins are applied to water-filled collagen fibrils, creating a porous hybrid layer[8,10] that is highly susceptible to water sorption and hydrolytic degradation.[1,2,11,12,13]

The poor durability of resin-dentin bonds is due, in part, to the inclusion of hydrophilic monomers, such as 2-hydroxy-ethil methacrylate (HEMA) into blends that are necessary to prevent phase changes when hydrophobic resins are applied to demineralized dentin matrices that are saturated with water.[8,14] It is know that incorporation of hydrophilic monomers into resins increases their water sorption and solubility[15,16,17,18] as well decreases their mechanical properties and durability.[5,15,16,19,20] Presumably, if dentin is bonded with more hydrophobic resins, they would absorb less water, plasticize less[15,17] and produce more durable bonds. The challenge is therefore how to coax hydrophobic monomers into a demineralized dentin matrix without inducing phase changes.[8]

Strategically, an alternative approach to make acid-etched dentin less hydrophilic, in such a way that it would be compatible with hydrophobic resin monomers has been proposed. This may be achieved by replacement the water in intrafibrillar spaces with ethanol.[14,21,22] Known as "ethanol-wet bonding technique" this new protocol prevents phase separation of the hydrophobic monomers as they are applied to ethanol-saturated dentin instead water-saturated dentin.[13,21,22,23] A recent study proofed that it is possible to coax an neat hydrophobic resin (2,2-bis[4(2-hydroxy-3-methacryloyloxy-propyloxy)-phenyl] propane (Bis-GMA) solvated in ethanol and used as a primer component of an experimental hydrophobic adhesive system into acid-etched dentin *via* the ethanol-wet bonding approach.[23]

Although dentin bonding with hydrophobic resins using the ethanol-wet bonding technique have shown promising initial results[21,22,23,24] there is no information on the effect of this protocol on long-term durability of resin-dentin bonds. The purpose of this study was to compare resin-dentin bond strength durability of different hydrophilic versus hydrophobic primers applied to acid-etched dentin saturated with water or ethanol. The tested null hypotheses were that: 1) primers hidrophilicity has no effect on μTBS, and 2) bonds made to dentin saturated with water are not different from those made to dentin saturated with ethanol over time.

Materials and Methods

Teeth selection and preparation

Twenty extracted noncarious human third molars stored in saline solution containing 0.1% thymol at 4 ^{0}C to prevent bacteria growth were used in this study within 6 months after extraction. The teeth were collected after patients' informed consent had been obtained under a protocol reviewed and approved by the Institutional Review Board of the Piracicaba School of Dentistry.

Flat surfaces were then created on deep-coronal dentin by sectioning the teeth 2 mm above the cementoenamel junction perpendicular to their longitudinal axes using a water-cooled slow-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA). A second parallel cut was made 3 mm below the cementoenamel junction to obtain crown segments. The coronal pulpal tissue was removed and the crown segments were ground wet with #240-grit silicon carbide paper until a remaining thickness of 0.5 -1 mm as measured from the highest pulp horn, indicating that the bonding interfaces were located in deep dentin. Dentin surfaces were further polished with #600-grit silicon carbide paper under running water for 20 sec to create a standardized and clinically relevant smear layer.

Experimental primers

Since manufacturers do not disclose the exact composition of their products, the Hoy's solubility parameters of commercially available dentin adhesives cannot be calculated.[13] Thus, two neat monomers were used in this study to prepare the experimental primers, 2-2-hydroxyethyl methacrylate (HEMA) and 2,2-bis[4(2-hydroxy-3-methacryloyloxy-propyloxy)-phenyl] propane (Bis-GMA), as representative hydrophilic and hydrophobic monomers, respectively. All monomers were American Chemical Society grade and were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). From these neat monomers, two experimental primers were formulated by combining 35% monomers solvated in 65% ethanol (Merck, Darmstadt, Germany) as follows: 35% HEMA/ 65% Ethanol and 35% Bis-GMA/ 65% Ethanol (in v/v%). These monomers/solvents blends, with known composition and concentration, were formulated in such way that they could be ranked in an increasing order of hydrophilicity, based on their Hoy's solubility parameters (δ). The Hoy's solubility parameters for both experimental primers and the water- and- ethanol saturated demineralized dentin[13,21,24] were calculated by summing the group molar attraction constants of their chemical structures according to the method of Hoy.[25] These values are presented in Table 1.

A fixed amount of each neat component, HEMA/Bis-GMA (7 µL) and ethanol (13 µL), was measured with a calibrated micropipette (Microman M 25, Gilson Medical Eletronics, S.A., Villiers le Bel, France) and mixed in a disposable vial. The freshly prepared monomer/solvent mixtures (20 µL) were ultra-sonicated (Maxiclean-Unique, Indaiatuba, SP, Brazil) for 5 min for ensure homogeneity. The experimental primers were used immediately after preparation and were not stored after each use.

Bonding Procedures

The dentin surfaces were acid-etched with 35% phosphoric acid gel (Scotchbond Multi Purpose, 3M/ESPE, St. Paul, MN, USA) for 15 sec and then were rinsed thoroughly for 20 sec with water. The acid-etched surfaces were randomly assigned to two groups of 10 specimens each, which were saturated with either water or ethanol. Five specimens in each group were bonded with one of the two experimental primers. There were two primers subgroups within the two surface moisture groups.

In water wet- bonding group, acid-etched dentin was blot-dried to leave a water moist surface immediately before bonding. In the ethanol wet- bonding group, acid-etched dentin surfaces were treated with a series of increasing ethanol concentrations (50%, 70% and 100%, three times for 30s each) based on the protocol described by Sadek *et al.* [22, 23] The ethanol/water mixtures or absolute ethanol was applied to dentin using a microbrush to cover the entire dentin surface, replacing 100% water or preceding ethanol solution that saturated the demineralized dentin matrix. Special care was taken to ensure that the dentin surface was always immersed in a liquid phase by keeping it visibly moist prior to the application of the subsequent solution with a higher ethanol concentration.[22] Two consecutives coats of each experimental primer were then applied to both water and ethanol saturated dentin surfaces using a microbrush under constant agitation for 30 sec and left undisturbed for additional 30 sec. Excess solvent was evaporated with a gentle air stream for 20 sec at a distance of 20 cm from dentin surface. Then, a layer of bonding agent (Adper Scotchbond Multi-Purpose adhesive, 3M/ESPE, St. Paul, MN, USA) was applied, thinned with a gently moisture-free air stream and light cured using a halogen light curing unit (XL 3000, 3M/ESPE, St. Paul, MN, USA) with a power output of 500 mw/cm^2 for 20 seconds. Resin composite build-ups of 5 mm in height were constructed on the bonded surface with 1-mm tchick increments of Z 100 (3M/ESPE, St. Paul, MN, USA) which were individually light-activated for 40 sec using the same light-curing unit and light intensity. The restored teeth were stored in deionized water at 37 ^{o}C for 24h.

Microtensile bond strength testing

In a precision cutting machine (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) with a water-cooled diamond saw, serial sections were done in a mesiodistal direction longitudinally to the tooth crown and perpendicular to the adhesive interface, maintaining a distance of 0.9 mm between sections, according to the "non-trimming" technique reported by Pashley *et al*.[26] The teeth were rotated 90 degrees and a new series of 0.9-mm-wide sections were done, obtaining stick-shaped specimens with a cross-sectional area of approximately 0.9 mm^2. The specimens from each tooth were either tested immediately or after storage in deionized water at 37^oC for 6 months. Each stick was individually fixed to a custom-made testing jig (Geraldeli's device)[27] with cyanoacrylate glue (Super Bonder Gel, Loctite Brazil Ltda, Itapevi, SP, Brazil) and subjected to microtensile strength in a universal testing machine (Instron 4411, Instron Corporation, Canton, MA, USA) running at a crosshead speed of 0.5 mm/min until failure. Immediately after testing, the debonded halves of each microtensile stick were removed from the jig and the load at failure (kgf) was divided by the cross-sectional area of the failed interface (cm^2) measured with a digital caliper (Starret 727, Starret Ltda, Itu, SP, Brazil) accurate to the nearest 0.01 mm. The result of μTBS was expressed in MPa.

Statistic Analysis

Since half of sticks obtained for each tooth was tested immediately and the other half after 6 months, the experimental unit in this study was the hemi-tooth. The mean μTBS of all sticks from the same hemi-tooth was averaged for statistical purposes. The μTBS mean for every testing group was expressed as the average of the five hemi-teeth used *per* group and expressed in MPa. The μTBS data was subjected to three-way ANOVA (experimental primers vs. bonding technique vs. storage time), followed by Holm- Sidak method for multiple comparisons. Statistical significance was set in advance at α=0.05%.

Results

The μTBS strength values (in MPa) for all tested groups are summarized in Table 2. The highest immediate and long-term (i.e. after 6 months of water storage) bond strength values were observed when both primers, HEMA/EtOH and Bis-GMA/EtOH, were applied to ethanol-wet acid-etched dentin. Theses values did not differ significantly from each other ($p>0.05$), but they were significantly higher than those obtained when both primers were applied to water-wet acid-etched dentin ($p<0.05$). When applied to water-wet acid-etched dentin, the HEMA/EtOH primer exhibited higher immediate μTBS than Bis-GMA/EtOH ($p<0.05$). Conversely, after 6 months of water storage, the μTBS values obtained with Bis-GMA/EtOH were higher than those recorded with HEMA/EtOH ($p<0.05$). Regardless of the bonding technique, significant decrease in μTBS was observed after 6 months of water storage ($p<0.05$) when HEMA/EtOH was used. In contrast, no statistically significant difference ($p>0.05$) in μTBS was observed for Bis-GMA/EtOH over 6 months for both water and ethanol-wet bonding techniques.

Discussion

The results of the present study confirm previous findings that resin dentin bond strengths are related to the solubility parameters of dentin adhesives.[28,29,30] In adhesive systems where etching precedes priming and bonding steps, the Hoy's solubility parameter compatibility of the primer formulation with that of demineralized dentin matrix may determine adhesive infiltration.[31] In expanded acid-etching dentin, regardless whether it was expanded with water or ethanol, the Hoy’s solubility parameter that is important is δt (i.e the parameter for the total cohesive energy) because of its relationship with miscibility.[13] The differences of δt values is thus important to characterize the ability of a primer monomer system to infiltrate into the demineralized dentin matrix.[31] It predicts that if there is less than 5 $(J/cm^3)^{1/2}$ between the δt of a primer/adhesive solution and demineralized dentin matrix, that the primer/adhesive solution will wet the demineralized dentin and cause it to swell enough to permit infiltration of the primer /adhesive solution.[13]

In the present study, ethanol-wet bonding clearly increased the immediate and long-term microtensile bond strength of HEMA and BisGMA-based primers tested. These results support the partial acceptation of the first null hypothesis, because primer hydrofilicity had a significant effect on µTBS under water-wet bonding. Since bonds made to ethanol-saturated dentin were significantly higher than those made to water-saturated dentin both immediately and after 6 months, the second null hypotheses must be rejected. Data from this study showed significantly higher immediate µTBS values for HEMA/EtOH compared to BisGMA/EtOH when water-wet bonding technique was employed. The hydrophilicity of HEMA, which makes this monomer compatible with water-saturated dentin[32], may explain these results. The Hoy's δ_t values for HEMA/EtOH and water-saturated dentin matrix are listed in Table 1 as 25.7 and 30.1 $(J/cm^3)^{1/2}$, respectively. The difference between their δt values of 4.4 suggest that they were compatible and miscible. By enhancing dentin wetting, the HEMA concentration (i.e. 35% solvated in ethanol) in HEMA/EtOH significantly improved bond strengths in water-saturated dentin.[33,34,35,36] This phenomenon was supposedly responsible for the immediate success of the HEMA/EtOH (δ_t= 25.7 $(J/cm^3)^{1/2}$) with the water-wet bonding technique in this study. However, µTBS of HEMA/EtOH was significantly lower than that of Bis-GMA/EtOH after 6 months of water storage. Presumably, it was partially due to HEMA molecule susceptibility to water sorption, which adversely influences its mechanical strength. The resulting low-quality flexible polymers and can have increased water sorption and solubility[15,37] and decreased mechanical properties[15,16] which undermine the long-term resin-dentin bond durability.[4,38]

Conversely, the Hoy's δt values for Bis-GMA/EtOH versus water-wet saturated collagen are 22.6 and 30.1 $(J/cm^3)^{1/2}$, respectively (Table 1). The 7.5 $(J/cm^3)^{1/2}$ difference in δt values indicated that the two were not miscible. Thus it is assumed that the Bis-GMA/EtOH applied to water-saturated dentin matrix underwent phase changes[10] because dimethacrylates like Bis-GMA are not water-soluble, thereby compromising monomer infiltration.[10,14] This shortcoming produced the lowest immediate μTBS value in this study. In spite of that, the μTBS value remained stable over 6 months. Bis-GMA is one of most frequently used cross-linker in adhesive system to provide mechanical strength by forming densely cross-linked polymers.[39] It is usually characterized by a hydrophobic behavior, which makes it only limitedly solvable in water[17,19] and could also prevent substantial water uptake after curing, resulting in polymers with better mechanical properties and improving the durability of resin-dentin bonds.[40]

The μTBS values obtained with the experimental HEMA/EtOH and HEMA/Bis-GMA primers applied to ethanol-saturated dentin were significantly higher than those obtained on the water-saturated dentin. These results could be related to controlled ambient changes in the demineralized dentin matrix when ethanol wet bonding was employed.[30] In essence, the ethanol-wet bonding consists in a modification of wet bonding using alcohol-saturated matrices instead of water-saturated matrices.[14] The hydrophilicity of the bonding substrate decreases when water is replaced by ethanol in the demineralized dentin. In contrast, the hidrophilicity of Bis-GMA increases when it is solvated in ethanol,[24] so that the dentin primer falls within the miscibility range of the ethanol saturated collagen mesh. Another example of How Hoy's solubility parameters theory could be used to explain the results obtained in the current study is that the differences between δt values for HEMA/EtOH (δt=25.7 $(J/cm^3)^{1/2}$) and Bis-GMA/EtOH (δt=22.6 $(J/cm^3)^{1/2}$) and demineralized dentin saturated with ethanol (δt=25.1 $(J/cm^3)^{1/2}$) are 0.6 and 2.5 $(J/cm^3)^{1/2}$, respectively. These small differences in δt demonstrate that they are completely miscible. The results of the present study clearly demonstrate that the bond strength for hydrophilic and hydrophobic primers can be increased by the use of ethanol instead of water in bonding procedures. An unexpected observation was that it is also possible to infiltrate hydrophilic primer into demineralized dentin saturated with ethanol. Additionally, wet bonding using ethanol rendered more stable bonds over 6 months of water storage for Bis-GMA/EtOH.

In view of theses results the ethanol-wet bonding appears as an alternative approach for coaxing hydrophobic monomers into demineralized matrices and enhance monomer uptake than compared to water wet bonding.[14,22,23] The uptake of monomers with hydrophobic characteristics into dentin should decrease water sorption and solubility and increase the durability of resin-dentin bonds.[15,17,18,19] Ethanol-saturated dentin may also enable decreasing in the amount of HEMA used in adhesives and increasing the amount of dimetacrylates, thus creating less hydrophilic hybrid layers that are expected to absorb less water.[14,22,23] If ethanol is used to saturate dentin matrices instead of water, dimetathacrylates like Bis-GMA may be able to diffuse through ethanol-filled interfibrillar spaces to reach the base of hybrid layer and create more uniform mechanical properties.[14] Most dental monomers, including Bis-GMA, are soluble in ethanol. With ethanol filling the interfibrillar spaces, the dental matrix becomes much more hydrophobic.[22,23] However, as ethanol replaces water, some interpeptide hydrogen bonding (H-bonding) develops, within collagen, that stiffens the matrix enough to minimize its shrinkage.[14,36,41,42] While this technique does not eliminate the shrinkage of demineralized collagen matrices completely, the collapse of collagen interfibrillar spaces, on the other hand, is not sufficient to prevent resin infiltration.[14,22]

Using hydrophilic and hydrophobic experimental primers associated with a relatively hydrophobic bonding agent, we found that the resin-dentin bonds created with an ethanol-wet bonding protocol were significantly higher than those obtained with water-wet bonding. In addition, no significant decrease in the μTBS of hydrophobic experimental primer was observed over a 6-month period when the ethanol-wet technique was used. These results provide strong evidence that the durability of the resin-dentin interface can be significantly increased by using relatively hydrophobic primers infiltrated into acid-etched ethanol-saturated dentin. Additionally, recent researches has reported that the application of hydrophobic resins may provide better sealing of acid-etched dentin if the substrate is saturated with ethanol instead of water.[43,44,45] Sauro et al.[45] reported that ethanol wet bonding seals dentin significantly better than water-wet dentin regardless of the adhesive in etch-and rinse systems. They founded significantly lower tracer uptake into ethanol-saturated hybrid layers analyzed by two- photon laser confocal microscopy images. The authors also underscored that the ethanol wet- bonding technique is not yet ready for clinical practice but it is evolving toward a practical technique by reducing of number of steps from original protocol. However, further research on the performance and technique sensitivity of the ethanol-wet bonding technique associated with hydrophobic adhesives are needed before this bonding protocol can be recommended for clinical use.[43]

Conclusions

The following conclusions can be drawn from the obtained results:

1) The ethanol wet-bonding promoted higher immediate and long-term bond strength for the Bis-GMA/EtOH.

2) Water storage resulted in lower bond strength for HEMA/EtOH, regardless of the employed bonding technique.

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Table 1. Composition and Hoy's solubility parameters for HEMA/Ethanol and BisGMA/Ethanol experimental model primers [$(J/cm^3)^{1/2}$] and the water- and- ethanol saturated demineralized dentin matrices (collagen).

Composition	Hoy's solubility parameters			
	δ_d	δ_p	δ_h	δ_t
HEMA 35%/ethanol65%	12.9	11.7	18.9	25.7
BisGMA 35%/ethanol65%	15.1	12.6	11.3	22.6
Water 30%/peptides70%	11.8	15.3	22.5	30.1
Ethanol 30%/ peptides70%	12.0	12.5	18.1	25.1

Percentages are all (v) percentage. Hoy's solubility parameters: $\delta_{d,}$ dispersives forces; $\delta_{p,}$ polar forces; $\delta_{h,}$ hydrogen bonding forces; $\delta_{t,}$ total cohesive forces. Bis-GMA: bisphenol A diglycidyl ether methacrylate; HEMA: 2-hydroxy-ethyl methacrylate.

Table 2. Microtensile Bond strengths (MPa) of experimental primers as a function of bonding technique and storage periods.

Experimental primers	**Water-wet Bonding**		**Ethanol-wet Bonding**	
	24 hours	***6 months***	***24 hours***	***6 months***
HEMA/EtOH	48.9 ± 9.7 A b	29.6 ± 6.3 B c	58.6 ± 6.8 A a	46.4 ± 7.1 A b
Bis-GMA/EtOH	41.6 ± 6.0 B b	37.0 ± 4.4 A b	54.1 ± 7.4 A a	50.9 ± 5.8 A a

Values are Mean ± SD (n=5).

Different upper-case letters indicate significant differences between values in each column ($p<0.05$).

Different lower- cases letters indicate significant differences between values in each row ($p<0.05$).

CONSIDERAÇÕES GERAIS

Durante mais de 15 anos, a técnica úmida tem sido recomendada para a união de materiais restauradores resinosos à dentina. A eficácia desta técnica deve-se a total expansão da matriz de dentina desmineralizada pela água, o que permite manter os espaços interfibrilares necessários para a infiltração dos monômeros resinosos presentes nos sistemas adesivos durante a formação da camada híbrida. Em razão disso os sistemas adesivos contemporâneos se tornaram cada vez mais hidrofílicos a fim de serem compatíveis com a umidade natural do substrato dentinário. O contraditório nesse caso é que, ao mesmo tempo em que o caráter hidrofílico dos monômeros presentes nos sistemas adesivos é desejável para infiltração em um substrato propositadamente saturado com água, ele também estabelece condições para a degradação do componente polimérico da camada híbrida. A despeito dos altos valores imediatos de RU alcançados com a técnica úmida, a camada hibrida formada em tais condições apresenta-se porosa e altamente suscetível aos efeitos deletérios da água. Assim, a resolução do problema da infiltração dos monômeros em substrato úmido gerou outro, o da degradação hidrolítica da união ao longo do tempo. As inquietações ainda presentes na literatura acerca desse problema motivaram a realização deste estudo.

No capítulo 1, a RU imediata e após 6 meses de armazenamento em água de *primers* experimentais a base de água e etanol foi avaliada em função do tratamento da dentina desmineralizada com oxalato de potássio e da sua umidade (seca com ar ou úmida com água). Os resultados obtidos demonstraram que o tratamento com oxalato de potássio diminuiu a RU imediata e após 6 meses para a maioria das condições estudadas. Portanto, a tentativa de melhorar a durabilidade da união resina-dentina através do bloqueio da umidade intrínseca do substrato dentinário e a utilização de *primers* com diferentes características de hidrofilia seguido de um adesivo com característica hidrofóbica em substrato úmido ou seco foi ineficiente. Estes resultados contrariam dados prévios da literatura que apontaram não haver diminuição da RU imediata quando se utilizou sistemas adesivos comerciais também a base de água e etanol com a técnica úmida. Por outro lado, corrobora com estudo prévio que avaliou a RU da associação de duas diferentes formulações de oxalato de potássio com um sistema adesivo a base de água e etanol. Apesar das diferenças na metodologia e materiais utilizados entre os trabalhos existentes na literatura e o presente estudo dificultarem a comparação, esses resultados controversos sugerem que mais pesquisas necessitam ser realizadas para melhorar a eficácia da união resina-dentina imediata e em longo prazo, assim como a reprodutibilidade dessa técnica, antes que ela possa ser recomendada para uso clínico, uma vez que implica no aumento do número de passos operatórios e pode não resultar no benefício esperado.

Em conseqüência dos resultados obtidos no estudo 1 em relação ao uso do oxalato de potássio para uma melhoria na obtenção da união resina-dentina e estabilidade ao longo do tempo, optou-se por suprimir essa variável no delineamento experimental do estudo 2. Os resultados encontrados no estudo 2 demonstraram claramente que a técnica úmida com etanol aumentou a RU imediata para os *primers* hidrofílico e hidrofóbico testados. Entretanto, quando analisados após 6 meses de armazenamento em água, somente o *primer* hidrofóbico manteve os valores de RU iniciais, evidenciando melhor desempenho ao longo do tempo, independente da técnica empregada (úmida com água ou etanol). Esses resultados demonstram que a estabilidade dos monômeros presentes no *primer* é fator primordial na durabilidade da união. Assim, de um ponto de vista clínico, pode-se especular que quando se utiliza sistemas adesivos que empregam *primers* com características hidrofílicas, quer sejam convencionais de 3 passos ou auto-condicionantes de 2 passos, o *primer* estará sempre mais vulnerável à degradação, em que pese a maior estabilidade do adesivo aplicado posteriormente, que geralmente apresenta característica mais hidrofóbica.

Os resultados obtidos no estudo 2 reforçam o consenso geral existente na literatura de que a formação da camada híbrida com monômeros como o Bis-GMA resulta em melhores propriedades mecânicas da mesma, devido à formação de um polímero com um maior número de ligações cruzadas. Além disso, o seu comportamento hidrofóbico limita a sorção de água e solubilidade, contribuindo para a melhoria da estabilidade da união resina-dentina ao longo do tempo. Portanto, os fabricantes de sistemas adesivos deveriam considerar a formulação de produtos que apresentassem características mais hidrofóbicas para serem utilizados conjuntamente com a técnica úmida utilizando etanol, ao contrário da maioria dos atualmente disponíveis, que apresentam características acentuadamente hidrofílicas.

Em vista dos resultados do estudo 2 e sem que pareça uma atitude precipitada, parece lícito afirmar que o uso de adesivos com características hidrofóbicas associados com a técnica úmida utilizando etanol apresenta potencial para uso clínico. Nesse sentido, esforços devem ser envidados para reduzir o número de passos durante a substituição da água da dentina desmineralizada pelo etanol, viabilizando tecnicamente a sua utilização clínica. Adicionalmente, pesquisas *in vitro* que avaliem a biocompatibilidade do etanol em dentina profunda devem ser realizadas antes da implementação clínica deste novo protocolo de união à dentina, assim como estudos *in vivo* poderiam também avaliar a durabilidade da união resina-dentina com a técnica úmida utilizando etanol em comparação com a técnica úmida convencional.

Os experimentos apresentados nos capítulos 1 e 2 deste estudo desafiaram alguns dos paradigmas atualmente vigentes relacionados à umidade do substrato dentinário na busca de uma união estável dos materiais restauradores à dentina. Os resultados obtidos corroboram achados prévios da literatura por um lado e contrastam por outro, evidenciando a necessidade de se realizar pesquisas adicionais *in vivo* e *in vitro* para a completa elucidação do problema. Entretanto, o conhecimento originado a partir desses experimentos pode contribuir para o desenvolvimento de novos sistemas adesivos e do aprimoramento da técnica de utilização, visando à obtenção de restaurações adesivas mais duradouras.

CONCLUSÕES

De acordo com a análise dos resultados obtidos a partir dos estudos realizados, as seguintes conclusões podem ser feitas:

1. De uma maneira geral a associação de oxalato de potássio com primers experimentais a base de água ou etanol resultou em RU significativamente menor, independente da composição do *primer* (água ou etanol) e da condição de umidade da dentina (técnica seca ou úmida) utilizada.

2. A técnica seca de união à dentina não afetou negativamente a RU imediata para o *primer* a base de água (HEMA/H_2O) e resultou em melhor desempenho após 6 meses de armazenamento em água quando o oxalato de potássio não foi utilizado.

3. A técnica úmida utilizando etanol proporcionou maior RU imediata e em longo prazo para o *primer* com característica hidrofóbica (Bis-GMA/EtOH).

4. O armazenamento em água resultou em menor RU para o *primer* hidrofílico (HEMA/EtOH), independente da condição de umidade da dentina (técnica úmida convencional ou com etanol).

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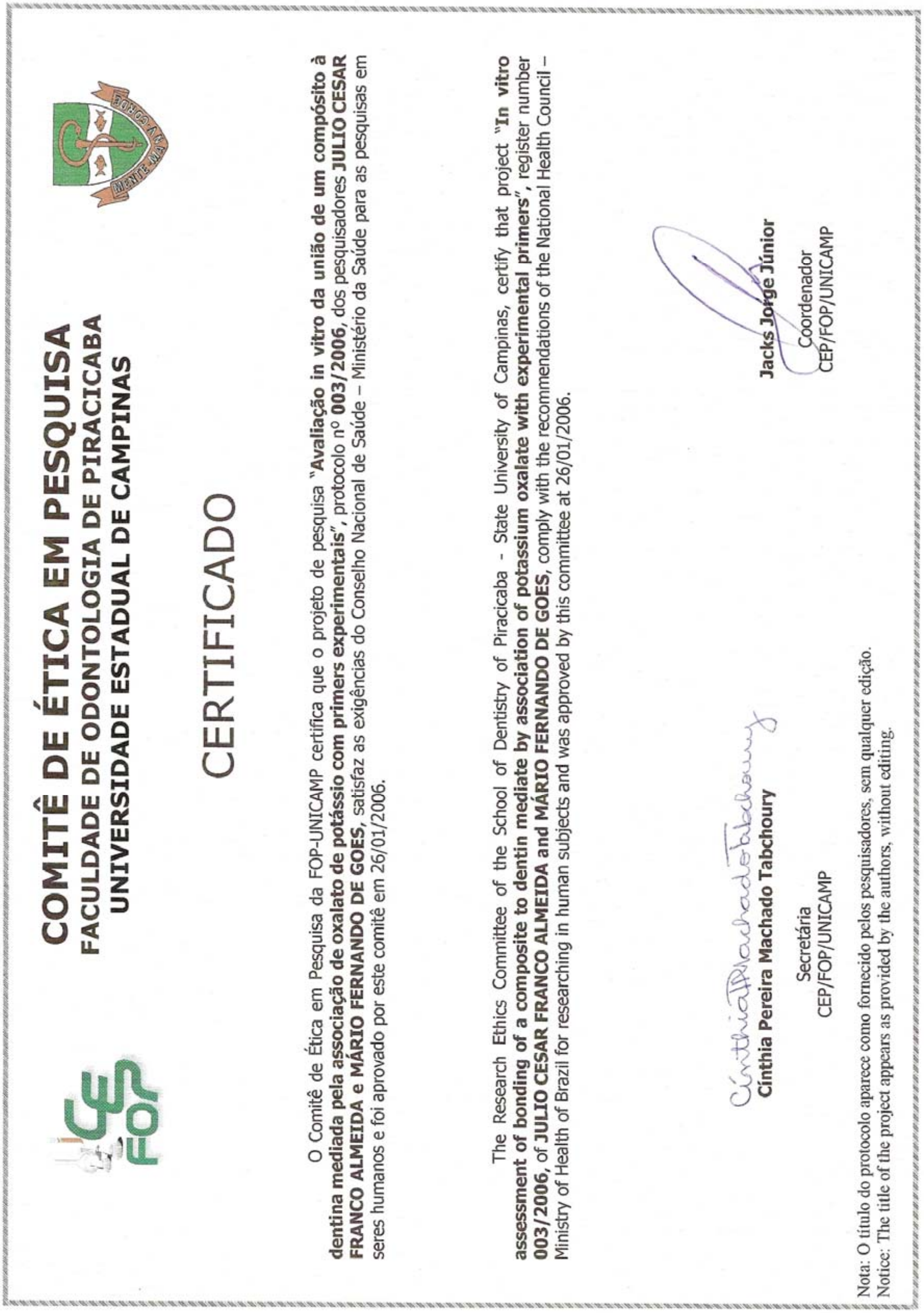
ANEXO 1- Certificado do comitê de ética



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 in vitro da união de um compósito à dentina mediada pela associação de oxalato de potássio com primers experimentais**", protocolo nº **003/2006**, dos pesquisadores **JULIO CESAR FRANCO ALMEIDA e MÁRIO FERNANDO DE GOES**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 26/01/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**In vitro assessment of bonding of a composite to dentin mediate by association of potassium oxalate with experimental primers**", register number **003/2006**, of **JULIO CESAR FRANCO ALMEIDA and MÁRIO FERNANDO DE GOES**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 26/01/2006.

CínthiaMachadoTabchoury
Cínthia Pereira Machado Tabchoury
Secretária
CEP/FOP/UNICAMP

Jacks Jorge Júnior
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 2- Comprovante da submissão do artigo*

Transcrição de mensagem recebida por email

jba@eastman.ucl.ac.uk escreveu:

"Dear Dr. Almeida:

Your manuscript entitled "Effect of wet versus dry bonding technique on the durability of water and ethanol-based primers bonded to oxalate-treated acid-etched dentin" has been successfully submitted online and is presently being given full consideration for publication in the Journal of Biomaterials Applications.

Your manuscript ID is JBA-09-0026.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to Manuscript Central at http://mc.manuscriptcentral.com/jba and edit your user information as appropriate.

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Sincerely,
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