SAFIRA MARQUES DE ANDRADE E SILVA

"Avaliação da qualidade da união resina composta-dentina obtida pelo emprego de materiais/técnicas que visam minimizar os efeitos da umidade dentinária sobre a longevidade das restaurações adesivas"

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de Doutor em Materiais Dentários.

Orientadora: Prof[a]. Dr[a]. Marcela Rocha de Oliveira Carrilho

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A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 12 de Dezembro de 2008, considerou a candidata SAFIRA MARQUES DE ANDRADE E SILVA aprovada.

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RESUMO

O objetivo geral desta pesquisa foi avaliar materiais e procedimentos empreendidos para incrementar a durabilidade das restaurações adesivas. Os objetivos específicos foram: 1) avaliar o efeito da aplicação de uma camada adicional de adesivo e de um agente de união com característica hidrofóbica e livre de solventes na permeabilidade da dentina hibridizada com adesivos convencionais de 2 passos; 2) avaliar os efeitos decorrentes da aplicação do oxalato de potássio na durabilidade da interface de união obtida com adesivos convencionais **e** 3) determinar se a aplicação prévia do oxalato de potássio na dentina condicionada interfere na dureza Knoop dos adesivos. No primeiro estudo, a permeabilidade dentinária foi mensurada utilizando-se um medidor automático de fluxo, antes e após a aplicação dos sistemas adesivos nas condições experimentais citadas. Os resultados mostraram que nenhum dos adesivos foi capaz de prevenir a passagem de fluidos através da sua estrutura polimerizada. Porém, a aplicação de uma camada de um agente de união hidrofobico e livre de solventes reduziu significantemente a permeabilidade da dentina hibridizada com adesivos convencionais de 2 passos. Já no segundo estudo, testes de resistência adesiva (RA) foram realizados 24 horas após o procedimento adesivo ou após 12 meses de armazenagem em água. Os espécimes foram preparados para observação em microscopia eletrônica de varredura para avaliação do modo de fratura. Os resultados mostraram que a associação do oxalato de potássio aos sistemas adesivos reduziu significantemente a RA dos mesmos. Após o período de armazenagem, a RA foi significantemente reduzida em todos os grupos. No entanto, esta queda foi significantemente menor para os grupos tratados com oxalato. Finalmente, no terceiro estudo, testes de dureza foram realizados em microdurômetro e expressos como dureza Knoop (KHN). Os resultados mostraram que a associação do oxalato de potássio aos sistemas adesivos convencionais reduziu a KHN inicial dos mesmos.

PALAVRAS CHAVES: adesivos dentinários, agente de união hidrofóbico, permeabilidade dentinária, dureza Knoop, resistência adesiva, oxalato de potássio

ABSTRACT

The general aim of this study was to evaluate materials and techniques that are thought to increase the longevity of adhesive restorations. The specific aims of this study were: 1) to test whether the application of an extra layer of the respective adhesive or the application of a hydrophobic, non-solvated bonding agent would interfere on the permeability of dentin hybridized with two-step etch-and-rinse adhesives; 2) to test whether the pretreatment of acid-etched dentin with potassium oxalate gel has an effect on the durability of resin-dentin interface created with etch-and-rinse adhesives; and 3) to determine whether the hardness of etch-and-rinse adhesives is affected by the pretreatment of acid-etched dentin with potassium oxalate gel. In the first study, dentin permeability was measured with an automatic record device, before and after dentin surfaces were bonded with the adhesives in the cited experimental conditions. The results showed that none of the adhesives or experimental treatments was capable to block completely the fluid transudation across the treated dentin; however, the application of the hydrophobic non-solvated bonding agent resulted in significant reductions in the fluid flow rate. In the second study, the resin-dentin bond strength of the adhesives was tested immediately (i.e. 24 hours) and after 12 months of storage. The results showed that the pretreatment of dentin with potassium oxalate affect negatively the baseline resin-dentin bond strength of dentin specimens. After storage, the bond strength of resin-bonded interfaces was significantly reduced for all tested groups. Nevertheless, the decrease in bond strength values was significantly lower for oxalate-treated specimens than for controls. Finally, in the third study, the specimens' hardness was determined with a hardness tester and express as Knoop Hardness (KHN). The results showed that the baseline KHN of the adhesives applied to acid-etched dentin pretreated with potassium oxalate was significantly lower than that exhibited by their respective controls.

KEYWORDS: dentin adhesives, dentin permeability, hydrophobic bonding agent, Knoop hardness, resin-dentin bond strength, potassium oxalate.

SUMÁRIO

INTRODUÇÃO GERAL

Nos últimos 20 anos, o desenvolvimento da *Odontologia Adesiva* esteve principalmente voltado à obtenção de dois objetivos cardeais: o estabelecimento de união resistente, formada entre os tecidos dentais e os materiais restauradores à base de resina e, a redução das taxas de desgaste sofrido por estes materiais. O aprimoramento das propriedades físico-mecânicas dos compósitos de resina, e a conseqüente diminuição de fracassos clínicos imediatos em função de fraturas e desgastes excessivos deste material viabilizaram procedimentos restauradores mais conservativos e estéticos, e ajudaram a disseminar o emprego corrente das restaurações adesivas.

Entretanto, a despeito do uso corriqueiro, os compósitos ainda representam um desafio sob o ponto de vista da longevidade, principalmente em cavidades amplas e que sofrem maior carga mastigatória (Mjör et al*.,* 2002; De Munck et al., 2005). O comprometimento relativo da estabilidade das restaurações adesivas é mais aparente quando a dentina é o substrato de união primordialmente envolvido. Esta prevalência, provavelmente, se deve ao emprego da técnica de adesão à dentina úmida, adotada na maioria dos protocolos de adesão, e que como solução de compromisso exige a utilização imperativa de sistemas adesivos hidrófilos a fim de promover a formação da zona de interdifusão ou camada híbrida (Kanca J, 1992). A despeito dos altos valores de resistência adesiva, obtidos com esta técnica (Gwinnett, 1992; Pashley et al., 1993), a obtenção de um selamento adequado e duradouro continua sendo imprevisível, principalmente quando se trata de estabelecer adesão com a dentina profunda de dentes vitais (Tay & Pashley, 2003; Shono et al., 1999), dada à contínua transudação de fluidos oriundos do substrato após a remoção da smear layer, e consequentemente à dificuldade em se determinar e controlar a umidade "ideal" requerida pelos procedimentos adesivos.

A condição de umidade excessiva do substrato também pode causar a diluição da mistura que constitui o sistema adesivo, promovendo a separação dos seus componentes (Spencer & Wang, 2002). Os componentes hidrofílicos do adesivo são capazes de se difundir mais rapidamente na intimidade da dentina desmineralizada, enquanto que os monômeros hidrofóbicos sofrem restrições em se infiltrar pelo substrato úmido e tendem a

se acumular no topo da zona desmineralizada (Spencer & Wang, 2002). Assim, em contraste à formação de uma barreira impermeável ideal, a camada híbrida formada pelos adesivos convencionais simplificados aplicados sobre a dentina condicionada e úmida tem sido caracterizada por ser uma estrutura porosa (Tay & Pashley, 2003; Tay et al., 2002; Silva at al., 2007), constituída por polímeros hidrofóbicos (Bis-GMA) irregularmente dispersos em uma matriz predominantemente hidrofílica (poli-HEMA), propensa a maior absorção de água e, conseqüentemente, mais suscetível à degradação ao longo do tempo (Yiu et al., 2006; Wang & Spencer, 2003).

A evolução tecnológica aos sistemas adesivos que objetiva primordialmente torná-los mais simples tecnicamente, não corresponde a uma melhoria na qualidade do produto; já que esta implica na ausência de uma camada adicional de resina hidrofóbica e livre de solvente e na inclusão de quantidades cada vez maiores de monômeros hidrofílicos (HEMA, BPDM, MDP) e solventes (etanol, acetona, água) na sua composição os tornando mais susceptíveis à sorção de fluidos intra e extra-orais e à hidrólise catalisada por enzimas salivares (Santerre et. al., 2001), o que pode também acelerar o processo de deterioração da interfaces adesivas formadas por esses materiais (Carrilho et al., 2004; Carrilho et. al., 2005).

O conhecimento das limitações decorrentes do procedimento adesivo e das características desses sistemas, aliado à busca pela estabilidade e durabilidade da união direciona os estudos atuais para um maior entendimento dos fenômenos que ocorrem durante a interação entre os sistemas adesivos e o substrato dentinário e também para alternativas que possam proporcionar melhores condições para a formação da camada híbrida.

Uma alternativa está baseada no emprego de soluções de oxalato de potássio em associação com os adesivos convencionais simplificados. A utilização do gel de oxalato de potássio após o condicionamento ácido da dentina faz com que as moléculas de oxalato de potássio se difundam através dos túbulos dentinários até que encontrem íons cálcio disponíveis para reação. Dessa maneira, os cristais de oxalato de cálcio se formam dentro dos túbulos dentinários (Pashley et al., 2001; Tay et al., 2003). A função primária desta técnica baseia-se no auxílio ao tratamento da sensibilidade dentinária, através da redução da

condutividade hidráulica da dentina após o procedimento adesivo (Pashley et al., 2001). No entanto, especula-se que esta associação possa trazer outros benefícios. Além de reduzir, ou eliminar, o volume de fluidos que poderia permear através da camada do adesivo polimerizado (Silva et al., 2007), os cristais de oxalato de cálcio formados na sub-superfície contribuiriam para a redução do movimento de fluidos dentinários durante a realização dos procedimentos adesivos, sejam estes estimulados pela pressão pulpar ou até mesmo na ausência da mesma. O "bloqueio" dos túbulos dentinários com os cristais de oxalato de cálcio permitiria um maior controle sobre a umidade presente no substrato, o que favoreceria a evaporação da água residual proveniente do substrato dentário e do solvente presente no sistema (Tay et al., 2005). A difusão dos monômeros resinosos na intimidade da dentina desmineralizada também seria facilitada, pois os monômeros hidrofóbicos sofreriam menores restrições em se infiltrar pelo substrato e poderiam contribuir para formação de um polímero mais coeso e com propriedades mecânicas melhoradas. Embora estas suposições pareçam ser plausíveis, requerem investigações mais profundas.

Diante do exposto, os objetivos deste trabalho foram:

1. Avaliar o efeito da aplicação de uma camada adicional de adesivo ou de agente de união com característica mais hidrofóbica e livre de solvente na permeabilidade da dentina hibridizada com adesivos convencionais simplificados.
2. Avaliar o efeito da utilização do oxalato de potássio na durabilidade da interface de união dentina/resina composta.
3. Avaliar o efeito da utilização do oxalato de potássio na dureza Knoop de adesivos convencionais.

CAPÍTULO 1:

Effect of an additional hydrophilic *versus* hydrophobic coat on the quality of dentinal sealing provided by two-step etch-and-rinse adhesives

ABSTRACT

Objective: To test the hypothesis that the quality of the dentinal sealing provided by two-step etch-and-rinse adhesives cannot be altered by the addition of an extra layer of the respective adhesive or the application of a more hydrophobic, non-solvated resin. Materials and Methods: full-crown preparations were acid-etched with phosphoric acid for 15 s and bonded with Adper Single Bond (3M ESPE), Excite DSC (Ivoclar/Vivadent) or Prime & Bond NT (Dentsply). The adhesives were used according to the manufacturers' instructions (control groups) or after application to dentin they were a) covered with an extra coat of each respective system or b) coated with a non-solvated bonding agent (Adper Scotchbond Multi-Purpose Adhesive, 3M ESPE). Fluid flow rate was measured before and after dentin surfaces were acid-etched and bonded with adhesives. Results: None of the adhesives or experimental treatments was capable to block completely the fluid transudation across the treated dentin. Application of an extra coat of the adhesive did not reduce the fluid flow rate of adhesive-bonded dentin ($p>0.05$). Conversely, the application of a more hydrophobic non-solvated resin resulted in significant reductions in the fluid flow rate ($p<0.05$) for all tested adhesives. Conclusions: The quality of the dentinal sealing provided by etch-and-rinse adhesives can be significantly improved by the application of a more hydrophobic, non-solvated bonding agent.

Uniterms: adhesives permeability, dentinal sealing, hydrophobic coating.

INTRODUCTION

In adhesive dentistry the concept of clinical progress has been often earmarked by procedural simplification. Classical multi-step adhesives have been, increasingly, replaced by simplified "single-step" systems that are, apparently, simpler and faster to use. Simplification of contemporary dental adhesives has occurred, however, at the expenses of an increasing incorporation of hydrophilic monomers (i.e. HEMA, BPDM, MDP, Phenyl-P). Not coincidentally, these contemporary hydrophilic adhesives have shown to draw water from hydrated dentin through an apparently intact, polymerized adhesive layer [7,11,24]. If the dentinal fluid passes through the adhesive, it may accumulate on its own surface, thereby interfering with coupling to the resin composites [4,27]. Thus, instead of creating a perfect sealing of dentin, polymerized hydrophilic adhesives actually behave as permeable membranes that potentially allow outward and inward fluid flow [4,6,7,10,11,24,27].

Deficient sealing of dentin may be analysed from several perspectives. One can argue that placing adhesive comonomer mixtures in an inhospitable environment such as the water-wet acid-etched dentin will inexorably result in the formation of porous polymers that are prone to absorb water [9,15]. Conversely, the use of more hydrophobic, less permeable resins such as the dentin bonding adhesives may supposedly prevent this problem and further improve the durability of resin-dentin bonds, because these materials tend to exhibit much lower ability to absorb water [15]. However, the use of hydrophobic monomers as bonding agents to dentin is not effective yet as they are not miscible to acid-etched dentin that is intentionally saturated with water [3,20].

While the water-wet bonding technique [13] is not modified to permit hydrophobic adhesives to be coaxed into acid-etched dentin, the intrinsic permeability of two-step etch-and-rinse adhesives[24,25] should be minimized, which is thought to be important in prolonging the integrity of the resultant resin-dentin bonds. Studies have demonstrated that three-step etch-and-rinse adhesives and two-step self-etching systems, which deliberately indicate the application of multiple coats of their primer solution and/or a coat of a more hydrophobic resin over the hybridized dentin, seem to constitute overlying adhesive layers and hybrid layers that are naturally less permeable than those formed when

using the most simplified, "single-step" systems [5,6,7]. Thus, the objective of this study was to evaluate whether the ability of two-step etch-and-rinse adhesives to seal dentin may be changed when these systems are covered with an extra coat of the same two-step etch-and-rinse system or a coat of a more hydrophobic non-solvated bonding agent. The null hypotheses tested were that the quality of the dentinal sealing provided by two-step etch-and-rinse adhesives cannot be altered by the application of: 1) an extra coat of the respective two-step etch-and-rinse system or 2) a coat of a more hydrophobic, non-solvated bonding agent.

MATERIAL AND METHODS

Teeth preparation

Fifty-four non-carious human third molars extracted for orthodontics reasons were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the Human Assurance Committee of University of São Paulo, Bauru. These teeth were stored in saline containing 1% thymol at 4°C and used within no longer than 6 months after extraction.

Crown preparations with chamfer margins located on the cementum/enamel junction (CEJ) were performed in the extracted teeth with diamond burs under copious air-spray (4137 KG SORENSEN, Barueri, São Paulo, Brazil). From prepared teeth, crown-segments were further obtained by transversally sectioning the teeth roots at 2 mm below the CEJ using a slow-speed diamond saw (IMPTECH PC 10, Boksburg, Republic of SA), under water cooling. The pulp tissue was carefully removed with a pair of small forceps. Care was taken to avoid touching the pulp chamber walls for consequently not crushing the predentine toward the dentinal tubules, which could alter the final permeability of dentin (David Pashley, personal communication). The resulting crown-segments were glued to Plexiglass slabs using a viscous cyanoacrylate (Zapit, Dental Ventures of American, Corona, CA, USA), which also covered the entire peripheral cementum. Each Plexiglass slab was penetrated by a short length of 18-gauge stainless steel tubing, which ended flush with the top of the Plexiglass slab. This tube permitted the pulp chamber to be filled with

water and to be connected to an automated flow-recording device (Flodec System, De Marco Engineering, Geneva, Switzerland) (Figure 1).

Bonding procedures

Three two-step etch-and-rinse adhesives were evaluated in this study: Adper Single Bond (SB - 3M ESPE, St. Paul, MN, USA), Excite DSC (EX – Ivoclar/Vivadent, Schaan, Liechtenstein) and Prime & Bond NT (PB - Dentsply DeTrey, Konstanz, Germany) (table 1).

The crown-segments were randomly divided into nine groups of six specimens each (n=6), corresponding to the three adhesives applied in three different conditions: 1) according to the manufacturers' instructions (controls); 2) with an additional coat of the respective adhesive and 3) with a coat of a more hydrophobic non-solvated resin bonding agent (Adper Scotchbond Multi-Purpose Adhesive, 3M ESPE, St Paul MN,USA).

During the bonding procedures, the hydrostatic pressure in the pulp chamber was null (0 cm H_2O) in order to avoid excessive fluid contamination of the bonding area [10]. The extra coat of the respective two-step etch-and-rinse adhesive (i.e. SB, EX or PB) or the more hydrophobic adhesive (i.e. Adper Scotchbond Multi-Purpose Adhesive) was always added right after the tested adhesives had been applied according to manufacturers' instructions. All dentin surfaces were checked to ensure complete covering with adhesives before the photo-activation was performed. Adhesives were, then, photo-cured for 10s with a light intensity of 500mW/cm^2 (Degulux Soft-start, DEGUSSA HÜLS).

Fluid flow measurement

An in vitro fluid transport model was used to measure the fluid conductance induced by hydrostatic pressure, following the general guidelines reported by Pashley and Depew [19] (Figure 1). Each crown-segment was connected via polyethylene tubing to the Flodec device (Flodec System, De Marco Engineering, Geneva, Switzerland) under a constant physiological hydrostatic pressure (20 cm H_2O)[8]. A pressure gradient between the water reservoir and the specimen induced fluid movement through the specimen. The rate of fluid movement was measured by following the displacement of a tiny air bubble that was introduced into a glass capillary located between the water reservoir and the specimen. Displacement of the air bubble was detected via a laser diode incorporated in the Flodec

device. The linear displacement was automatically converted to fluid flow ($\mu L\ min^{-1}$) via the computer software program. The rate fluid flow across dentin was measured two times, sequentially, as follows: 1) after the dentin surface was acid-etched with 35% phosphoric acid gel (3M ESPE) for 15 s for the determination of maximum baseline conductance, and 2) after dentin was hybridized following one of the described bonding procedures. All fluid flow measurements were made with the specimen immersed in water to minimize interferences derived from evaporative water fluxes [14]. For each specimen, the fluid flow ($\mu L.min^{-1}$) across the adhesive-bonded dentin was expressed as a percentage of the maximum permeability derived from the acid-etched dentin (assigned as 100%). This allowed each specimen to serve as its own control, since the same surface area was used in all two measurements [6,18].

Statistic analysis

The percentage fluid flow across the adhesive-bonded dentin for control and experimental groups were analyzed by a two-way ANOVA, having as main factors: the adhesives (i.e. SB, EX and PB) and the experimental dentin treatments (i.e. adhesives application according to manufacturers' instructions, with an extra coat of the respective adhesive or with a coat of a more hydrophobic resin). Post hoc multiple comparisons were performed using Tukey's tests. Statistical significance was preset at $\alpha = 0.05$

RESULTS

Fluid conductance results expressed as percentages of the maximum permeability that occurred in the baseline acid-etched dentin are summarized in table 2. None of the adhesives or experimental bonding treatments was able to interrupt completely the transudation of fluid across the adhesive-bonded interface. Reduction in the fluid conductance of acid-etched dentin after adhesives/treatment application was in the range of 53% to 78%. Regardless of the adhesive system, the application of an extra coat of the respective two-step etch-and-rinse system did not reduce significantly the fluid conductance of dentin when compared to that exhibited by the control groups ($p>0.05$). Conversely, the application of a relatively more hydrophobic non-solvated bonding resin reduced

significantly the fluid conductance of dentin ($p<0.05$), irrespectively to the applied adhesive.

DISCUSSION

The present results indicated that the application of an extra coat of the respective two-step adhesive did not reduce the fluid flow across the adhesive-bonded dentin and, thus, did not improve the quality of dentinal sealing. Nevertheless, the simulated conversion of two-step etch-and-rinse adhesives into three-step adhesives, by applying a relatively hydrophobic bonding agent over the primed dentin, significantly reduced the fluid flow across adhesive-bonded interfaces, thereby improving the quality of dentinal sealing. In concert, these results determine the acceptance of the first anticipated null hypothesis and the rejection of the second anticipated null hypothesis.

Ideally, the dental adhesives should render dentin impermeable, or at least they should reproduce the natural permeability of dentin when this is covered with enamel and/or cementum. However, our results showed that even the most effective treatment used for blocking fluid transudation across the adhesive-bonded dentin (i.e. adhesive + hydrophobic resin) was not able to seal perfectly the dentin. Actually, several previous studies have reported the deficient ability of dental adhesives to keep dentin perfectly sealed [2,6,7]. In addition, some of these studies showed that the smear layer/smear plugs formed in dentin as a result of the operative procedures may be, at short-terms, more effective in sealing the dentinal tubules than the majority of the current dental adhesives [3,18,19]. This suggests that an impervious dentinal sealing may not be easily achieved if bonding procedures are performed after the removal of smear layer/smear plugs by either acid etchants or calcium-chelant agents [3,10,29]. Supposedly, at short-terms, the channels/pathways around the resin tags or the overlying adhesive layer seem to offer less resistance to water movement than do the contiguous channels through the smear plugs/smear layer complex [3]. According to our previous findings, the resin tags seem not to hybridize perfectly with the surrounding water-filled interfibrillar spaces [26].

Dentinal tubules that become freely unobstructed by the acid etching can readily permit the transudation of dentinal fluid from the pulp toward the surface. Whenever this dentinal fluid transudation occurs, that is, during the infiltration of hydrophilic adhesives [11] or after adhesive polymerization [7,24], it may create nanoleakage channels within adhesives [25] by inducing phase separation of adhesive components [23], interfering with resin monomers conversion [12,23] and resin polymers crosslinking, frequently permitting the elution of residual monomers from the polymerized adhesive [9]. Depending on the number and density of these channels and porosities, they may account for a significant increase in the hole-free volume of dental adhesives, which in turn is thought to be intimately related with the extent and rate of water diffusion in these polymeric materials [15]. If the dentinal fluid passes through the adhesive, it will accumulate on top of hybrid layer and interfere with coupling to resin composites [4,11]. Nanoleakage channels reported for most of adhesives [27,31] are, therefore, not only an evidence of material permeability and corrosion, but also a virtual sign of the inability of these adhesives to provide a perfect sealing to dentin.

Deficient sealing of dentin can be also analyzed from the perspective of adhesives polarity [7,14]. The two-step etch-and-rinse adhesives used in this study are basically composed of highly hydrophilic monomers (i.e HEMA, PENTA). Studies on the kinetics of water diffusion have shown a positive correlation between the magnitude of water sorption and the degree of hydrophilicity of experimental adhesives [15]. It is suggested that the absorbed water may form hydrogen bonds with the hydrophilic and ionic domains (i.e. hydroxyl, carboxyl and phosphate groups) present in these hydrophilic adhesives [28]. The water molecules that attach to the polymer chain via hydrogen bonding, referred as "bound" molecules, it is thought to disrupt the interchain hydrogen bonding, induce swelling, and plasticize the polymer [1]. Thus, while it is alarming, it is not surprising to notice that an additional coat of the two-step etch-and-rinse adhesives was not enough to prevent fluid transudation across adhesive-bonded dentin. In fact, in a recent study on the water permeation through multiple layers of organic resin coatings, Nguyen and co-workers [16] observed that by increasing the number of coats of a hydrophilic resin can only extend

the required time for water to permeate completely these coatings, but it did not impede water to move across them.

Clearly, the present results indicated that the permeability of two-step etch-and-rinse dental adhesives can be reduced if they are coated with a resin relatively more hydrophobic. The bonding agent of the Adper Scotchbond Multi-Purpose is based not only on HEMA, but on Bis-GMA (60-70% - 3M ESPE Technical Profile) and its application over the tested two-step etch-and-rinse adhesives not only increased the time necessary for water to permeate the bonded interfaces, but it may also reduce the percentage of fluid transudation across such interfaces, thereby improving the acid-etched dentin sealing. Undoubtedly, this result corroborates with the notion that three-step etch-and-rinse adhesives, which recommend a separate application of a bonding agent composed of hydrophobic and crosslinking monomers to the primed tooth surface, produce resin-dentin bonds that are more durable than those formed with two-step etch-and-rinse ones [29]. Again, the trend to simplification of adhesives showed to be incompatible with a good dentinal sealing.

Although Bis-GMA forms a polymeric backbone is relatively more hydrophobic, less prone to absorb water than do the highly hydrophilic monomers such as HEMA, PENTA, BPDM, Phenyl-P and others [16]; neither Bis-GMA nor its derivatives co-momonors (i.e. Bis-GMA-E) and other more hydrophobic monomers (i.e.UDMA) used in dentistry exhibit functional groups (i.e. hydroxyls) that are prone to form hydrogen-bond with water molecules [22], thereby being able to absorb and retain in their resultant polymers a certain amount of water [9] . This could explain why the application of the bonding agent of the Adper Scotchbond Multi-Purpose cannot completely annul the permeability of bonded dentin [6,15]. In fact, the first *in vitro* study that quantified fluid movement across dentin before and after adhesive bonding showed that the application of Adper Scotchbond Multi-Purpose to acid-etched dentin, followed by a 2-mm thick layer of resin composite (Z350, 3M ESPE) did not reduce completely the permeability of dentin, allowing a residual hydraulic conductance of 17% [2]. Since in the present study we did not apply a resin composite over the bonded dentin, we speculate that the residual fluid movement observed for the groups wherein Adper Scotchbond Multi-Purpose was applied (22-32%; Table 2)

would indirectly correspond to the maximum capacity of this material to reduce the permeability of the acid-etched dentin that has been previously primed with a more hydrophilic, two-step etch-and-rinse adhesive system.

Recently, it has been pointed out that the defective dentinal sealing and fluid conductance across resin-bonded dentin may be significantly affected by the solvent used to saturate the acid-etched dentin [20]. The high permeability of dental adhesives bonded to water-saturated dentin (i.e. water-wet bonding technique) probably occur at the expense of a sub-optimal resin infiltration into collagen interfibrillar spaces, both within the intertubular dentin and the circumferential dentin surrounding resin tags [3]. Accordingly, the combined use of water-wet bonding technique and hydrophilic resins compromise the requirements for perfect sealing and durable coupling between resin composites and adhesive-bonded dentin [3]. Thus, the development of a bonding technique that combines the use of hydrophobic resins applied to acid-etched dentin that is saturated with an anhydrous solvent, such as ethanol, could be effective to alleviate the water sorption seen in dentin bonded with hydrophilic simplified adhesives [21]. Application of hydrophobic experimental dental adhesives to ethanol-saturated dentin has shown strong evidences of reduction in bonded dentin permeability and [3] improvements in the durability of resin-dentin bonds [17]. While it is a promising clinical approach, additional appraisals on the performance and technique sensitivity of ethanol-wet bonding technique associated with hydrophobic adhesives are undoubtedly necessary.

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REFERENCES

1 Adamson MJ. Thermal expansion and swelling of cured epoxy resin used in graphite/epoxy composite materials. J Mater Sci. 1980;15:1736-45.

2 Bouillaguet S, Duroux B, Ciucchi B, Sano H. Ability of adhesive systems to seal dentin surfaces: an in vitro study. J Adhes Dent. 2000;2(3):201-8.

3 Carrilho MR, Tay FR, Sword J, Donnelly AM, Agee K, Nishitani Y, Sadek FT, Carvalho RM, Pashley DH. Dentin sealing provided by smear layer/smear plugs versus adhesive resins/resin tags. Eur J Oral Sci. 2007;115(4):321-9.

4 Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR, Pashley DH. Adhesive permeability affects coupling of resin cements that utilize self-etching primers to dentine. J Dent. 2004;32(1):55-65.

5 Cheong C, King NM, Pashley DH, Ferrari M, Toledano M, Tay FR. Incompatibility of self-etch adhesives with chemical/dual-cured composites:
two-step vs one-step systems. Oper Dent. 2003;28(6):747-55.

6 Chersoni S, Acquaviva GL, Prati C, Ferrari M, Grandini S, Pashley DH, et al. In vivo fluid movement through dentin adhesives in endodontically treated teeth. J Dent Res. 2005;84(3):223-7.

7 Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, et al. In vivo and in vitro permeability of one-step self-etch adhesives. J Dent Res. 2004;83(6):459-64.

8 Ciucchi B, Bouillaguet S, Holz J, Pashley D. Dentinal fluid dynamics in human teeth, in vivo. J Endod. 1995;21(4):191-4.

9 Ferracane, JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater. 2006;22(3):211-22.

10 Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res. 2004;83(11):843-8.

11 Itthagarun A, Tay FR. Self-contamination of deep dentin by dentin fluid. Am J Dent. 2000;13(4):195-200.

12 Jacobsen T, Soderholm KJ. Some effects of water on dentin bonding.

Dent Mater. 1995;11(2):132-6.

13 Kanca, J 3rd. Effect of resin primer solvents and surface wetness on resin composite bond strength to dentin. Am J Dent. 1992;5(4):213-5.

14 King, NM, Tay FR, Pashley DH, Hashimoto M, Ito S, Brackett WW, et al. Conversion of one-step to two-step self-etch adhesives for improved efficacy and extended application. Am J Dent. 2005;18(2):126-34.

15 Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK, Carrilho MR. Water sorption/solubility of dental adhesive resins. Dent Mater. 2006; 22(10):973-80.

16 Nguyen T, Byrd E, Bentz D, Lin CJ. In situ measurement of water at the organic coating/substrate interface. Proc Org Coat. 1996;27:181-93.

17 Nishitani Y, Yoshiyama M, Donnelly AM, Agee KA, Sword J, Tay FR, Pashley DH. Effects of resin hydrophilicity on dentin bond strength. J Dent Res. 2006;85(11):1016-21.

18 Pashley DH, Carvalho RM, Pereira JC, Villanueva R, Tay FR. The use of oxalate to reduce dentin permeability under adhesive restorations. Am J Dent. 2001;14(2):89-94.

19 Pashley DH, Depew DD. Effects of the smear layer, Copalite, and oxalate on microleakage. Oper Dent. 1986;11(3):95-102.

20 Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, Donnelly A, Garcia-Godoy F. "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(1): 7-20.

21 Sadek FT, Pashley DH, Ferrari M, Tay FR. Tubular occlusion optimizes bonding of hydrophobic resins to dentin. J Dent Res. 2007;86(6):524-8.

22 Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med. 2001;12(2):136-51.

23 Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet

bonding conditions. J Biomed Mater Res. 2002;62(3):447-56.

24 Tay FR, Frakenberger R, Kreijci I, Bouillaguet S, Pashley DH, Carvalho RM, Lai CNS. Single-bottle adhesives behave as permeable membranes after polymerization. I. In vivo evidence. J Dent. 2004;32:611-21.

25 Tay FR, Pashley DH. Water treeing--a potential mechanism for degradation of dentin adhesives. Am J Dent. 2003;16(1):6-12.

26 Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, Tay KC. Bonding BisGMA to dentin--a proof of concept for hydrophobic dentin bonding. J Dent Res. 2007;86(11):1034-9

27 Tay FR, Suh BI, Pashley DH, Prati C, Chuang SF, Li F. Factors contributing to the incompatibility between simplified-step adhesives and self-cured or dual-cured composites. Part II. Single-bottle, total-etch adhesive. J Adhes Dent. 2003;5(4):91-105.

28 Van Landuyt KL, Snauwaert J, De Munck J, Coutinho E, Poitevin A, Yoshida Y, Suzuki K, et al. Origin of interfacial droplets with one-step adhesives. J Dent Res. 2007;86(8):739-44.

29 Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K,Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003;28(3):215-35.

30 Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M et al. Solvent and water retention in dental adhesive blends after evaporation. Biomaterials 2005;26(34):6863-72.

31 Yoshida E, Uno S, Nodasaka Y, Kaga M, Hirano S. Relationship between water status in dentin and interfacial morphology in all-in-one adhesives. Dent Mater. 2007;23(5):556-60.

Table 1- Composition, manufacture's instructions and batch number of the adhesives used in this study.

Adhesives	Composition	**Manufacture 's recommended protocol**	**Batch number**
Excite	HEMA, phosphoric acid, acrylate, Bis GMA, dimethacrylate, highly dispersed silica, catalysts, stabilizers, ethanol	Apply a generous amount of Excite. Gently agitate the adhesive for at least 10 s. Dry gently for 1-3 s. Cure for 10 s.	E30108
Adper Single Bond	HEMA, BisGMA, dimethacrylate, copolymer with methacrylic function, polyacrylic & polyitaconic acids, photoinitiators, water and ethanol	Apply 2 consecutive coats of Single Bond, dry gently for 2-5 s. Cure for 10 s.	3HW
Prime & Bond NT	Resin di and tri methacrylate, amorphous functional silica, PENTA, cetylamine hydrofluoride, photoinitiators, stabilizers, acetone	Apply 3 consecutive coats of Prime & Bond NT. This surface should remain fully wet for 20 s. Gently dry with air for at least 5 s. Cure adhesive for 10 s.	534320
Adper Scotchbond Multi Purpose Adhesive	Bis-GMA, HEMA, initiators	Apply adhesive and light cure for 10 s.	5PH

Table 2- Fluid flow (in percentage) across bonded-dentin when using two-step etch-and-rinse adhesives that were applied as recommended by manufacturers (controls) or coated with an extra coat of the respective two-step etch-and-rinse adhesive or with a more hydrophobic bonding resin.

	TREATMENTS		
Adhesives	**Manufacturer's recommended**	**Extra coat of the respective adhesive**	**Coat of a hydrophobic resin**
SB	38 ± 14 **A**	45 ± 11 **A**	22 ± 9 **B**
EX	47 ± 9 **A**	41 ± 15 **A**	27 ± 10 **B**
PB	39 ± 13 **A**	38 ± 19 **A**	32 ± 14 **B**

Values (Mean ± SD) are expressed as a percentage of the maximum permeability (acid-etched dentin). Groups identified by different letters were statically different ($p<0.05$).

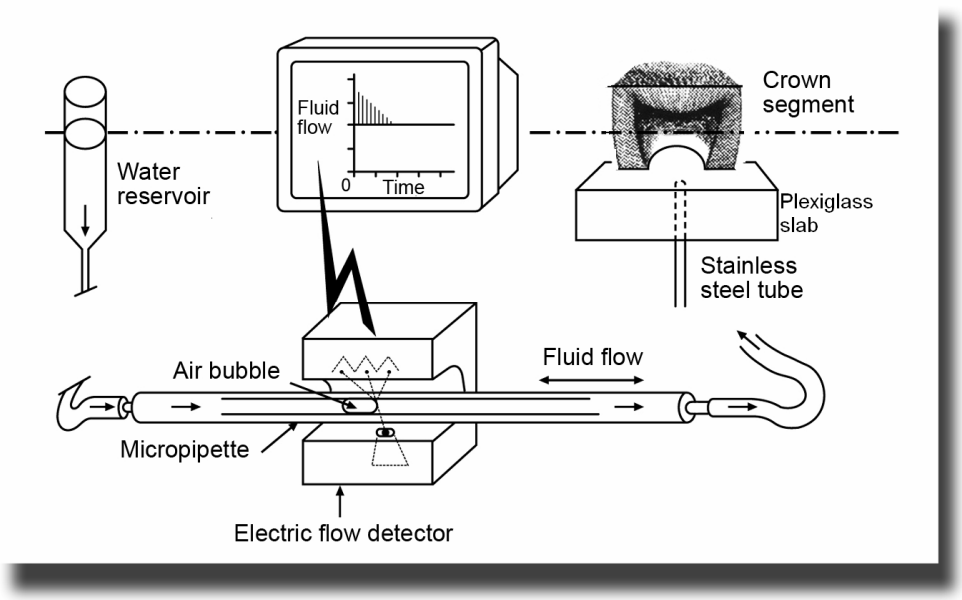

Figure 1. Schematic of the fluid-filtration apparatus used to perform repeated measurements of the permeability of dentin surfaces after acid-etching and after resin-bonding.

CAPÍTULO 2:

Effect of oxalate desensitizer on the durability of resin-bonded interfaces

ABSTRACT

Oxalate desensitizers have to reduce the permeability of dentin, thereby favoring the control of dentin moisture previously to etch-and-rinse adhesives. The objective of this study was to evaluate if the effect of combined application of oxalate with etch-an-rinse adhesives interferes on the durability of resin-dentin bonds when using two two-step and one three-step etch-and-rinse adhesives (Single Bond, One-Step and Scotchbond Multi-purpose, respectively). The resin-dentin bond strength of the adhesives was tested immediately (i.e. 24 hours) and after 12 months of storage in water. The adhesives were used either according to the manufacturers' instructions (control groups) or after the treatment of acid-etched dentin with a potassium oxalate gel (BisBlock - Bisco Inc., Schaumburg, IL, USA). Treatment of dentin with potassium oxalate was shown to affect negatively the baseline resin-dentin bond strength of dentin specimens, regardless the employed adhesive ($p<0.05$). After storage, the bond strength of resin-bonded interfaces was significantly reduced for all tested groups ($p<0.001$). Nevertheless, the decrease in bond strength values was significantly lower for oxalate-treated specimens than for controls ($p<0.05$).

Clinical Relevance: Although oxalate desensitizer may affect the initial bond strength of etch-an-rinse adhesives to dentin, it showed to slow down the rate of resin-dentin bonds degradation.

INTRODUCTION

It is generally accepted that the efficiency and quality of adhesive bonding to dentin depends on a homogeneous and complete hybridization between the exposed collagen fibrils and resin monomers. The current approach for achieving this goal is to keep the demineralized dentin saturated in water so that the nanospaces between collagen fibrils can be expanded and, theoretically, more prone to uptake resin.[1] Conversely, the water content on the acid-etched dentin surface when using the water-wet bonding technique makes the hybridization of dentin with hydrophobic resins impractical, forcing manufactures to include hydrophilic monomers into dental adhesives formulation.

Among many critical issues, the excess of residual water within the demineralized dentin has shown to interfere with the conversion of resin monomers[2-4] and to cause the phase separation between hydrophobic and hydrophilic components of the dental adhesives,[5-6] which results in formation of porous hybrid layers.[7-8] Inopportunely, after the adhesives polymerization, a consistent outward fluid flow of dentinal tubules is observed.[9-11] This chain of events compromises the perfect sealing of acid-etched dentin,[12-13] increasing the prevalence of microleakage and/or post-operative dentin sensitivity.

The application of an acidic solution of oxalate has been used in clinical dentistry to desensitize dentin. Potassium oxalate desensitizers react with ionized calcium in dentin to form insoluble calcium oxalate crystals that can occlude the dentinal tubules.[14-16] When applied on acid-etched dentin, calcium oxalate crystals tend to be formed only into the tubules, leaving the dentin surface unobstructed and available to be bonded with dental adhesives.[17] Thus, it has been considered that the use of oxalate during bonding procedure could enhance solvent evaporation by decreasing the amount of water entrapped within these adhesives, thus allowing better control of the adequate moisture and facilitating the penetration of adhesives into wet demineralized dentin.[18-19]

Although recent studies have shown that oxalate desensitizers seems not to compromise the early bond strength (over short periods, i.e. 24 hours) of relatively neutral adhesives to dentin, none of these studies tested the effect of this combination over time. The objective of this study was to evaluate if the effect of combined application of oxalate

with etch-an-rinse adhesives interferes on the durability of resin-dentin bonds when using two- and three-step etch-and-rinse adhesives. The null hypotheses tested were that there are no differences in bond strength to dentin that was treated or not with potassium oxalate and, then, bonded with two or three-step etch-and-rinse adhesives in the following conditions: 1) when tested immediately after the restorative procedures and 2) when tested after 1 year of storage in phosphate-buffered saline (PBS)with 0.02% sodium azide.

MATERIAL AND METHODS

▶ *Teeth selection and preparation*

Thirty freshly extracted human third molars were collected, stored in saline containing 1% thymol at 4°C and used within no longer than 6 months after extraction (Figure 1). 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 pulpal tissue was carefully removed with a pair of small forceps, avoiding crushing the predentin. The dentin surface was further abraded with 600-grit silicon carbide paper, until a remaining dentin thickness of 1.5 ± 0.2 mm was achieved from the ground surface to the highest pulp horn. The resulting crown-segments were glued to Plexiglass slabs (1.8 x 1.8 x 0.7 cm) using viscous cyanoacrylate (Zapit, Dental Ventures of American, Corona, CA, USA), which also covered the entire peripheral cementum. Each Plexiglass slab was penetrated by a short length of 18-gauge stainless steel tubing, which ended flush with the top of the slab. This tube permitted the pulp chamber to be filled with a neutral pH fluid consisting of PBS supplemented with 0.02% sodium azide (Figure 2).

▶ *Specimens preparation and storage for microtensile bond testing*

Three etch-and-rinse adhesive systems were used in this study: two two-step etch-and-rinse adhesives, Adper Single Bond 2 (SB – 3M ESPE, St Paul, MN, USA), One-Step (OS – Bisco Inc., Schaumburg, IL, USA), and one three-step etch-and-rinse system, Adper Scothbond Multi-Purpose (MP-3M ESPE, St Paul, MN, USA). These materials

were used either according to the manufacturers' instructions (control groups) or after the treatment of acid-etched dentin with a potassium oxalate gel (BisBlock - Bisco Inc., Schaumburg, IL, USA) (Table 1). Briefly, the potassium oxalate gel was applied on the acid-etched dentin for 30 s and abundantly rinsed off (60 s). The enamel margins were re-etched for 15 seconds and rinse thoroughly. After potassium oxalate application, adhesives were applied according to manufacturers' instructions **(**Figure 3**).** Surfaces were checked to ensure complete covering with adhesives before light-activation (Degulux Soft-start, DEGUSSA HÜLS, Germany) that was performed under a power density of 500 mW/cm^2 (Figure 4). The specimens received four layers of a resin composite (Z-250 - 3M ESPE) to build up a "crown" approximately 4 mm high. Each 1.0-mm increment was light-cured for 40 s (Degulux Soft Start, DEGUSSA HÜLS, Germany) **(**Figure 5).

After storage in distilled water at 37°C for 24 hours, each tooth was longitudinally sectioned into two halves (Figure 6); one was prepared to be immediately tested in tension and the other had its resin-bonded surface protected with a varnish and, then, it was stored in 0.02% NaN_3-containing phosphate-buffered saline at 37˚C for 1 year. Right before the microtensile testing (immediately or after 1 year of water storage), each hemi-tooth was vertically, serially sectioned with a diamond impregnated disk (Labcut 1010, Extec, Corp., Enfield, CT, EUA) to obtain at least 8 resin-bonded beams, with a cross-sectional area of approximately 0.8 mm^2 **(**Figure 7). These specimens were individually fixed to a custom-made testing jig with cyanoacrylate glue and subjected to microtensile testing at crosshead speed of 0.5mm/min until failure (Model 4411, Instron Corporation, Canton, MA, USA). The fractured specimens were sputter-coated with gold/palladium and examined with a scanning electron microscope (JEOL-5600 LV, Tokyo, Japan) at 15 Kv. The failure modes were classified as cohesive failures in bonding resin and/or in resin composite (CR), in dentin (CD), in hybrid layer (CHL) or as mixed failures (M).

▶ ***Statistical Analysis***

The experimental unit in this study was the hemi-tooth as half of the specimens were tested immediately and the other half after 1 year. A three-way ANOVA and *post hoc* Bonferroni tests were used to analyze the effects of "adhesives" (MP, SB and OS), "dentin

treatment" (control and with oxalate) and "storage time" (immediate *vs* 1 year) on bond strength to dentin. Student's t-tests were used to compare the effect of the water storage on the reduction of bond strength in control *versus* oxalate-treated specimens within each used adhesive. In this analysis adhesives were not compared. Statistical significance was preset at α=0.05.

RESULTS

The means bond strength and standard deviations for all tested groups are shown in figure 8. At an immediate analysis, the treatment of dentin with potassium oxalate was shown to affect negatively the resin-dentin bond strength of dentin specimens, regardless the employed adhesive ($p<0.05$). After 12 months of water storage, the bond strength of resin-bonded interfaces was significantly reduced for all tested groups ($p<0.001$). The decrease in bond strength values was significantly higher for control groups than for experimental groups ($p<0.05$), that is, for those wherein dentin was treated with potassium oxalate. The percentage of bond strength reduction for oxalate-treated groups varied between 18 – 31%, while for controls it ranged between 36 – 52% (Table 2).

When tested immediately, the most prevalent fracture pattern observed for control specimens was mixed failure (M); while for oxalate-treated specimens the failures were almost equally distributed in mixed, cohesive in bonding resin and within the hybrid layer (Table 3). After water storage, CBR was the most frequently mode of failure observed for oxalate-treated specimens; while for control specimens, CHL was the most prevalent failure for SB and CBR was the most prevalent failure for MP and OS (Table 3). Failure mode analyses of representative specimens are shown in figure 9.

DISCUSSION

The previous application of potassium oxalate on acid-etched dentin significantly affected the initial performance of the tested adhesives. In addition, although the decrease in bond strength has been observed for most of specimens submitted to

prolonged storage in water, the percentage of reduction in bond strength for those treated with potassium oxalate was significantly lower than for controls (Table 2). These results, therefore, do not support the acceptance of the null hypotheses that there were no differences in bond strength to dentin when it was treated or not with potassium oxalate and tested immediately or after 1 year of storage in water.

According to previous studies, free fluoride ions released from fluoride-containing adhesives may potentially interact with calcium to form spherical lousily bond calcium fluoride crystals.[20-21] These spherical calcium fluoride crystals are probably derived from dissolution of the calcium oxalate crystals that are also formed by the application of potassium oxalate to acid-etched dentin. Calcium oxalate crystals are located deeper inside the dentinal tubules and their solubility is sensitive to pH changes. That is why adhesives with relatively low pH are thought to create conditions that favor the formation of calcium fluoride crystals. The point is that the calcium fluoride crystals may be located more superficially in the dentinal tubules and they could somehow interfere with the adhesives' infiltration/polymerization,[20-21] compromising the hybrid layer formation.

The adhesives used in the present study were chosen exactly because they exhibit low fluoride content,[20-21] relatively higher pH (3.3 for MP, 3.6 for SB and for 4.5 for OS) (3M ESPE/BISCO - Technical Product profile) and, mainly, for not having affected the immediate bond strength to dentin when used along with oxalates.[17-18,21] Two of the adhesives tested in the present study (SB and OS) were precisely those that, in the mentioned previous studies, showed not to have their bond strength altered by the use of an oxalate desensitizer.[20-21] Despite our results had not confirmed these data, we feel encourage to state that the combined application of this oxalate desensitizer with etch-and-rinse adhesives is not unconditionally reproducible. In a parallel study, we observed that, when applied to acid-etched dentin under similar conditions of the current study, all tested adhesives (OS, SB and MP) had their microhardness significantly compromised by the previous application of BisBlock on acid-etched dentin (Silva et al.,unpublished data).Thus, although the oxalate solution had been thoroughly rinsed before adhesives' application, we believe that residual oxalic acid or other by-products of Bisblok had not been completely

removed from the surface after the rinsing, interfering with the proper polymerization of adhesives and, ultimately, compromise their bonding performance.

Despite BisBlock has affected the initial bond strength to dentin, it seemed to play an important role in decelerating the long-term degradation of the resin-dentin bonds (Figure 8 and Table 2). The decrease in bond strength to oxalate-treated specimens was significantly lower than for controls, regardless of the tested adhesive. Significant reductions of the bond strength to dentin have been observed after middle to long-term water storage.[22-23] Water sorption swells the polymer and reduces the frictional forces between the polymer chains, causing a decrease of their mechanical properties.[24-25] Hydrophilic resins, such as those used in the current study, are highly prone to absorb water,[26-28] having their intrinsic strength damaged, immediately, by plasticization[29-30] and, eventually, by hydrolytic degradation.[31-32] The presence of calcium oxalate crystals partially blocking the fluid transudation across the dentinal tubules may have prevented the adhesives to prematurely absorb water, decelerating their mechanical disruption by the plasticizing effects of water.

In a recent study, Vachiramon et al., (2008)[33] also reported a detrimental effect of oxalate desensitizer on the bond strength to dentin that had been bonded with Adper Single Bond and stored for 3 months under simulated pulpal pressure. TEM micrographs showed that the hybrid layers formed in oxalate-treated dentin were heavily impregnated with silver deposits.

In theory, hybrid layers created with three-step etch-and-rinse adhesives, which indicate the separate application of a resin relatively more hydrophobic over the acid-etched primed-dentin, may be less susceptible to absorb water than those formed by the use of simplified, two-step etch-and-rinse adhesives. However, our results showed that the decrease in the long-term bond strength for specimens bonded with three-step etch-and-rinse adhesive (i.e. MP) was not significantly lower than the bond strength reduction exhibited for specimens bonded with simplified two-step etch-and-rinse adhesives (i.e. SB and OS) (Figure 8). Adhesives that indicate a separate application of hydrophobic co-monomers over the primed dentin may be more resistant to the diffusion of water that comes from external sources than to diffusion of water that is present into the dentinal

tubules and/or in the intertubular dentin. Similarly to the two-step etch-and-rinse adhesives, the hydrophilic components of the primer solutions of three-step etch-and-rinse adhesives[34] responds to the formation of hybrid layer in the acid-etched dentin that is intentionally saturated with water. Thus, the resin layer that is in direct contact with dentin and dentinal tubules using the current adhesives is essentially a hydrophilic structure prone to be permeated by intratubular dentinal fluid.[8,10-11] Fluid movement across resin-bonded dentin presumably travels from fluid-filled dentinal tubules, around the interface between resin tags surrounding the dentin matrix and then through fluid-filled porosities in the overlying adhesive or across the interface between the overlying adhesive and the top of hybrid layer.[13] If the fluid passes through the adhesive, it may accumulate on top of hybrid layer and interfere with coupling to resin composites.[7,35]

Despite the present protocol has been performed with extracted teeth, the bonding procedures were done while the pulp chamber was filled with an aqueous solution and during water storage the margins of resin-dentin interfaces were sealed with varnish so that the water exchange between the teeth and the storage medium was probably limited. These factors may have kept the dentinal tubules and intertubular dentin under a certain degree of hydration. Our results showed that application of potassium oxalate was the only factor that really slowed the rate of resin-dentin bonds degradation (Figure 8 and Table 2). These findings, in concert with the fact that the use of three-step etch-and-rinse adhesive did not prevent the bond strength reduction over time, lead us to speculate that the residual water present into the dentinal tubules can be more relevant to the long-term degradation/stability of hybrid layers than that derived from sources that are external to the tooth.

It has been shown that water in acid-etched dentin can be largely replaced by ethanol. This makes possible the use of more hydrophobic resin blends for dentin bonding.[36] It has been recently found that the permeability of resin-dentin bonds can be significantly reduced if the acid-etched dentin is saturated with ethanol and subsequently bonded with hydrophobic adhesives.[13,37] The ultimate goal of the "ethanol-wet bonding"[36] is to infiltrate the interfibrillar spaces and dentinal tubules with hydrophobic dimethacrylate resins that do not absorb much water. In addition, infiltration of relatively hydrophobic

monomers in ethanol-filled interfibrillar spaces have shown to produce similar or higher initial bond strengths to dentin if compared to those achieved by the infiltration of hydrophilic monomers in water-saturated acid-etched dentin.[36,38-39] These results were obtained when dentin specimens were bonded in the absence of fluid contamination from dental pulp, as the ethanol wet-bonding protocol was found to be very technique-sensitive in the presence of water.[40] Recently, another study showed that it is possible to achieve high bond strengths to dentin when experimental hydrophobic adhesives are used with the adjunctive use of potassium tetroxalate prior to the dehydration of dentin by ethanol saturation.[39] It was suggested that as the dentinal tubules were patently blocked with oxalate crystals, the fluid contamination of dentin surface could have been prevented during the application of hydrophobic adhesives,[40] thereby forming resistant hybrid layers with better quality. Future studies should be conducted to check whether the application of oxalates to acid-etched dentin saturated with ethanol and bonded with hydrophobic adhesives can form resin-dentin bonds that are more stable over time.

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REFERENCES

1 Gwinnett AJ & Kanca JA 3rd (1992) Micromorphology of the bonded dentin interface and its relationship to bond strength *American Journal of Dentistry* 5(2) 73-77.

2 Paul SJ, Leach M, Rueggeberg FA, Pashley DH (1999) Effect of water content on the physical properties of model dentine primer and bonding resins. *Journal of Dentistry* 27(3) 209-214.

3 Wang Y, Spencer P (2005) Continuing etching of an all-in-one adhesive in wet dentin tubules *Journal of Dental Research* 84(4) 350-354.

4 Hashimoto M, Tay FR, Svizero NR, de Gee AJ, Feilzer AJ, Sano H, Kaga M, Pashley DH (2006) The effects of common errors on sealing ability of total-etch adhesives *Dental Material*s 22(6) 560-568.

5 Spencer P, Wang Y (2002) Adhesive phase separation at the dentin interface under wet bonding conditions *Journal of Biomedical Materials Research* 62(3) 447-456.

6 Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P, Van Meerbeek B (2005) Monomer-solvent phase separation in one-step self-etch adhesives *Journal of Dental Research* 84(2) 183-188.

7 Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH (2004) Fluid movement across resin-dentin interface during and after bonding *Journal of Dental Research* 83(11) 843-848.

8 Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A (2002) Single-step adhesives are permeable membranes *Journal of Dentistry* 30(7-8)371-382.

9 Ciucchi B, Bouillaguet S, Holz J, Pashley D (1995) Dentinal fluid dynamics in human teeth, in vivo *Journal of Endodontics* 21(4) 191-194.

10 Bouillaguet S, Duroux B, Ciucchi B, Sano H (2000) Ability of adhesive systems to seal dentin surfaces: an in vitro study *Journal of Adhesive Dentistry* 2(3) 201-

208.

11 Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, Huang C, Prati C, Breschi L, Ferrari M, Pashley DH, Tay FR (2004) In vivo and in vitro permeability of one-step self-etch adhesives *Journal of Dental Research* 83(6) 459-464.

12 Tay, F.R., Gwinnett, A.J., Wei, S.H (1996) The overwet phenomenon: a transmission electron microscopic study of surface moisture in the acid-conditioned, resin-dentin interface American *Journal of Dentistry* 9(4) 161-166.

13 Carrilho MR, Tay FR, Sword J, Donnelly AM, Agee KA, Nishitani Y, Sadek FT, Carvalho RM, Pashley DH (2007) Dentine sealing provided by smear layer/smear plugs vs. adhesive resins/resin tags *European Journal of Oral Science* 115(4)321-329.

14 Gillam DG, Mordan NJ, Sinodinou AD, Tang JY, Knowles JC, Gibson IR (2001) The effects of oxalate-containing products on the exposed dentine surface: an SEM investigation *Journal of Oral Rehabilitation* 28(11) 1037-1044.

15 Muzzin K.B, Johnson R (1989) Effects of potassium oxalate on dentin hypersensitivity in vivo *Journal of Periodontology* 60(3) 151-158.

16 Pashley D.H; Galloway S.E (1985) The effects of oxalate treatment on the smear layer of ground surfaces of human dentine *Archives of Oral Biology* 30(10) 731-737.

17 Pashley DH, Carvalho RM, Pereira JC, Villanueva R, Tay FR (2001) The use of oxalate to reduce dentin permeability under adhesive restorations *American Journal of Dentistry* 14(2) 89-94.

18 Tay FR, Pashley DH, Mak YF, Carvalho RM, Lai SC, Suh BI (2003) Integrating oxalate desensitizing agents with a total-etch two-step adhesive *Journal of Dental Research* 82(9) 703-707.

19 de Andrade e Silva SM, Marquezini L Jr, Manso AP, Garcia FP, Carrilho MR, Pashley DH, Tay FR, Carvalho RM (2007) Effects of a combined application of potassium oxalate gel/adhesive agent on dentin permeability in vitro *Journal of Adhesive Dentistry* 9(6) 505-512.

20 Yiu CK, King NM, Suh BI, Sharp LJ, Carvalho RM, Pashley DH, Tay FR (2005) Incompatibility of Oxalate Desensitizers with Acidic, Fluoride-containing Total-etch Adhesives *Journal of Dental Research* 84(8) 730-735.

21 Yiu CK, Hiraishi N, Chersoni S, Breschi L, Ferrari M, Prati C, King NN, Pashley DH, Tay FR (2006) Single-bottle adhesives behave as permeable membranes after polymerisation. II.Differential permeability reduction with an oxalate desensitizer *Journal of Dentistry* 34(2) 106-16.

22 Beatty MW, Swartz ML, Moore BK, Phillips RW, Roberts TA (1993) Effect of crosslinking agent content, monomer functionally, and repeat unit chemistry on properties of unfilled resins *Journal of Biomedical Materials Research* 27(3) 403-413.

23 Ferracane JL, Berge HX, Condon JR (1998) In vitro aging of dental composites in water – Effect of degree of conversion, filler volume, and filler/matrix coupling *Journal of Biomedical Materials Research* 42(3) 465-472.

24 Söderholm KJ (1984) Water sorption in a bis(GMA)/TEGDMA resin. *Journal of Biomedical Materials Research* 18(3) 271-279.

25 Kalachandra S, Turner DT (1987) Water sorption of polymethacrylate networks: bis-GMA/TEGDM copolymers *Journal of Biomedical Materials Research* 21(3) 329-338.

26 Ito S, Hashimoto M, Wagdaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitane Y, Yoshiyama M, Tay FR, Pashley DH (2005) Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity *Biomaterials* 26 6449-6459.

27 Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK, Carrilho MR (2006) Water sorption/solubility of dental adhesive resins *Dental Material*s 22(10) 973-980.

28 Reis AF, Giannini M, Pereira PN (2007) Influence of water-storage time on the sorption and solubility behavior of current adhesives and primer/adhesive

mixtures *Operative Dentistry* 32(1) 53-59.

29 Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR (2004) Effect of resin hydrophilicity and water storage on resin strength *Biomaterials* 25(26) 5789-5796.

30 Carrilho MR, Tay FR, Pashley DH, Tjäderhane L, Carvalho RM (2005) Mechanical stability of resin-dentin bond components *Dental Materials* 21(3) 232-241.

31 Göpferich A (1996) Mechanisms of polymer degradation and erosion *Biomaterials* 17(2) 103-114.

32 Ferracane J (2006) Hygroscopic and hydrolytic effects in dental polymer networks *Dental Material*s 22(3) 211-222.

33 Vachiramon V, Vargas MA, Pashley DH, Tay FR, Geraldeli S, Qian F, Armstrong SR (2008) Effects of oxalate on dentin bond after 3-month simulated pulpal pressure *Journal of Dentistry* 36(3) 178-185.

34 Van Landuyt KL, Snauwaert J, De Munck J, Coutinho E, Poitevin A, Yoshida Y, Suzuki K, Lambrechts P, Van Meerbeek B (2007) Origin of interfacial droplets with one-step adhesives *Journal of Dental Research* 86(8) 739-744.

35 Itthagarun A, Tay Fr, Pashley Dh, Wefel Js, Garcia-Godoy F, Wei Shy (2004) Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part III. Evidence from fluid conductance and artificial caries inhibition *American Journal of Dentistry* 17(6) 394-400.

36 Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee K, Carrilho MR, Donnelly A, García-Godoy F (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 *American Journal of Dentistry* 20(1) 7-21.

37 Carrilho MR, Tay FR, Donnelly AM, Agee KA, Carvalho RM, Hosaka K, Reis A, Loguercio AD, Pashley DH (2007) Membrane permeability properties of dental adhesive films *Journal of Biomedical Materials Research Part B: Applied*

Biomaterials 27. In press.

38 Nishitani Y, Yoshiyama M, Donnelly AM, Agee KA, Sword J, Tay FR, Pashley DH (2006) Effects of resin hydrophilicity on dentin bond strength *Journal of Dental Research* 85(11) 1016-21.

39 Sadek FT, Pashley DH, Ferrari M, Tay FR (2007) Tubular occlusion optimizes bonding of hydrophobic resins to dentin *Journal of Dental Research* 86(6) 524-8.

40 Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, Tay FR (2008) Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique *Journal of Biomedical Materials Research* 84(1) 19-29.

Table 1. Composition and application mode of materials employed in this study.

Material (Manufacture)		Composition (Batch #)	Application
Gel Potassium Oxalate desensitizer	**BisBlock** (Bisco Inc., Schaumburg, IL, USA)	Oxalic acid, potassium salt and water (0500009787)	Apply for at least 30 s on the acid etched dentine, rinse and leave moist for bonding. If enamel is present, re-etch the enamel, then rinse and leave moist for bonding.
Adhesives	**Adper Scotchbond Multi Purpose** (3 M ESPE, St Paul, MN, USA)	Primer: aqueous solution of HEMA and a polyalkenoic acid copolymer. Adhesive: Bis-GMA, HEMA, initiators (5PH)	Apply primer and dry gently for 5 s. Apply adhesive and light cure for 10 s.
	Adper Single Bond (3M ESPE, St Paul, MN, USA)	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water and ethanol (4BC)	Apply 2 consecutives coats, dry gently for 2 to 5 s and light cure for 10 s.
	One Step (Bisco Inc., Schaumburg, IL, USA)	HEMA, BPDM, initiator and acetone (0600001383)	Apply 2 coats of adhesive and dry for 10 seconds. If the surface is not glossy, apply additional coats and dry.
Resin Composite	**Filtek Z-250**	Bis-GMA, UDMA, Bis-EMA, initiator and zircon/silica filler	Insert four 1.0-mm layers and light cure them, individually, for 40s.

Abbreviations: BIS-GMA: Bisphenol A diglycidyl ether dimethacrylate; Bis-EMA: Bisphenol A polyethylene glycol diether dimethacrylate; HEMA 2-Hydroxyethyl methacrylate, BPDM: Biphenyl dimethacrylate; UDMA: urethane dimethacrylate.

Table 2. Reduction (%) of the bond strength (as a function of the baseline results) for control versus oxalate-treated specimens after 1 year of storage in water.

Adhesives	Control groups	Oxalate-treated groups
MP	41% A	31% B
SB	36% a	18% b
OS	52% α	30% β

Analysis in row: Different case letters indicate statistically significant differences between Control- and Oxalate-treated groups within the factor "adhesive" ($p<0.05$; Student's t-test). Comparisons between adhesives were not performed. MP= Adper Scothbond Multi-purpose system; SB= Adper Single Bond 2 system and OS= One-step system.

Table 3. Percentage distribution of cohesive failure mode.

	Control								Oxalate							
	Immediate				1 year				Immediate				1 year			
	CR	CD	CHL	M	CR	CD	CHL	M	CR	CD	CHL	M	CR	CD	CHL	M
MP	18	0	30	52	54	0	46	0	24	0	38	38	26	0	32	42
SB	7	0	35	57	0	0	77	23	38	0	29	32	59	0	14	18
OS	38	3	0	59	64	0	4	32	32	4	46	18	50	0	27	23

CR: cohesive in bonding resin and/or resin composite, CD: cohesive in dentin, CHL: within the hybrid layer and M: mixed. MP= Adper Scothbond Multi-purpose system; SB= Adper Single Bond 2 system and OS= One-step system.

FIGURES

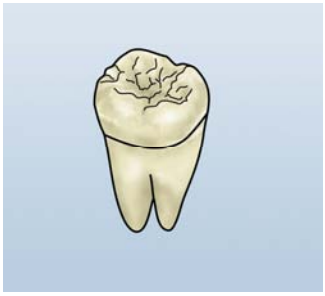

Fig.1 Extracted human third molars.

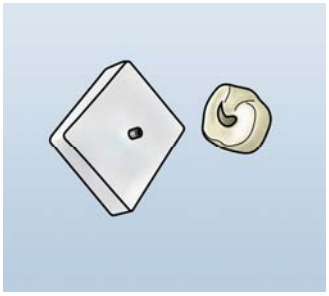

Fig.2 Crown-segments prepared after removal of occlusal enamel, roots and pulpal tissue.

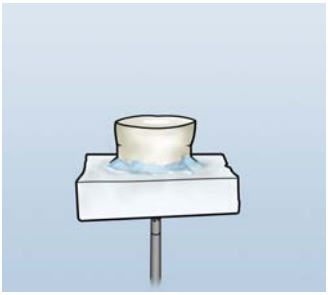

Fig. 3 Crown-segments glued to Plexiglass slabs.

Fig.4 Adhesive application associated or not with potassium oxalate**.**

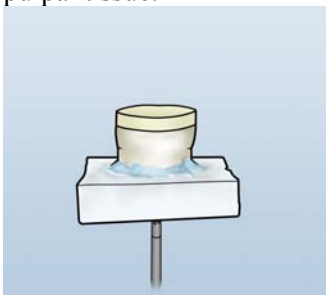

Fig.5 Crown built up with resin composite to permit microtensile tests**.**

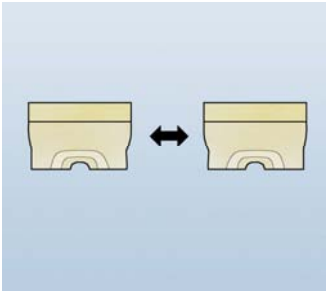

Fig.6 Tooth sectioned into two halves after storage for 24h in PBS containing sodium azide.

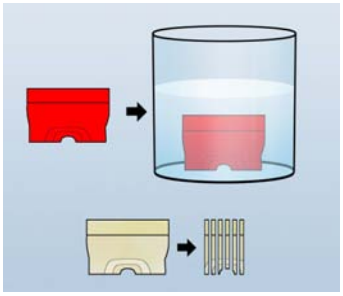

Fig.7 One half of the teeth prepared to be immediately tested and the other half had its resin-bonded interface protect with a nail varnish and then it was stored in PBS containind sodium azide for 12 months.

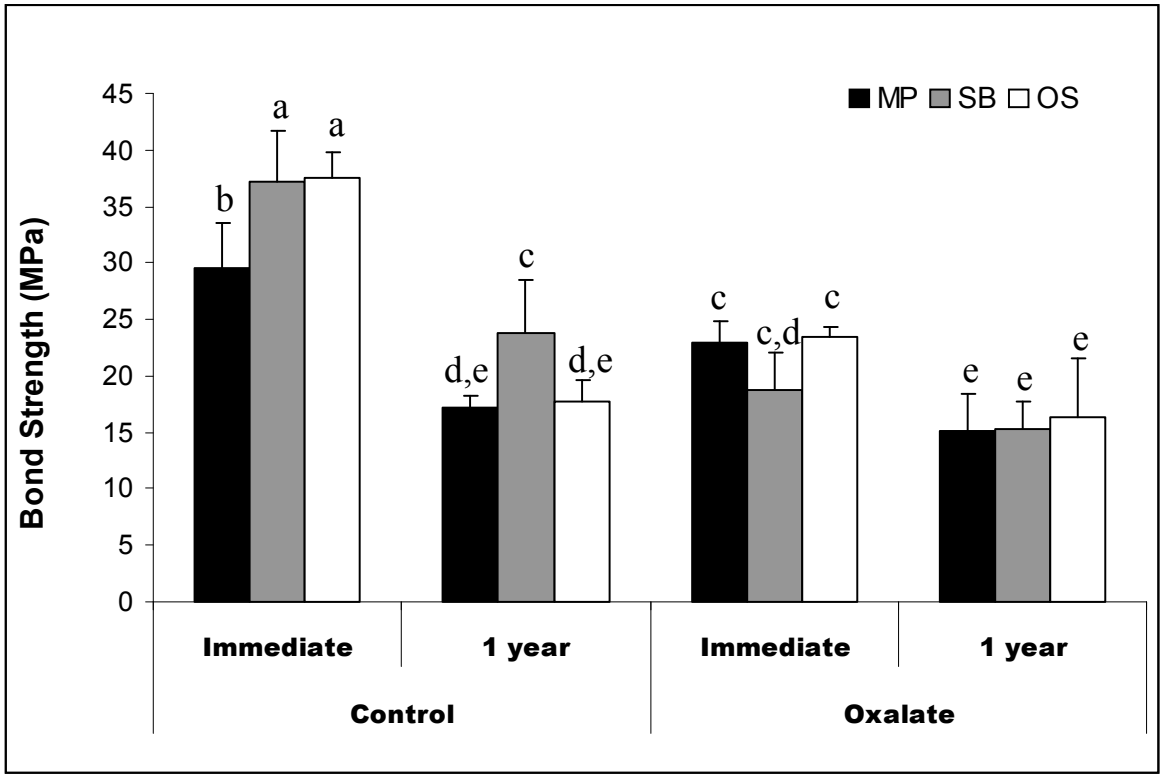

Figure 8. The means bond strength and standard deviations for all tested groups (n^{o} specimens = 5/ experimental condition. **MP**= Adper Scothbond Multi-purpose system;**SB**= Adper Single Bond 2 system and **OS**= One-step system. The height of the bars is the bond strength mean; half-brackets indicate plus one standard deviation. Groups identified with the same case letters did not differ statistically ($p>0.05$).

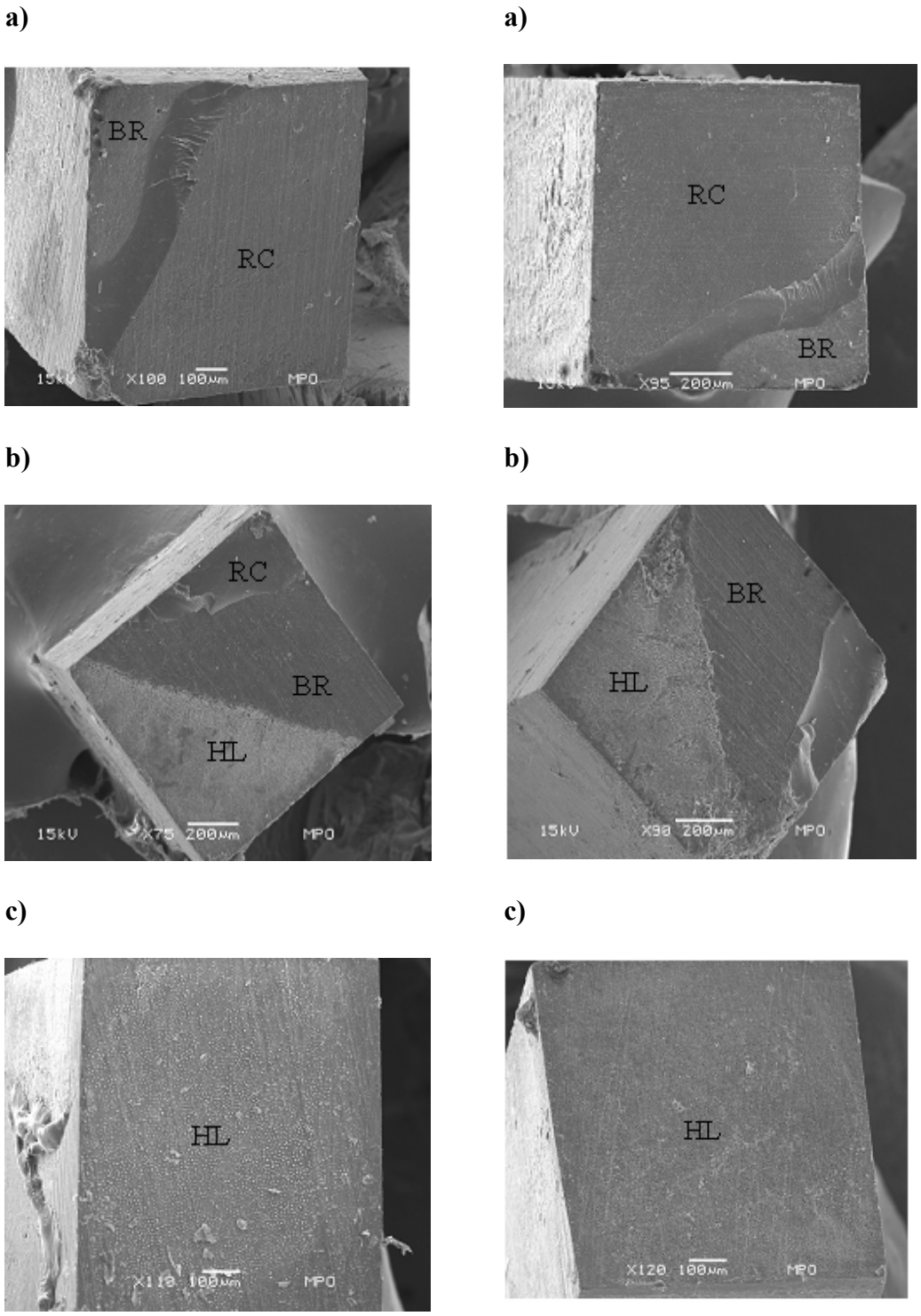

Figure 9. SEM observations of the fractured dentin surfaces. (a) Cohesive in bonding resin. (b) Mixed failure, at the hybrid layer, bonding resin and resin composite (c) Cohesive in hybrid layer. RC, resin composite; BR, bond resin; HL, hybrid layer.

CAPÍTULO 3:

Effects of potassium oxalate on Knoop hardness of etch-and-rinse adhesives

SUMMARY

Objectives: To determine whether the hardness of etch-and-rinse adhesives is affected by the pretreatment of acid-etched dentin with potassium oxalate gel. **Methods:** Unerupted human third molars were cut into crown segments by removing the occlusal enamel and roots. The pulp chamber of these crown segments was connected to a syringe barrel filled with phosphate buffered saline so that the moisture of dentin was maintained during the bonding procedures. Three etch-and-rinse adhesives – two two-step systems (Adper Single Bond 2 - SB, One-Step - OS) and one three-step system (Adper Scothbond Multi-Purpose - MP) were applied to acid-etched dentin that had been pretreated (experimental groups) or not (control groups) with potassium oxalate (BisBlock). The Knoop hardness (KHN) of adhesives was taken in different sites of the outer surface of adhesive-bonded dentin. **Results:** The KHN of the three tested adhesives applied to acid-etched dentin pretreated with potassium oxalate was significantly lower than that exhibited by their respective controls ($p<0.05$). Regardless of the pretreatment with potassium oxalate, the adhesive OS exhibited the lowest KHN when compared to MP and SB systems ($p<0.05$). **Conclusions** Application of potassium oxalate on acid-etched dentin may interfere with the properties of adhesives that are subsequently applied to dentin.

Keywords: etch-and-rinse adhesives, potassium oxalate, Knoop hardness, adhesives polymerization.

INTRODUCTION

In contemporary dental adhesives, high concentrations of relatively hydrophilic methacrylate monomers (i.e. HEMA, BPDM, PENTA) are blended with relatively hydrophobic monomers (i.e. Bis-GMA, UDMA) and solvents (i.e. ethanol, acetone) to enhance bonding to intrinsically water-wet dentin. The presence of hydrophilic monomers and volatile solvents improves the wetting performance of dental adhesives when applied to acid-etched dentin that is intentionally saturated with water. Volatile solvents facilitate the displacement of water from the acid-etched dentin matrix,[1] ensuring better monomers penetration into the micro- and nanoporosities left between the collagen fibrils[2] and, thus, improving their micro-retention to the tooth substrate.[3-4]

Although the water-wet bonding technique may facilitate the infiltration of hydrophilic resin monomers into demineralized dentin, the presence of residual solvent/water before the photo-activation of adhesives has thought to be responsible for producing localized areas of incomplete monomer polymerization, [5-6] which correspond with the sites of porosities revealed by silver deposition in nanoleakage studies.[7] These porous interfaces/polymers are prone to permeation by water[7] and, to a certain extent, by small solutes.[8-9] Polymers containing a mixture of hydrophilic and/or ionic domains become swollen by absorbed water [10] and allow fluid transport in and out of the cross-linked polymer network.[6-7] The hydrophilic adhesives, therefore, behave as permeable membranes[11] that cannot achieve the requirement for perfect sealing of dentin. [12-13]

During bonding procedures, most of the water that is trapped within the adhesive layer or accumulated on its surface originates from the underlying hydrated dentin.[14] Studies have suggested that the solvent evaporation procedures (i.e. air blasts) or even the hypertonicity of solvated adhesive may be responsible for creating an osmotic gradient that induces outward water movement from the underlying hydrated dentin into the adhesive. [6,12]

One way of avoiding the negative consequences caused by the permeability of hydrophilic adhesives is to take advantage of the use of oxalate desensitizers prior to adhesives' application.[15-16] Oxalate solutions or gels react with ionized calcium in dentin to

form insoluble crystals of calcium oxalate. [17-18] Due to its ability to occlude the dentinal tubules, oxalate-based desensitizers are considered as being potent agents in the treatment of dentin sensitivity.[18] The calcium oxalate crystals formed into the dentinal tubules were shown to reduce the fluid conductance of dentin,[19-20] reducing the pain sensation.[21] As "side 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 the 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.

When applied to acid-etched dentin, the calcium oxalate crystals are formed beyond the acid-etched surface and, in theory, they should not interfere with the subsequent bonding procedures.[15] However, the data on the effect of oxalate desensitizers on the bonding performance of adhesives is scant and unclear. While the oxalates can constitute an alternative to prolong the longevity of resin-dentin bonds, we have recently observed that the application of potassium oxalate to the acid-etched dentin affected the baseline bond strength of two- and three-step etch-and-rinse adhesive systems [Silva et al., unpublished data]. We speculated that despite the oxalate solution has been thoroughly rinsed before the application of adhesives to dentin, residual oxalic acid or any other of its by-products (i.e. thickening agent) could have remained on the dentin surface then interfering with the proper polymerization of the adhesives and, consequently, compromising their bonding performance.

The aim of this study was to determine whether the polymerization of adhesives can be affected by the pretreatment of acid-etched dentin with a potassium oxalate desensitizer. Since hardness measurements have been accepted as a good predictor of the degree of polymerization of dental resins,[22-24] we tested the hardness of three etch-and-rinse adhesives after their application to acid-etched dentin that was pre-treated or not with potassium oxalate gel. The null hypothesis tested was that: the hardness of adhesives applied to acid-etched dentin pretreated with potassium oxalate did not differ of that hardness exhibited by adhesives applied to acid-etched dentin with no oxalate pretreatment.

MATERIALS AND METHODS

Teeth preparation

Thirty non-carious human third molars extracted for orthodontics reasons were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the Ethics Committee of the University of Campinas (Figure 1). These teeth were stored in saline containing 0.02% sodium azide and used within no longer than 6 months after extraction.

Crown-segments were prepared by removing the occlusal enamel and roots of these teeth, using a slow-speed diamond saw (Labcut 1010®, Extec, Corp., Enfield, CT, EUA) under water-cooling. The pulp tissue was carefully removed with a pair of small forceps. Care was taken to avoid touching the pulp chamber walls for consequently not crushing the predentine toward the dentinal tubules, which could alter the final permeability of dentin (David Pashley, personal communication). The dentin surface was further abraded with 600-grit silicon carbide paper, until a remaining dentin thickness of 1.5 ± 0.2 mm was achieved from the ground surface to the highest pulp horn. The resulting crown segments were glued to Plexiglass slabs (1.8 x 1.8 x 0.7 cm) using viscous cyanoacrylate (Zapit, Dental Ventures of American, Corona, CA, USA), which also covered the entire peripheral cementum (Figure 2). Each Plexiglass slab was penetrated by a short length of 18-gauge stainless steel tubing, permitting the pulp chamber to be filled with a neutral pH fluid consisting of phosphate buffered saline supplemented with 0.02% sodium azide, simulating the natural water content of dentin and guarantying a moist environment during the bonding procedures (Figure 3).

Bonding procedure

The exposed dentin surfaces were polished with a # 600 grit SiC paper during 30 s and, then, they were acid-etched with 35% phosphoric acid gel (3M ESPE, St Paul, MN, USA) for 15 s and rinsed thoroughly with tap water for 30 s. The specimens were divided into two groups for which the bonding procedures were performed on acid-etched dentin (control group) or acid-etched potassium oxalate-treated dentin (experimental

group). For the experimental group, the potassium oxalate gel, Bisblock (BISCO Inc., USA), was applied on the surface for 30 s and rinsed off with tap water for 60 s (Figure 4).

Three etch-and-rinse adhesive systems were selected for this study: the two-step systems: Adper Single Bond (SB – 3M ESPE, St Paul, MN, USA) and One-Step (OS – Bisco, Schaumburg, IL, USA) and the three-step system: Adper Scothbond Multi-Purpose (MP-3M ESPE, St Paul, MN, USA). In principle, these adhesives were applied to the acid-etched dentin surfaces of control and experimental groups while dentin was visibly moist with water as recommended by manufacturers. Nevertheless, this condition created such a soft bonded-surface that the hardness could not be recorded even after storing the specimens in dry conditions and testing those 48 h after the adhesives' polymerization. Thus, adhesives were applied vigorously to the acid-etched dentin of control and experimental groups after the dentin surface was air-dried for 30 s with an oil-free compressed air.[25] This "dry bonding" technique ensured that the only source of water that could potentially interfere with adhesives hardness was the one that was present in the pulp chamber by outward dentinal fluid flow during bonding procedures.

Surfaces were checked to ensure complete covering with adhesives and a glass cover slip was placed on the top of the adhesive in order to create a flat adhesive surface avoiding excessive contact with the atmospheric oxygen during light-activation [26] (Figure 5). The adhesives were light-cured for 20 s using a halogen-tungsten unit (Degulux, DEGUSSA HÜLS) operated at 500mW/cm^2. Once polymerized, the specimens were stored in dry conditions at 37^{o}C until hardness measurement was taken.

Hardness measurement

Twenty-four hours after the bonding procedures was completed, the specimens' hardness were determined with a Shimadzu HMV-2 hardness tester (Shimadzu Corporation, Kyoto- Japan), equipped with a Knoop indenter at 25 g of load and 6 s dwell time. Six indentations were performed on the top of the adhesive-bonded dentin surfaces, over the sites that correspond to the pulp horns, where the intrinsic wetness may pose a severe challenge to dentin bonding systems. At least three indentations in each specimen were performed in enamel bonded substrate, simulating a situation where the adhesive cannot be influenced by the surface moisture (negative controls) (Figure 6).

Knoop hardness was determined by examining the surface with an optical microscope (40 X) and expressed as Knoop Hardness Number (KHN).

Statistical analysis

The KHN determined for control and experimental groups were analyzed by two-way ANOVA tests, having as main factors: the adhesives (i.e. SB, OS and MP) and the substrate treatments (i.e. control versus oxalate-treated), with the data derived from dentin being analyzed separately from those derived from enamel. Post-hoc multiple comparisons were performed using Tukey's tests. Statistical significance was preset at $\alpha = 0.05$.

RESULTS

The mean KHN of the adhesive-bonded dentin and adhesive-bonded enamel are seen Figures 7 and 8, respectively. The pretreatment of dentin or enamel with potassium oxalate was shown to affect significantly the KHN of the three adhesives, when compared to their respective controls ($p<0.05$) (Figures 7 and 8). In general, the KHN obtained for enamel substrate (10 - 16.6) was higher than those for dentin (7.6 – 15.0).

For both tested substrates (i.e. dentin and enamel), the OS system exhibited the lowest KHN when compared to the other tested systems (SB and MP) ($p<0.05$), regardless of the surface pretreatment (control or oxalate-treated). The mean KHN for OS adhesive when applied to dentin varied between 8.6 (control) and 7.6 (oxalate-treated dentin). For MP adhesive system, the mean KHN tested on dentin ranged from 14.2 (control) to 12.4 (oxalate-treated dentin). These values did not differ significantly ($p>0.05$) from those observed for the SB adhesive (for comparisons between correspondent groups, i.e. MP control *vs* SB control; MP experimental *vs* SB experimental), for which the mean KHN varied between 15.0 (control) and 13.2 (oxalate-treated dentin).

For enamel substrate, the differences in the KHN were significant only for the factor "substrate treatment" ($p<0.05$), while statistic significance for the main factor "adhesives" and the interaction between two main factors (adhesives X substrate treatment) were not observed ($p>0.05$) (Figure 8).

DISCUSSION

The results of the present study demonstrated that when applied to oxalate-treated acid-etched dentin, all tested etch-and-rinse adhesives (OS, SB and MP) had their KHN significantly compromised, which lead us to reject the anticipated null hypothesis that the hardness of these adhesives applied to acid-etched dentin versus oxalate-treated acid-etched do not differ.

Several studies have found out a positive correlation between hardness and degree of conversion.[22-24] While this may not be a full consensus in the literature, [28-29] as hardness may reflect the degree of crosslinking between the polymer chains,[30] it has been conveniently used to compare the extent of polymerization exhibited by different dental resins under numerous testing conditions (i.e. varying the light-curing time, the amount of energy delivered during photo-activation, the type of light source, the distance between the light-curing unit and the sample, etc).[23,29-30] The density and distribution of crosslinks between polymer chains [31-32] play an important role in the final cohesion of polymers.[33] Polymer networks with homogeneous packing density (i.e. restricted free volume and high level of polymer's chain crosslinking) tend to exhibit higher mechanical properties.[34] According to Rueggeberg 1988[26], hardness is sensitive in detecting small changes in polymer crosslinking so that reductions in its KHN may suggest the existence of a less densely cross-linked polymer.[30] If this assumption was extrapolated to explain the present results, we could presume that potassium oxalate interfered with adhesives polymerization and mechanical properties by favoring the formation of linear, poorly crosslinked adhesives.

Earlier studies that evaluated the effectiveness of oxalate desensitizers in reducing the permeability of the resin-bonded dentin also indicated that these oxalates may have a variable effect on the bond strength of resin-bonded dentin , [15-16,35] depending on which adhesive they were combined to.[35] Yiu and co-workers (2005) [35] showed that fluoride ions released from fluoride-containing adhesives with relatively low pH may potentially interact with calcium-oxalate crystals to form spherical loosely bound calcium fluoride crystals that could interfere with the adhesives' infiltration/polymerization, thereby

compromising the hybrid layer formation. Based on this study [35], the pH of the adhesives SB, OS and MP was not low enough to cause the solubility of calcium oxalates. For this reason, we decided to use adhesives that exhibit low fluoride content and relatively higher pH (3.3 for MP, 3.6 for SB and for 4.5 for OS) [3M ESPE - Technical Product Profile, respectively). Thus, we assume that the decrease in KHN of the adhesives applied on oxalate-treated dentin that was observed could not be caused by the low pH and or high fluoride content of the tested adhesives.

At first, it might be speculated that despite the oxalate solution had been thoroughly rinsed before adhesives' application, residual oxalic acid may have remained to react with calcium, causing crystal precipitation on the dentin surface, thereby compromising the adhesives hardness. However, since the dentin surface was probably deprived of calcium phosphate due to its phosphoric acid-etching,[36] we are forced to consider that the decrease in adhesives hardness was unlikely caused by crystals precipitation. One could argue that indeed the instantaneous dissolution of calcium oxalate crystals present in the dentinal tubules may potentially provide free calcium for reacting with residual oxalic acid, thus causing the precipitation of calcium oxalate on the acid-etched dentin surface. Nevertheless, we believe this was also improbable. In fact, it is likely that other by-products of Bisblock had not been completely removed from the surface after the rinsing, thereby interfering with the proper polymerization of adhesives. This speculation may also explain the decrease in KHN in enamel, as manufacture recommends a second etch with phosphoric acid gel before adhesive application in order to remove the calcium oxalate crystals from the surface.

An unexpected finding of this study was that the three-step adhesive (i.e. MP) did not show a higher KHN value, when compared to SB, a two-step adhesive. Supposedly, the inclusion of solvent and hydrophilic components in two-step etch-and-rinse adhesives should make these softer than non-solvated adhesives.[5,37] This is because residual solvent, which cannot be completely eliminated from the adhesive before light-curing, [38] may plasticize the polymer network and reduce its mechanical strength. Thus, the adhesive MP that indicates a separate application of a relatively more hydrophobic, non-solvated resin over the acid-etched primed-dentin was supposed to exhibit the highest KHN values.

However, by assuming that a Bisblock by-product has remained in tested specimens, one may speculate that these by-products could have also contamined the bonding agent of MP adhesive as this was partially mixed with the unpolymerized primer solution that was set on the oxalate-treated dentin.

Although the results of the present study showed that potassium oxalate applied on acid-etched dentin affected the baseline hardness of the tested adhesives, a parallel study that we recently concluded showed that potassium oxalate played an important role in decelerating the long-term degradation of the resin-dentin bonds created using the same adhesives [Silva et al.,unpublished results]. Most likely, the presence of calcium oxalate crystals partially blocking the fluid transudation across the dentinal tubules may have prevented the adhesives to prematurely absorb water, decelerating their mechanical disruption by the plasticizing effects of water and then prolonging their lifetime.

The use of potassium oxalate on acid-etched dentin could also be useful in the "ethanol-wet bonding" technique.[39] The objective of this technique is to use more hydrophobic resin blends for dentin bonding, in order to reduce adhesive permeability and dentin-bonds degradation. As hydrophobic monomers do not bond well to the water-saturated dentin, ethanol is used to replace rinse-water from acid-etched matrices.[40-41] However, as ethanol wet-bonding protocol was found to be very technique-sensitive in the presence of water, the dentinal tubules could be patently blocked with calcium oxalate crystals in order to prevent fluid contamination during the application of hydrophobic adhesives.[42]

Recently studies have shown that infiltration of relatively hydrophobic monomers in ethanol-filled interfibrillar spaces produced similar or higher initial bond strengths to dentin if compared to those achieved in "wet-bonding" technique.[41-42] If the concept of ethanol wet-bonding is not as far as it seems, application of oxalates to acid-etched dentin saturated with ethanol will be necessary in order to bond hydrophobic adhesives on dentin substrate producing more durable resin-dentin bonds. However, futures studies are still necessary to access the influence of potassium oxalate gels on adhesion realized on ethanol-saturated dentin prior the clinical implementation of this approach.

CONCLUSIONS

Although potassium oxalate has previously shown to produce resin-dentin bonds that are more stable over time, it may adversely affect the baseline hardness of etch-and-rinse adhesives.

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REFERENCES

1 Jacobsen T, Söderholm KJ. Effect of primer solvent, primer agitation, and dentin dryness on shear bond strength to dentin. *American Journal of Dentistry* 1998; **11**:225-8.

2 Tay FR, Gwinnett AJ, Wei SH. Ultrastructure of the resin-dentin interface following reversible and irreversible rewetting. *American Journal of Dentistry* 1997;**10**:77-82.

3 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. *Dental Materials* 2005; **21**:864-81.

4 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. *Dental Materials* 2008; **24**:90-101.

5 Paul SJ, Leach M, Rueggeberg FA, Pashley DH. Effect of water content on the physical properties of model dentine primer and bonding resins. *Journal of Dentistry* 1999; **27**:209-214.

6 Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentin interface during and after bonding *Journal of Dental Research* 2004; **83**:843-8.

7 Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. *Journal of Dentistry* 2002; **30**:371-82.

8 Tay FR, Pashley DH, Yiu C, Cheong C, Hashimoto M, Itou K, Yoshiyama M, King NM. Nanoleakage types and potential implications: evidence from unfilled and filled adhesives with the same resin composition. *American Journal of Dentistry* 2004; **17**:182-90.

9 Pashley DH. Dentine permeability and its role in the pathobiology of dentine sensitivity. *Archives of Oral Biology* 1994; **39**:73S-80S.

10 Unemori M, Matsuya Y, Matsuya S, Akashi A, Akamine A. Water absorption of poly(methyl methacrylate) containing 4-methacryloxyethyl trimellitic anhydride. *Biomaterials* 2003; **24**:1381-7.

11 Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, Huang C, Prati C, Breschi L, Ferrari M, Pashley DH, Tay FR. In vivo and in vitro permeability of one-step self-etch adhesives. *Journal of Dental Research* 2004; **83**:459-64.

12 Tay FR, Frankenberger R, Krejci I, Bouillaguet S, Pashley DH, Carvalho RM, Lai CN. Single-bottle adhesives behave as permeable membranes after polymerization. I. In vivo evidence. *Journal of Dentistry* 2004; **32**:611-21.

13 Carrilho MR, Tay FR, Donnelly AM, Agee KA, Carvalho RM, Hosaka K, Reis A, Loguercio AD, Pashley DH. Membrane permeability properties of dental adhesive films. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2007 (in press).

14 Moll K, Haller B. Effect of intrinsic and extrinsic moisture on bond strength to dentine. *Journal of Oral Rehabilitation* 2000; **27**:150-65.

15 Pashley DH, Carvalho RM, Pereira JC, Villanueva R, Tay FR. The use of oxalate to reduce dentin permeability under adhesive restorations. *American Journal of Dentistry* 2001; **14**:89-94.

16 Tay FR, Pashley DH, Mak YF, Carvalho RM, Lai SC, Suh BI. Integrating oxalate desensitizers with total-etch two-step adhesive. *Journal of Dental Research* 2003; **82**:703-7.

17 Pashley DH, Livingston MJ, Reeder OW, Horner J. Effects of the degree of tubule occlusion on the permeability of human dentine in vitro. *Archives of Oral Biology* 1978; **23**:1127-33

18 Pashley DH. Dentin permeability, dentin sensitivity, and treatment through tubule occlusion. *Journal of Endodontics* 1986; **12**:465-74.

19 Yiu CK, Hiraishi N, Chersoni S, Breschi L, Ferrari M, Prati C, King NN, Pashley DH, Tay FR. Single-bottle adhesives behave as permeable membranes after polymerisation. II. Differential permeability reduction with an oxalate desensitiser. *Journal of Dentistry* 2006; **34**:106-16.

20 de Andrade e Silva SM, Marquezini L Jr, Manso AP, Garcia FP, Carrilho MR, Pashley DH, Tay FR, Carvalho RM. Effects of a combined application of potassium oxalate gel/adhesive agent on dentin permeability in vitro. *Journal of Adhesive*

Dentistry 2007; **9**:505-12.

21 Brännström M. Ethiology of dentin hypersensitivity. *Proceedings of the Finnish Dental Society* 1992; **88**: 7-13.

22 Ferracane JL. Correlation between hardness and degree of conversion during the setting reaction of unfilled dental restorative resins. *Dental Materials* 1985; **1**:11-4.

23 Rode KM, Kawano Y, Turbino ML. Evaluation of curing light distance on resin composite microhardness and polymerization. *Operative Dentistry* 2007; **32**:571-8.

24 Bouschlicher MR, Rueggeberg FA, Wilson BM. Correlation of bottom-to-top surface microhardness and conversion ratios for a variety of resin composite compositions. *Operative Dentistry* 2004; **29**:698-704.

25 Reis A, Loguercio AD, Carvalho RM, Grande RH. Durability of resin dentin interfaces: effects of surface moisture and adhesive solvent component. *Dental Materials* 2004; **20**:669-76.

26 Rueggeberg FA, Craig RG. Correlation of parameters used to estimate monomer conversion in a light-cured composite. *Journal of Dental Research* 1988; **67**:932-7.

27 Ferracane JL, Berge HX, Condon JR. In vitro aging of dental composites in water--effect of degree of conversion, filler volume, and filler/matrix coupling. *Journal of Biomedical Materials Research* 1998; **42**:465-72.

28 Chung KH, Greener EH. Correlation between degree of conversion, filler concentration and mechanical properties of posterior composite resins. *Journal of Oral Rehabilitation* 1990; **17**:487-94.

29 da Silva EM, Poskus LT, Guimarães JG. Influence of light-polymerization modes on the degree of conversion and mechanical properties of resin composites: a comparative analysis between a hybrid and a nanofilled composite. *Operative Dentistry* 2008; **33**:287-93.

30 Calheiros FC, Daronch M, Rueggeberg FA, Braga RR. Degree of conversion and mechanical properties of a BisGMA:TEGDMA composite as a function of the applied radiant exposure. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2008; **84**:503-9.

31 Andrady AL, Sefcik MD. Transport of hydrogen and carbon monoxide in highly

crosslinked poly(propylene glycol) networks Journal Polymeric Science Polymer Physics. 22nd ed.1984. p.237-243.

32 Ajithkumar S, Patel NK, Kansara SS. Sorption and diffusion of organic solvents through interpenatrating polymer networks (IPNs) based on polyurethane and unsaturated polyester *European Polymer Journal* 2000; **36**: 2387-2393.

33 Sideridou I, Tserki V, Papanastasiou G. Study of water sorption, solubility and modulus of elasticity of light-cured dimethacrylate-based dental resins. *Biomaterials* 2003; **24**: 655-65.

34 Elliott JE, Lovell LG, Bowman CN. Primary cyclization in the polymerization of bis-GMA and TEGDMA: a modeling approach to understanding the cure of dental resins *Dental Materials* 2001; **17**: 221-9.

35 Yiu CK, King NM, Suh BI, Sharp LJ, Carvalho RM, Pashley DH, Tay FR. Incompatibility of Oxalate Desensitizers with Acidic, Fluoride-containing Total-etch Adhesives. *Journal of Dental Research* 2005; **84**:730-5.

36 Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Operative Dentistry* 2003;**28**:215-35.

37 Sideridou ID, Karabela MM, Vouvoudi ECh. Volumetric dimensional changes of dental light-cured dimethacrylate resins after sorption of water or ethanol. *Dental Material* 2008 **24**:1131-6.

38 Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, Pashley DH. Effects of HEMA on water evaporation from water-HEMA mixtures. *Dental Materials* 1998; **14**:6-10.

39 Sadek FT, Pashley DH, Ferrari M, Tay FR. Tubular occlusion optimizes bonding of hydrophobic resins to dentin. *Journal of Dental Research* 2007; **86**: 524-8.

40 Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee K, Carrilho MR, Donnelly A, García-Godoy F. 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. *American Journal of Dentistry* 2007; **20**:7-

21.

41 Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, Tay FR. Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique *Journal of Biomedical Materials Research* 2008*;* **84**:19-29.

42 Nishitani Y, Yoshiyama M, Donnelly AM, Agee KA, Sword J, Tay FR, Pashley DH. Effects of resin hydrophilicity on dentin bond strength *Journal of Dental Research 2006;* **85**:1016-21.

FIGURES

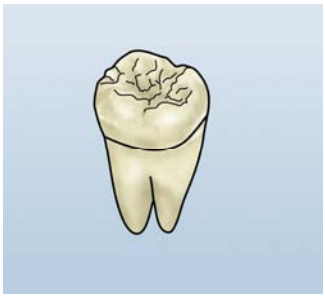

Fig.1 Extracted human third molars.

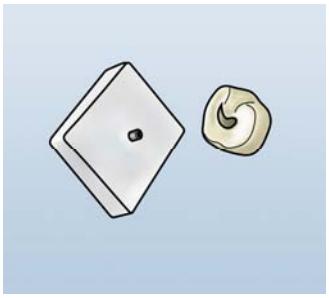

Fig.2 Crown-segments prepared after removal of occlusal enamel, roots and pulpal tissue.

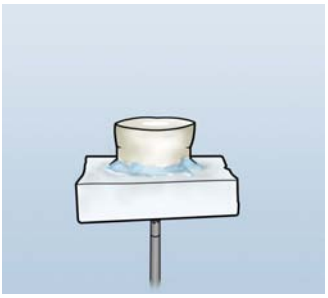

Fig. 3 Crown-segments glued to Plexiglass slabs.

Fig.4 Adhesive application associated or not with potassium oxalate**.**

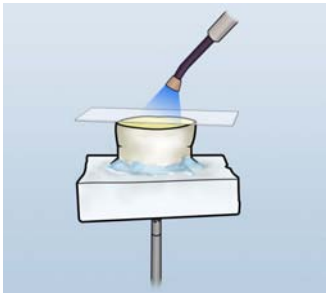

Fig.5 Glass cover slip placed on the top of the adhesive during light activation.

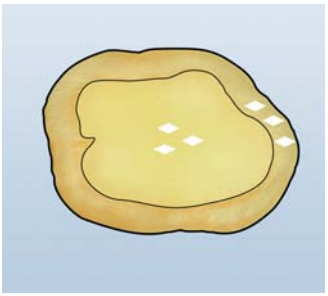

Fig.6 Indentations performed in enamel and dentin bonded substrate.

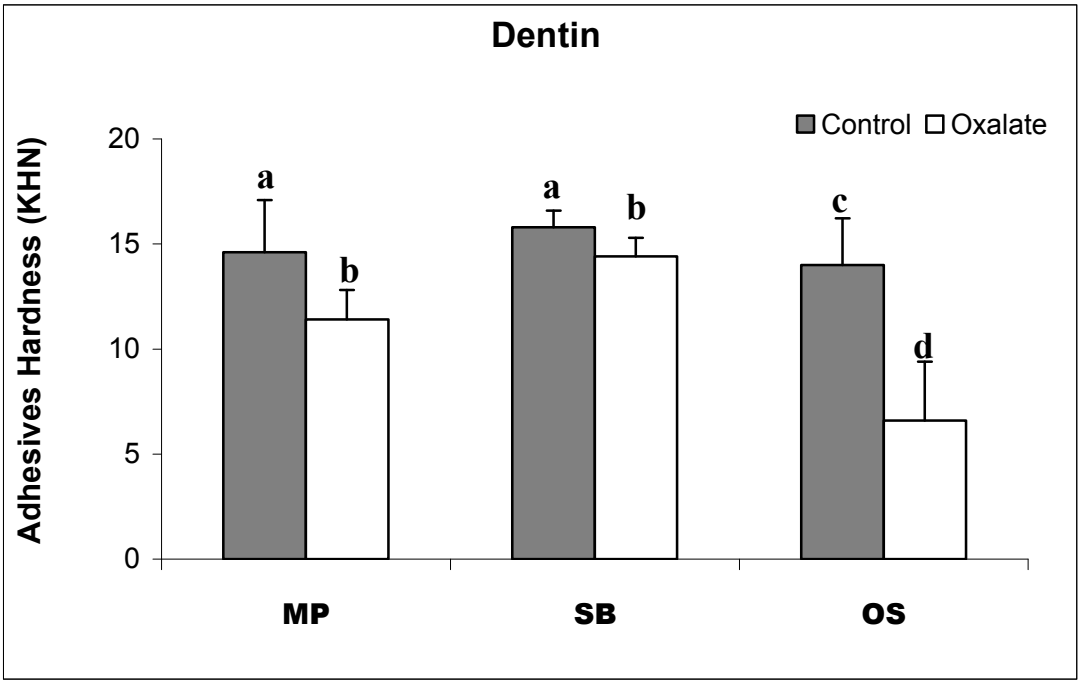

Figure 7. The mean KHN and standard deviation (in KHN) of the tested groups on dentin (n=30). **MP**= Adper Scothbond Multi-purpose system; **SB**= Adper Single Bond 2 system and **OS**= One-step system. The height of the bars is KHN mean; half-brackets indicate plus one standard deviation. Groups identified with the same case letters did not differ statistically ($p>0.05$).

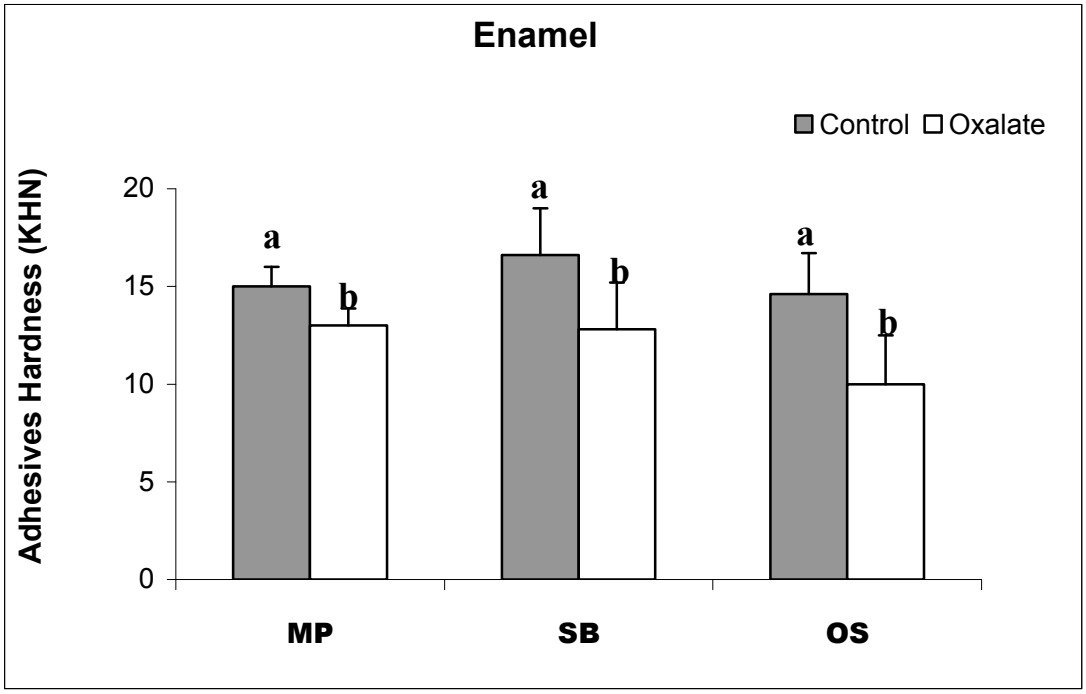

Figure 8. The KHN and standard deviations of the tested groups on enamel substrate (n=30). **MP**= Adper Scothbond Multi-purpose system; **SB**= Adper Single Bond 2 system and **OS**= One-step system. The height of the bars is KHN mean; half-brackets indicate plus one standard deviation. Groups identified with the same case letters did not differ statistically ($p>0.05$).

CONSIDERAÇÕES GERAIS

No primeiro capítulo deste trabalho observou-se que os adesivos não garantem o selamento hermético da dentina e que por isto funcionam como membranas permeáveis, permitindo que fluidos permeiem através da sua estrutura polimerizada. A aplicação de uma camada adicional do próprio adesivo não resultou em redução significativa da permeabilidade, já que, por conter a mesma quantidade de monômeros hidrofílicos e solventes, resultou na formação de mais uma camada de adesivo altamente permeável. Portanto, podemos concluir com base neste estudo, que o aumento da espessura da camada do adesivo de passo único não resulta na formação de um polímero mais coeso e menos permeável, pois, previsivelmente, os solventes continuam retidos nesta interface, prejudicando a conversão dos monômeros e formação das ligações cruzadas entre as cadeias poliméricas. Ao contrário, a aplicação de uma resina com característica mais hidrofóbica e livre de solventes sobre a dentina hibridizada reduziu significantemente a permeabilidade dentinária, pois estes tiveram melhores condições de se converter em um polímero mais coeso, tornando-os deste modo menos susceptível a permeação de fluidos.

Este estudo confirma os achados de outros trabalhos que também demonstraram que a permeabilidade dos adesivos simplificados se deve às modificações de formulação que foram introduzidas, no sentido de tona-los hidrofílicos e tecnicamente simples. A conversão de sistemas de 2 passos para 3 passos, apesar de aumentar o tempo clínico, parece ser benéfica no que cerne a qualidade desta adesão. No futuro, os fabricantes deveriam considerar o fornecimento de um frasco de adesivo livre de solventes nos seus kits para uso opcional pelo profissional.

No segundo capítulo deste estudo a resistência adesiva imediata e em longo prazo destes mesmos adesivos em associação com o oxalato de potássio foi avaliada. Os resultados mostraram que esta associação causou uma queda na resistência adesiva imediata dos adesivos, quando comparados ao grupo controle (sem oxalato). O resultado deste estudo vai de encontro a outros trabalhos na literatura, que, ao contrário, demonstraram que o oxalato de potássio, não causa diminuição da resistência de união de adesivos que tenham as mesmas características dos adesivos estudados (de não serem muito

ácidos e que de não conter flúor na sua composição). No entanto, a despeito dos baixos valores de resistência imediata, também pudemos observar que o oxalato foi capaz de desacelerar a degradação da interface após o período de armazenagem, já que apesar de todos os grupos terem a resistência de união diminuída após este período, esta queda foi menos acentuada para os grupos onde o oxalato foi aplicado previamente. Este achado nos levou a especular que provavelmente estes cristais formados nos interior dos túbulos são capazes de impedir o maior contato da umidade oriunda dos fluidos dentinários com a camada hibrida, influenciando na sorção de água, plastificação do polímero e consequentemente nos resultados favoráveis de longevidade.

O objetivo do terceiro capítulo deste estudo foi avaliar os efeitos da associação do oxalato de potássio e sistemas adesivos nas propriedades mecânicas dos mesmos, através da análise de dureza Knoop dos adesivos colocados sob a dentina tratada ou não com o oxalato. Especulava-se que os cristais de oxalato de potássio formados no interior dos túbulos dentinários pudessem impedir a migração de fluidos durante o procedimento adesivo, resultando na formação de um polímero mais coeso e com propriedades mecânicas melhoradas. Os resultados deste trabalho demonstraram que o gel de oxalato interfere negativamente na dureza destes adesivos.

Acreditamos que os resultados destes trabalhos possam estar interligados. Provavelmente, o gel de oxalato de potássio ou algum subproduto desta reação, mesmo após a sua lavagem, tenha permanecido na superfície interferindo na polimerização do adesivo e conseqüente formação do polímero, reduzindo desta forma sua dureza e resistência adesiva.

Estudos futuros ainda são necessários para comprovação das especulações feitas mediante os resultados deste trabalho e para que a associação do oxalato de potássio com sistemas adesivos possa ser preconizada clinicamente, trazendo os benefícios esperados.

CONCLUSÕES FINAIS

Com base nos resultados destes estudos podemos concluir que:

1. A aplicação do agente de união hidrofóbico livre de solventes reduziu significantemente a permeabilidade da dentina hibridizada com adesivos convencionais simplificados;

2. A aplicação de oxalato de potássio na dentina reduziu significantemente a resistência de união imediata dos adesivos, porém desacelerou a degradação da interface adesiva;

3. A aplicação de oxalato de potássio na dentina condicionada reduziu significantemente a dureza Knoop dos adesivos.

REFERÊNCIAS BIBLIOGRÁFICAS*

1 Carrilho MR, Carvalho RM, Tay FR, Yiu C, Pashley DH. Durability of resin-dentin bonds related to water and oil storage. Am J Dent. 2005;18(6):315-9.

2 Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Effects of storage media on mechanical properties of adhesive systems. Am J Dent. 2004;17(2):104-8.

3 De Andrade E Silva SM, Marquezini L Jr, Manso AP, Garcia FP, Carrilho MR, Pashley DH, Tay FR, Carvalho RM. Effects of a combined application of potassium oxalate gel/adhesive agent on dentin permeability in vitro. J Adhes Dent. 2007;9(6):505-12.

4 De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005;84(2):118-32.

5 Gwinnett AJ. Moist versus dry dentin: its effect on shear bond strength. Am J Dent. 1992;5(3):127-9.

6 Mjör IA, Gordan VV. Failure, repair, refurbishing and longevity of restorations. Oper Dent. 2002;27(5):528-34.

7 Pashley DH, Carvalho RM, Pereira JC, Villanueva R, Tay FR. The use of oxalate to reduce dentin permeability under adhesive restorations. Am J Dent. 2001;14(2):89-94.

8 Pashley EL., Tao L, Pashley, DH. Effects of oxalate on dentin bonding. Am J Dent.1993; 6(3):116-8.

9 Pashley DH, Ciucchi B, Sano H, Horner JA. Permeability of dentin to adhesive agents. Quintessence Int. 1993;24(9):618-31.

10 Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med. 2001;12(2):136-51.

11 Shono Y, Terashita M, Shimada J, Kozono Y, Carvalho RM, Russell CM., Pashley DH. Durability of resin-dentin bonds. J Adhes Dent. 1999;1(3):211-8.

12 Spencer P; Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. J Biomed Mater Res. 2002; 62(3): 447-56.

13 Tay FR, Pashley DH. Water treeing--a potential mechanism for degradation of dentin adhesives. Am J Dent. 2003;16(1):6-12.

14 Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent. 2002 ; 30(7-8) :371-82.

15 Tay FR, Pashley DH, Mak YF, Carvalho RM, Lai SC, Suh BI. Integrating oxalate desensitizing agents with a total-etch two-step adhesive. J Dent Res. 2003; 82 (9): 703-7.

16 Tay FR, Pashley DH, Hiraishi N, Imazato S, Rueggeberg FA, Salz U, Zimmermann J, King NM. Tubular Occlusion Prevents water-treeing and through-and through fluid movement in a Single-bottle, One –step Self-etch Adhesive Model. J Dent Res. 2005; 84(10): 891-96.

17 Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding J Dent Res. 2003; 82(2):141-5.

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* De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Committee of Medical Journal Editors- Grupo de Vancuver. Abreviatura dos periódicos em conformidade com o Medline.

ANEXOS

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 "**Efeito dos procedimentos adesivos em combinação a uma solução de oxalato de potássio na indução da movimentação de fluidos pela dentina: estudo da qualidade e estabilidade da interface de união**", protocolo nº **130/2005**, dos pesquisadores **SAFIRA MARQUES DE ANDRADE E SILVA** e **MARCELA ROCHA DE OLIVEIRA CARRILHO**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 04/01/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Effects of a potassium oxalate gel/adhesive agent combined application on the hydraulic conductance of the dentin: study of the quality and stability of the adhesive/dentin interface**", register number **130/2005**, of **SAFIRA MARQUES DE ANDRADE E SILVA and MARCELA ROCHA DE OLIVEIRA CARRILHO**, 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 04/01/2006.

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.

Warning: preg_split() expects parameter 4 to be long, string given
in **/usr/local/www/squirrelmail/functions/imap_messages.php** on line **808**

Warning: Invalid argument supplied for foreach()
in **/usr/local/www/squirrelmail/functions/mime.php** on line **52**

De: "Carlos Ferreira dos Santos" <cebola@usp.br>
Assunto: Re: [JAOS] Editorial Review of Article - Accept
Data: Qui, Outubro 23, 2008 10:39 pm
Para: safira@fop.unicamp.br

Prezada Safira,

Obrigado pela mensagem.

Parabéns pela conclusão do seu doutorado em dezembro.

Você pode incluir o referido artigo como um dos trabalhos de sua tese.
A mensagem anterior que lhe enviamos e esta servem como documento
comprobatório de minha autorização como Editor-Chefe do Journal of
Applied Oral Science.

Cordialmente,

Prof. Carlos Santos

Citando safira@fop.unicamp.br:

>
> Prezado Prof. Carlos,
>
> Vou defender o meu doutorado em Dezembro e gostaria de solicitar uma carta
> de autorização da JAOS para incluir este artigo como um dos trabalhos da
> na minha tese; ja agora detem os direitos autorais sobre o mesmo.
>
> Gostaria também de reinterar a nossa satisfação com a publicação do nosso
> trabalho na JAOS.
>
>
> Grata,
> Safira Andrade
>
>> Dear Authors,
>>
>> I am pleased to inform you that your manuscript "JAOS-280 - EFFECT OF
>> HYDROPHOBIC COATING ON THE QUALITY OF DENTIN SEALING PROVIDED BY
>> SIMPLIFIED
>> ETCH-AND-RINSE ADHESIVES" has been accepted for publication in the Journal
>> of Applied Oral Science.
>>
>> Soon you will receive the galley proofs for your corrections.
>>
>> Thank you for choosing our journal to publish your work.
>>
>> Please, do not hesitate to contact me for any further assistance or
>> clarification
>>
>> Yours sincerely,
>> _____
>> Carlos F. Santos, DDS, PhD, Associate Professor
>> Editor-in-Chief

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
PABX (0XX14)235-8000 – FAX (0XX14)223-4679

Comitê de Ética em Pesquisa

Processo nº 71/2003

Bauru, 2 de outubro de 2003

Senhor Professor,

O projeto de pesquisa encaminhado a este Comitê de Ética em Pesquisa em Seres Humanos, denominado **"Avaliação in vitro dos efeitos da aplicação de sistemas adesivos, do oxalato de potássio, ou a combinação de ambos, na permeabilidade dentinária"**, de autoria de *Safira Marques de Andrade e Silva*, que será desenvolvido sob sua orientação, após o envio da documentação pendente e dos esclarecimentos prestados considerou o trabalho APROVADO em reunião de 24 de setembro de 2003.

Este Comitê gostaria de esclarecer ao pesquisador que:

1- *O CEP-FOB-USP assim como todos os comitês credenciados pela CONEP em nosso país têm como objetivo primeiro resguardar e proteger o Sujeito da pesquisa, por este motivo pode e dever verificar informações que não sejam esclarecedoras.*
2- *Pesquisas que utilizam dentes humanos obtém estes dentes de sujeitos, então, com certeza os envolve. Nesta pesquisa especificamente os termos que nos foram enviados estão preenchidos por pacientes, entretanto apresentam na porção inferior do termo o número de dentes extraídos anotados por uma só pessoa e isso pode com certeza gerar dúvidas e mesmo assim o CEP aceitou os termos, pois acredita na conduta ética do pesquisador responsável e não no respaldo de revistas científicas que muitas vezes não solicitam os pareceres dos CEP e aceitam apenas um nº de protocolo, infelizmente.*
3- *Nós do CEP-FOB-USP trabalhamos arduamente avaliando muitos projetos mensalmente com parecer final consubstanciado e temos a certeza de que grande parte das pesquisas realizadas na FOB-USP está sendo desenvolvida dentro de preceitos éticos nunca antes considerados.*

Informamos que após o envio do trabalho concluído, este comitê enviará o parecer final, que deverá ser utilizado na publicação do trabalho

Atenciosamente,

Profª Drª Ana Lúcia Álvares Capelozza
Coordenadora

Ilmº Sr. Prof. Dr. **Ricardo Marins de Carvalho**
DD. Docente do Departamento de Dentística, Endodontia e Materiais Dentários