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Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba

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Cirurgião-Dentista

AVALIAÇÃO DA RESISTÊNCIA DE UNIÃO, NANOINFILTRAÇÃO E PROPRIEDADES FÍSICAS DE SISTEMAS ADESIVOS: COMPORTAMENTO AO LONGO DO TEMPO

Tese apresentada à Faculdade de Odontologia de Piracicaba - UNICAMP, como parte dos requisitos para obtenção do título de Doutor em Clínica Odontológica, Área de Dentística.

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Orientador: Prof. Dr. Marcelo Giannini

Banca Examinadora:
Prof. Dr. Patrícia Nóbrega Rodrigues Pereira
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por iluminar o caminho, e me amparar
nesta jornada...

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às vezes calmaria,

outras vezes tempestade.

Seu rumo depende dos ventos dos resultados e

das correntes da informação.

Você almeja voltar à terra firme.

Mas quando se vê, finalmente seguro, em terra firme...

Sente o desejo de voltar ao mar,

o grande oceano da pesquisa.

SUMÁRIO

PREFÁCIO

Esta tese está baseada nos seguintes artigos:

1. *Comparison of microtensile bond strength to enamel and dentin of human, bovine and porcine teeth*. **Journal of Adhesive Dentistry 2004 Summer; 6(2):117-121.**

2. *The effect of organic solvents on one-bottle adhesives bond strength to enamel and dentin*. **Operative Dentistry 2003; 28(6):700-706.**

3. *Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives. Nanoleakage expression*. **Journal of Biomedical Materials Research Part B: Applied Biomaterials 2004 Oct 15; 71B(1):90-98.**

4. *Water-Induced Nanoleakage Prevention by Bonding Strategies*.

5. *Interfacial Ultramorphology of Single-step Adhesives: Nanoleakage as a Function of Time*.

6. *Influence of water-storage time on the sorption and solubility behavior of current adhesives and primer/adhesive mixtures*.

7. *Effects of water-storage on the mechanical and ultramorphological characteristics of current adhesives and primer/adhesive mixtures.*

8. *Effects of water-exposure on the long-term effectiveness of different bonding procedures*

RESUMO

A efetividade dos procedimentos restauradores adesivos promovida através de diferentes protocolos de aplicação tem sido questionada com relação à durabilidade da união ao substrato dentinário. Os objetivos deste estudo foram avaliar: a resistência de união em diferentes substratos dentais; a importância dos solventes orgânicos na união produzida por adesivos que utilizam o condicionamento ácido prévio; a habilidade de diferentes adesivos em evitar a nanoinfiltração na interface resina-dentina; a sorção de água e solubilidade, a resistência à tração, o módulo de elasticidade e características ultraestruturais dos adesivos. Foram utilizados sistemas que empregam diferentes estratégias para condicionamento e infiltração no substrato dental: adesivos que utilizam condicionamento ácido prévio, adesivos de dois passos que empregam *primers* autocondicionantes e adesivos autocondicionantes de passo único. Os testes foram realizados após 24 h ou até o período de 12 meses de armazenagem em água e/ou óleo mineral. A resistência de união, foi avaliada através do ensaio de microtração. Para avaliação da nanoinfiltração foi utilizado $AgNO_3$ amoniacal como agente traçador, para evidenciar espaços nanométricos e sinais de degradação nas interfaces. Os espécimes foram preparados para observação em Microsopia Eletrônica de Transmissão e/ou Varredura. Para avaliação da sorção de água e solubilidade, a variação de massa dos espécimes foi aferida. A resistência de união, a resistência máxima à tração e o módulo de elasticidade foram avaliados em máquina de ensaio universal. Os resultados mostraram que nenhum adesivo foi capaz de evitar a nanoinfiltração. A maioria dos materiais mostrou-se eficiente logo após a sua aplicação; no entanto, o comportamento da união ao longo do tempo frente a presença de água variou consideravelmente. Os adesivos autocondicionantes de passo único foram os mais susceptíveis à degradação após armazenagem em água. De acordo com as propriedades estudadas, conclui-se que: dentes bovinos são melhores substitutos para dentes humanos em testes de união; a presença de solventes orgânicos é essencial na adesão à dentina; e, a degradação da união está relacionada à hidrofilia dos materiais testados.

ABSTRACT

The effectiveness of adhesive restorative procedures promoted by different application protocols has been questioned with regard to the durability of adhesion to dentin substrate. The aims of this study were to: evaluate the bond strengths to different dental substrates; determine the importance of organic solvents in the adhesion promoted by etch-and-rinse adhesives; evaluate the ability of different adhesive systems to prevent nanoleakage in resin-dentin interfaces; and determine the water sorption and solubility, ultimate tensile strength, elastic modulus and ultrastructural characteristics of the bonding resins. Adhesive systems that use different etching and infiltration strategies were used: etch-and-rinse systems, two-step self-etching primers, and one-step self-etching adhesives. Tests were performed after 24 hours or after a storage period of up to 12 months in water and/or mineral oil. Bond strengths were analyzed by means of the microtensile bond test. Ammoniacal $AgNO_3$ was used as a tracer for the nanoleakage test, in order to disclose nanometer-sized spaces and degradation signs within interfaces. Specimens were prepared for Transmission and/or Scanning Electron Microscopy. For water sorption and solubility analysis, the mass variation of specimens was determined. Microtensile bond strength, ultimate tensile strength and elastic modulus were analyzed in a universal testing machine. Results showed that no adhesive system was able to prevent nanoleakage. Most adhesives proved to be effective right after application; however, the behavior of bonded interfaces after long-term water-storage varied considerably. Single-step self-etching adhesives were the most susceptible to degradation after storage in water. Based on the tests performed and properties analyzed, it can be concluded that: bovine teeth are the best substitutes for human teeth in adhesion tests; organic solvents are essential in dentin adhesion; and, degradation of resin-dentin bonds is related to the hydrophilicity of the materials tested.

1. INTRODUÇÃO GERAL

A qualidade da adesão está diretamente relacionada à eficiência da penetração dos monômeros nos espaços interfibrilares, ao completo envolvimento pela solução adesiva das fibrilas colágenas expostas pelo condicionamento ácido, e ao grau de conversão do adesivo (PASHLEY et al., 2000). Apesar dos avanços alcançados pelos adesivos dentinários, trabalhos apontam para uma possível degradação da união da resina composta aos tecidos dentais ao longo do tempo na presença de água (SANO et al., 1999; HASHIMOTO et al., 2000; DE MUNCK et al, 2003; GIANNINI et al., 2003). A redução da resistência de união de sistemas adesivos à dentina é atribuída à degradação das fibrilas colágenas e/ou da resina adesiva (TAY et al., 2003a). Fatores como a viscosidade da solução do adesivo e o colapso da malha de fibrilas colágenas podem dificultar a infiltração do agente de união. Somando-se a este fato, a degradação hidrolítica dos polímeros sintéticos é um evento previsível, já que eles são naturalmente degradáveis (GÖPFERICH, 1996). O fator que diferencia um polímero degradável de um não-degradável é o período de tempo que o processo de degradação leva para acontecer (GÖPFERICH, 1996). A degradação de um polímero é definida como o processo de cisão da corrente polimérica, durante o qual esta é quebrada em segmentos menores (oligômeros), e em circunstâncias especiais, até em monômeros novamente (ÖRTENGREN, 2000).

A adesão ao substrato dentinário pode ser realizada através de duas técnicas: condicionamento ácido prévio ou auto-condicionamento. A primeira técnica baseia-se na aplicação de um ácido semiforte como o fosfórico, para remoção da *smear layer* e desmineralização da dentina subjacente a uma profundidade de 3 a 6 µm (PERDIGÃO et al., 1996). No entanto, esta técnica tem sido considerada crítica (PEREIRA et al., 1999; SPENCER et al., 2000), pois uma infiltração deficiente pode ocorrer se a dentina estiver demasiadamente seca, ou o "*overwet phenomenon*" pode ocorrer na presença de umidade excessiva (KANCA, 1992; TAY et al., 1996). Existem evidências da infiltração incompleta e separação de fases dos monômeros adesivos, o que pode ser prejudicial à formação da união (SPENCER et al., 1999; SPENCER et al., 2000; HASHIMOTO et al., 2002). Com o intuito de reduzir as dificuldades da técnica e simplificar os procedimentos de aplicação dos adesivos, uma segunda técnica foi desenvolvida, na qual *primers* auto-condicionantes compostos de monômeros ácidos são aplicados sobre a dentina coberta pela *smear layer* sem a necessidade de remoção do material com água subsequentemente. Após este passo simultâneo de condicionamento e infiltração, uma camada de resina adesiva é então aplicada sobre a dentina tratada. Acredita-se que os sistemas adesivos auto-condicionantes desmineralizam a dentina e infiltram seus monômeros simultaneamente, evitando o colapso das fibrilas de colágeno pela secagem com ar e também, a ocorrência de fibrilas desprotegidas pela resina aplicada (WATANABE et al., 1994; TAY & PASHLEY, 2001; CARVALHO et al., 2005). Recentemente, uma técnica auto-condicionante de adesão que consiste em um passo único de aplicação foi introduzida. Os adesivos de passo único reúnem as etapas de condicionamento, infiltração e adesão em um único procedimento (TAY & PASHLEY, 2001).

Apesar destes sistemas de união serem comercializados como simplificados, devido ao menor número de passos de aplicação, eles são na realidade misturas complexas de monômeros resinosos hidrófilos e hidrófobos, solventes, água e outros aditivos (TAY & PASHLEY, 2001). Acredita-se que a absorção de água pelo polímero formado tanto na camada híbrida, quanto na camada de adesivo pode contribuir para a degradação da resistência de união à dentina ao longo do tempo (TANAKA et al., 1999; HASHIMOTO et al., 2000). Este fenômeno é agravado pela incorporação de quantidades ainda maiores de componentes resinosos hidrófilos nos adesivos auto-condicionantes de passo único, pois hidrofilia e estabilidade hidrolítica são propriedades antagônicas (TAY et al., 2002a).

A busca por um agente de união que promova uma união duradoura ao substrato dentinário e seja de fácil aplicação tem sido um desafio. Além de estabilidade hidrolítica, propriedades antimicrobianas e liberação de Flúor são desejáveis aos produtos aplicados nas estruturas dentais (IMAZATO et al., 1997;

SAITO, 1996). Recentemente, NAKAJIMA et al. (2003) relataram que a resistência de união produzida por um sistema de união que utiliza um monômero antibacteriano (MDPB – 12-metacriloyloxidodecilpyridiniumbrometo) na composição do seu *primer* auto-condicionante e um agente de união que libera flúor não apresentou evidências de degradação após seis meses de armazenagem em água. Por outro lado, quando se utilizou somente o *primer* antibacteriano sem o agente de união contendo flúor, verificou-se uma tendência da degradação da união, mostrando possíveis efeitos benéficos da incorporação de flúor aos sistemas adesivos.

O termo “nanoinfiltração” foi introduzido para se descrever a ocorrência de espaços nanométricos dentro da camada híbrida, mesmo na ausência de uma fenda na interface de união (SANO et al., 1994; SANO et al., 1995a). Esta técnica utiliza um traçador de baixo peso molecular como o nitrato de prata ($AgNO_3$) para evidenciar tais porosidades na interface. Posteriormente, esta área de união é observada em microscocopia eletrônica (SANO et al., 1995b). A deposição de grãos de prata na camada híbrida dos sistemas que utilizam o condicionamento ácido prévio é atribuída à existência de regiões onde as fibrilas colágenas não foram totalmente envolvidas pela resina adesiva, ou onde a resina não foi adequadamente polimerizada. A degradação da união tem sido atribuída à penetração de fluidos nestas porosidades (SANO et al., 1999; HASHIMOTO et al., 2001). Recentemente, uma nova forma de manifestação da nanoinfiltração nas interfaces foi relatada (TAY et al., 2002a; LI et al., 2000). A deposição de grãos de prata foi observada não só na camada híbrida, mas também na camada de adesivo (TAY et al., 2003a; TAY et al., 2003b).

Sabe-se que as propriedades mecânicas dos adesivos podem influenciar as propriedades da interface de união e, conseqüentemente, podem estar relacionadas com a longevidade das restaurações (TAKAHASHI et al., 2002). Uma vez que o mecanismo de degradação das interfaces ainda não foi totalmente elucidado e controlado, a investigação em longo prazo das propriedades físicas dos sistemas adesivos, da resistência de união e da nanoinfiltração pode trazer informações importantes, pois os procedimentos adesivos são ferramentas indispensáveis na prática de uma Odontologia mais conservadora, além de estética.

2. PROPOSIÇÕES GERAIS

Os objetivos deste trabalho foram:

1. Analisar a resistência de união ao esmalte e à dentina de origem humana, bovina e suína, e comparar as características morfológicas destes substratos após o condicionamento com ácido fosfórico;
2. Analisar os efeitos da presença de solventes orgânicos na resistência de união ao esmalte e à dentina e analisar o padrão de fratura dos espécimes testados;
3. Analisar em Microscopia Eletrônica de Transmissão e de Varredura, os efeitos da armazenagem em água por 6 meses nos padrões de nanoinfiltrção das interfaces de união resina-dentina produzida por diferentes sistemas de união;
4. Examinar a efetividade de diferentes sistemas de união na prevenção da nanoinfiltração, e avaliar os efeitos de diferentes meios de armazenagem na estabilidade das interfaces de união ao longo do tempo utilizando Microscopia Eletrônica de Transmissão;
5. Comparar a capacidade de sistemas adesivos auto-condicionantes de passo único na prevenção da nanoinfiltração após armazenagem em água, e avaliar a ultramorfologia da união produzida por estes adesivos;
6. Analisar os efeitos do tempo de armazenagem em água na sorção de água e solubilidade de sistemas de união;
7. Analisar os efeitos do tempo de armazenagem em água nas propriedades mecânicas e características ultramorfológicas dos sistemas de união;
8. Analisar os efeitos do tempo e condições de armazenagem em água na resistência de união à dentina.

3. MATERIAL E MÉTODOS

CAPITULO 1:

Comparison of microtensile bond strength to enamel and dentin of human, bovine and porcine teeth

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Keywords: enamel, dentin, porcine teeth, bovine teeth, bond strength.

ABSTRACT

Purpose: To determine the bond strengths promoted by an adhesive system to human, bovine and porcine enamel and dentin, and compare their etched micromorphology by scanning electron microscopy.

Materials and Methods: Thirty sound freshly extracted teeth were used in this study: ten human third molars, ten bovine incisors and ten porcine molars. Human (H), bovine (B) and porcine (P) teeth had their crowns flattened with 600-grit SiC paper to expose either enamel (E) or middle dentin (D) surfaces. After application of the adhesive resin, composite crowns approximately 8 mm high were built up with TPH Spectrum composite. After 24-hour water-storage, specimens were serially sectioned in the buccal-lingual direction to obtain 0.8 mm slabs, which were trimmed to an hourglass shape of approximately 0.8 mm^2 at the bonded interface. Specimens were tested in tension in a universal testing machine (0.5 mm/min). Results were statistically analyzed by ANOVA and Tukey test at the 95 % confidence level.

Results: Tukey test showed significant differences between bond strengths obtained on enamel and dentin ($p<0.05$). However, there were no statistically significant differences on μTBS among human, bovine and porcine teeth. SEM observations revealed a similar dentinal morphology for the three species. However, porcine enamel specimens presented a very different characteristic of enamel prisms distribution.

Conclusion: Bovine teeth proved to be possible human teeth substitutes either for dentin or enamel bond testing. However, even though porcine teeth provided enamel and dentin bond strengths similar to human and bovine teeth, enamel morphology presented a very different configuration pattern.

INTRODUCTION

Since the beginning of the adhesive era with Buonocore's paper in 1955,[2] hundreds of studies have been carried out in an attempt to elucidate and overcome the drawbacks of bonding resins with the development of better restorative materials and techniques. Many researchers need human teeth to be used as test substrates. The preferred and most commonly used teeth are third molars[5,13] or premolars,[16] which are normally extracted for surgical, periodontal, orthodontic or pathological reasons. While an increasing need for human sound teeth is verified in research centers, extracted human teeth are becoming increasingly difficult to obtain due to progresses in conservative dental treatment during the last decades.[11] In addition, the requirement of informed consent of the donors by ethic committees has made it even more difficult. Besides that, risk of cross-contamination is another concern about using human teeth.

Researchers have been seeking substitutes for human dentin for a long time. In 1979, Fusayama et al.[4] tested the possibility of using ivory as a substitute for human dentin in tensile adhesion measurements. However, the authors concluded that bonding to ivory was quite different from bonding to human dentin. Other mammalian teeth are best suited to be used as human teeth substitutes because their composition and morphology are very similar[3,18] despite some obvious anatomical and permeability differences.[19] Canine and monkey teeth have been used for *in vivo* studies.[12,15] Nevertheless, the adhesion conditions and composition of bovine teeth are easier to standardize for *in vitro* studies, as they are very similar to human teeth.[21] Schmalz et al.[17] reported that the variability in the permeability characteristics of bovine dentin is about half that of human origin. Moreover, bovine teeth are not only readily available, but the age of the cattle can also be easily controlled.[14,19] Another possibility could be the use of porcine teeth as substitutes for human teeth, due to the ease of obtaining them from abattoirs.

Several studies have assessed the possibility of substituting nonhuman mammalian for human teeth in adhesion tests.[11,15,16] However, there is no common agreement among authors. Most of the previous studies were carried out with adhesive systems applied according to concepts and techniques that are not commonly used anymore. Thus, the purpose of this study was to compare the morphological characteristics and microtensile bond strength of resin to enamel and dentin of human, bovine and porcine teeth. The null hypothesis was that there are no differences in resin-dentin or resin-enamel bond strengths among human, bovine and porcine teeth.

MATERIALS AND METHODS

Thirty teeth were used in this study: ten human freshly extracted sound third molars (stored in 0.05% thymol), which were obtained by protocols that were analyzed and approved by the appropriate institutional review board of the Piracicaba Dental School – University of Campinas and with the informed consent of the donors (process # 17/2001); ten bovine incisors and ten porcine molars that had been freshly extracted. Five teeth of each species were used for enamel and five for dentin bond testing. After being cleaned and pumiced, human (H), bovine (B) and porcine (P) teeth had their crowns flattened with 600-grit SiC paper in running water to expose either enamel (E) or middle dentin (D) surfaces, and create a standard smear layer. Human third molars and porcine molars had their occlusal surfaces flattened, while bovine incisors had their labial surfaces flattened to expose either enamel or middle dentin. Teeth were randomly assigned to six experimental groups (n=5): group 1 – human enamel, group 2 – human dentin, group 3 – bovine enamel, group 4 – bovine dentin, group 5 – porcine enamel and group 6 – porcine dentin.

Teeth were acid etched with 35% H_3PO_4 (3M Scotchbond etchant - 3M ESPE, St. Paul, MN, USA) for 15 s and rinsed for another 15 s. The dentin surfaces were left visibly moist, before application of two consecutive coats of the one-bottle ethanol/water-based Single Bond adhesive system (3M ESPE), specimens were light-cured for ten seconds. After application of the adhesive resin, composite crowns of approximately 8 mm were built up with TPH Spectrum composite resin (Dentslpy De Trey, Konstanz, Germany). An XL2500 (3M ESPE) light curing unit with an output of 650 mW/mm^2 was used in this study. Teeth were stored in distilled water for 24 h to permit the termination of the polymerization process. Afterwards, specimens were vertically serially sectioned in the buccal-lingual direction to obtain 0.8 mm thick slabs (ISOMET 1000 – Buehler Ltd, Lake Bluff, IL, USA). Four slabs were selected from each tooth. Each slab was trimmed with a diamond bur mounted in a high-speed handpiece to an hourglass shape with a cross-sectional area of approximately 0.8 mm^2 at the bonded interface. Specimens were positioned in a microtensile testing device and tested in tension in an Instron universal testing machine (4411 - Instron Corp, Canton, MA, USA) at a cross-head speed of 0.5 mm/min until failure. After testing, specimens were carefully removed from the grips with a scalpel blade and the cross-sectional area at the site of fracture measured to the nearest 0.01 mm with a digital caliper (Starret 727-6/150 – Starret Ltd, Itu, SP, Brazil) to calculate the tensile bond strength and express results in MPa. A mean bond strength value was calculated for each tooth. Differences in microtensile bond strengths (μTBS) were evaluated for statistical significance using a two-way analysis of variance ANOVA (species vs. hard tissue substrate), and Tukey test at the 0.05 level of significance. All statistical analysis was done using SAS for the personal computer (SAS Institute, Cary, NC, USA).

In order to observe the morphology of human, bovine and porcine etched substrates, additional enamel and dentin specimens were wet-abraded to expose either superficial enamel or middle dentin, prior to being etched with 35% H_3PO_4 to remove the smear layer and expose enamel prisms and dentin structures. Specimens were fixed, post-fixed, dehydrated in ascending acetone concentrations up to 100%, critical point dried (CPD 030 - BAL-TEC, Furstentum, FL, Liechtenstein), sputter-coated with gold (MED 010 - BAL-TEC) and observed under a SEM (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, United Kingdom). Representative areas of dentin and enamel surfaces were photographed at 20,000 and 15,000X magnification, respectively.

RESULTS

The mean μTBS are summarized in Table 1. Two-way ANOVA revealed that there were statistically significant differences for the factor “substrate” ($p=0.00012$), but failed to identify any significant differences for the factor “species” ($p=0.13$) or for the interaction between the two factors ($p=0.10$). Tukey test showed significant differences between bond strengths obtained on enamel and dentin for all species ($p<0.05$). However, there were no statistically significant differences in μTBS among human, bovine and porcine teeth.

SEM observations revealed that the dentinal morphology of the three species were quite similar, with respect to tubules number and diameter in the regions studied (Fig. 1). However, porcine enamel morphology differed greatly from human and bovine substrates (Fig. 2). Porcine enamel presents an alternating thick interprismatic matrix with crystallites at an angle of about 90^o to the direction of prisms, forming “inter-row sheets”.

DISCUSSION

Although adhesion studies to vital and non-vital teeth in the oral environment should preferably be carried out using human teeth to evaluate dental adhesives,[21] it is nearly impossible. In 1983 Nakamichi et al.[11] had already reported an increasing difficulty of obtaining human teeth to be used as test substrates in adhesion measurements. This reduction in the availability of human sound teeth is due, in part, to advances in conservative dental treatment.[11,14,16,21] This study analyzed resin bond strengths and enamel and dentin morphology of two possible substitutes for human teeth in adhesion tests.

Results of the present study revealed no significant differences on bond strength values among human, bovine and porcine teeth to either enamel or dentin. Thus, the results support the null hypothesis that there were no differences in resin-dentin and resin-enamel bond strengths among human, bovine or porcine teeth. Even though statistical analysis detected no significant difference, porcine enamel bond strengths were notably higher than those obtained with human and bovine enamel. Lack of statistical significance was probably a result of sample size being small. Dentin bond strength values for the three species were statistically lower than enamel bonds. It is worthy of note that no significant differences were detected among the three tested species. In addition, dentin bonds were very similar among them. According to Nakamichi et al.,[11] only the superficial layer close to enamel of bovine teeth could be substituted for human teeth. However, in the present study, similar values were obtained in middle dentin.

Despite some anatomical and permeability differences,[19] histochemical and comparative morphological studies have revealed that all mammalian teeth are essentially similar.[3,11,18] Scanning electron microscopy observations showed that human, bovine and porcine dentin substrates are very much alike with regard to number and diameter of tubules in middle dentin, with approximately 25,000 tubules/mm^2 (Fig 1A-C), which support the theory that mammalian teeth present almost the same micromorphology. The diameter and number of dentinal tubules in rats, cats, dogs and monkeys were compared to human dentin by Forssell-Ahlberg et al.[3] at different depths, showing that these species present similar dentinal morphology.

On the other hand, the comparison of enamel morphology after acid etching with 35% H_3PO_4 revealed that species presented different prism orientation patterns. Human and bovine enamel presented apparently similar aspects (Fig 2A and 2B). However, in porcine teeth, enamel prisms arrangement was quite different from human and bovine enamel (Fig 2C). In addition, bovine and porcine enamel prism cores look more porous than human's (Fig 2A-C). Boyde[1] showed that the cross-sections of prisms are dependent upon the shape of the Tomes process of the ameloblast. In terms of enamel formation, changing prism cross-sections can be understood as reflecting changing shapes of the Tomes process during the passage of the ameloblasts from the EDJ towards the outer surface. Phylogenetic analyses have shown that there is a differentiation in enamel prism patterns according to the type of diet of species.[20] Porcine enamel presents characteristics particular to herbivores. However, even though bovids are also herbivores, their enamel pattern is very similar to that of humans.

The bonding mechanism of adhesive systems to dental substrates is apparently the same for the three investigated species, which might have accounted for similar bond strengths. For dentin, the adhesive resin infiltrates into demineralized intertubular dentin surface, forming a hybrid layer, and into dentin tubules, forming resin tags.[9] The bonding mechanism to enamel is quite different from that of dentin. Enamel acid etching produced a Type I conditioning pattern for the three species studied (Fig 2A-C). Mineral removal of the interprismatic area exposed prism cores, resulting in an increase in surface area and surface energy. The retentive ability of etched enamel depends on micromechanical retention of resin tags to the etched enamel surface.[6,7]

The difference in enamel prisms morphology might have contributed for the highest bond strengths for the porcine teeth, because it can promote a larger surface area with more irregularities contributing for stronger micromechanical retention of the resin tags. It has been reported that the speed in tooth development before and after eruption influence enamel characteristics.[8,11] Thus, enamel comparisons should be considered with caution, especially when using porcine teeth.

Adhesion tests, while not perfect, have enabled the development of improved bonding systems and techniques. Studies performed with bovine teeth have already generated and will continue to generate important information with regard to adhesion concepts.[10] Within the limits of this study, it can be concluded that although human teeth are preferable, bovine teeth are better substitutes than porcine teeth in testing resin adhesion.

AKNOWLEDGMENTS

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REFERENCES

1. Boyde A. The structure of developing mammalian enamel. In: Strak MV & Fearnhead RW (ed), Tooth enamel. Bristol: Wright 1965;163-194
2. Buonocore M. A simple method of increasing adhesion of acrylic filling materials to enamel surfaces. J Dent Res 1955;34:849-853.
3. Forssell-Ahlberg K, Brännström M, Edwall L. the diameter and number of dentinal tubules in rat, cat, dog and monkey. Acta Odont Scand 1975;33:243-250.
4. Fusayama T, Nakamura M, Kurosaki N, Masaaki I. Non-pressure adhesion of a new adhesive restorative resin. J Dent Res 1979;58(4):1364-1370.
5. Giannini M, Carvalho RM, Martins LRM, Dias CTS, Pashley DH. The influence of tubule density and area of solid dentin on bond strength of two adhesive systems to dentin. J Adhes Dent 2001;3:315-324.
6. Gwinnett AJ. Histologic changes in human enamel following treatment with acidic adhesive conditioning agents. Arch Oral Biol 1971;16:731-738.
7. Miyazaki M, Sato M, Onose H. Durability of enamel bond strength of simplified bonding systems. Oper Dent 2000;25(2):75-80.
8. Eastoe JE. The changing nature of developing dental enamel. Br Dent J 1966;15;121(10):451-454.
9. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982;16:265-273.
10. Nakabayashi N, Takarada K. Effect of HEMA on bonding to dentin. Dent Mater 1992;8:125-130.

11. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible human teeth substitutes. J Dent Res 1983;62:1076-1081.
12. Pashley EL, Tao L, Mackert JR, Pashley DH. Comparison of in vivo vs in vitro bonding of composite resin to the dentin of canine teeth. J Dent Res 1988;67:467-470.
13. Pashley DH, Zhang Y, Agee KA, Rouse CJ, Carvalho RM, Russell CM. Permeability of demineralized dentin to HEMA. Dent Mater 2000;16:7-14.
14. Retief DH, Mandras RS, Russell CM, Denys FR. Extracted human versus bovine teeth in laboratory studies. Am J Dent 1990;3:253-258.
15. Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999;78:906-911.
16. Saunders WP. The shear impact retentive strengths of four dentine bonding agents to human and bovine dentine. J Dent 1988;16:233-238.
17. Schmalz G, Hiller K-A, Nunez LJ, Stoll J, Weis K. Permeability characteristics of bovine and human dentin under different pretreatment conditions. J Endodon 2001;27:23-30.
18. Robinson C, Briggs HD, Atkinson PJ, Weatherell JA. Matrix and mineral changes in developing enamel. J Dent Res 1979;58(Spec Issue B):871-82.
19. Tagami J, Tao L, Pashley DH, Horner JA. The permeability of dentine from bovine incisors in vitro. Archs Oral Biol 1989;34:773-777.
20. von Koenigswald W. Tooth enamel of the cave bear (Ursus spelaeus) and the relationship between diet and enamel structures. Ann Zool Fennici 1992;28:217-227.
21. Watanabe I, Nakabayashi N. Measurement methods for adhesion to dentine: current status in Japan. J Dent 1994;22:67-72.

LEGENDS

Figure 1. Representative SEM micrograph of human (A), bovine (B) and porcine (C) dentin. (DT) dentin tubules, (ID) intertubular dentin. Note the collagen web exposed after etching with phosphoric acid. Original magnification X 20,000.

Figure 2. Representative SEM micrograph of human (A), bovine (B) and porcine (C) enamel. Note a thick layer of interprismatic matrix (IPM) with crystallites at an angle of 90^o to the direction of prisms (PC) forming "inter-row sheets" in porcine enamel. Original magnification X 15,000.

Table 1. Mean bond strengths in MPa ± SD of Single Bond adhesive system to enamel and dentin for human, bovine and porcine teeth.

	Dentin		Enamel	
Human	17.34 ± 4.93	B	25.43 ± 6.60	A
Bovine	15.64 ± 4.95	B	21.25 ± 7.08	A
Porcine	15.52 ± 3.68	B	31.33 ± 3.69	A

Means designated by different letters are significantly different by Tukey test at the 0.05 level of significance. (n=5)

FIGURE 1

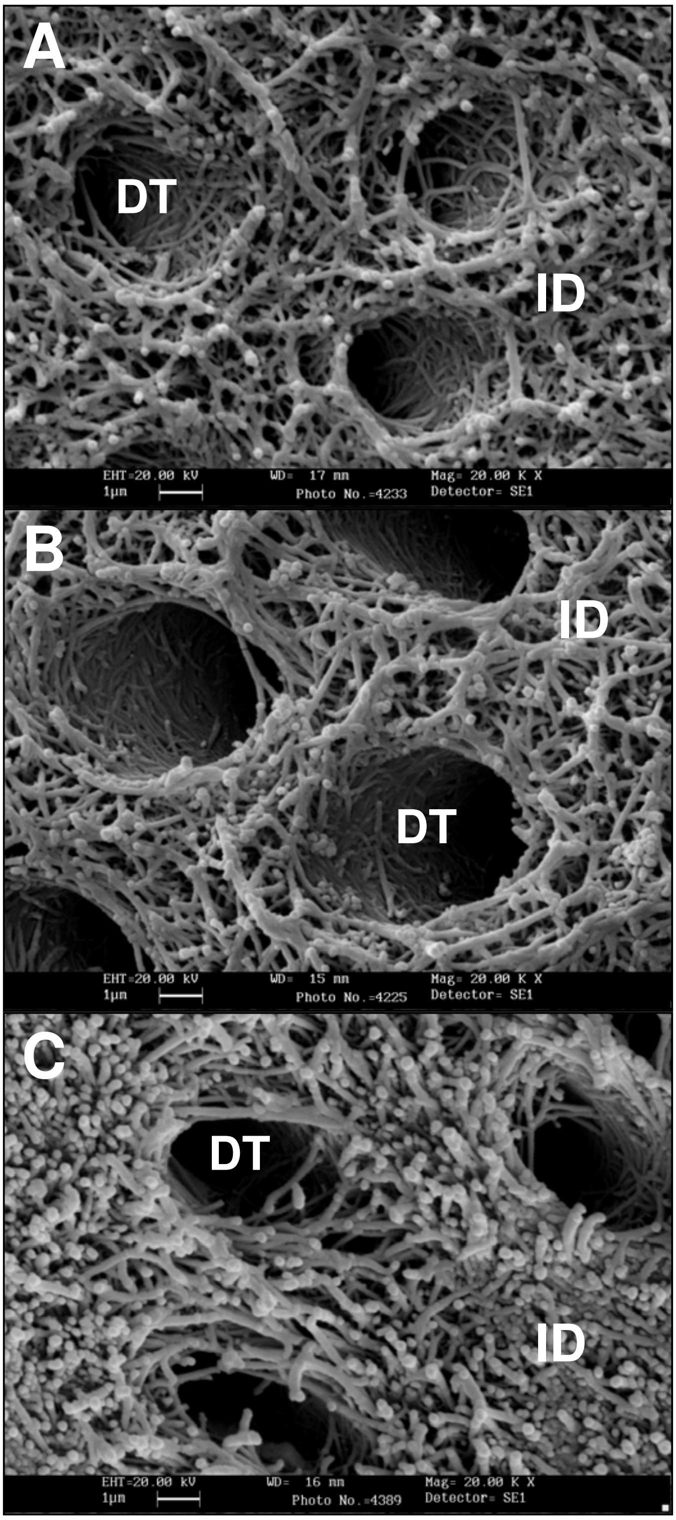
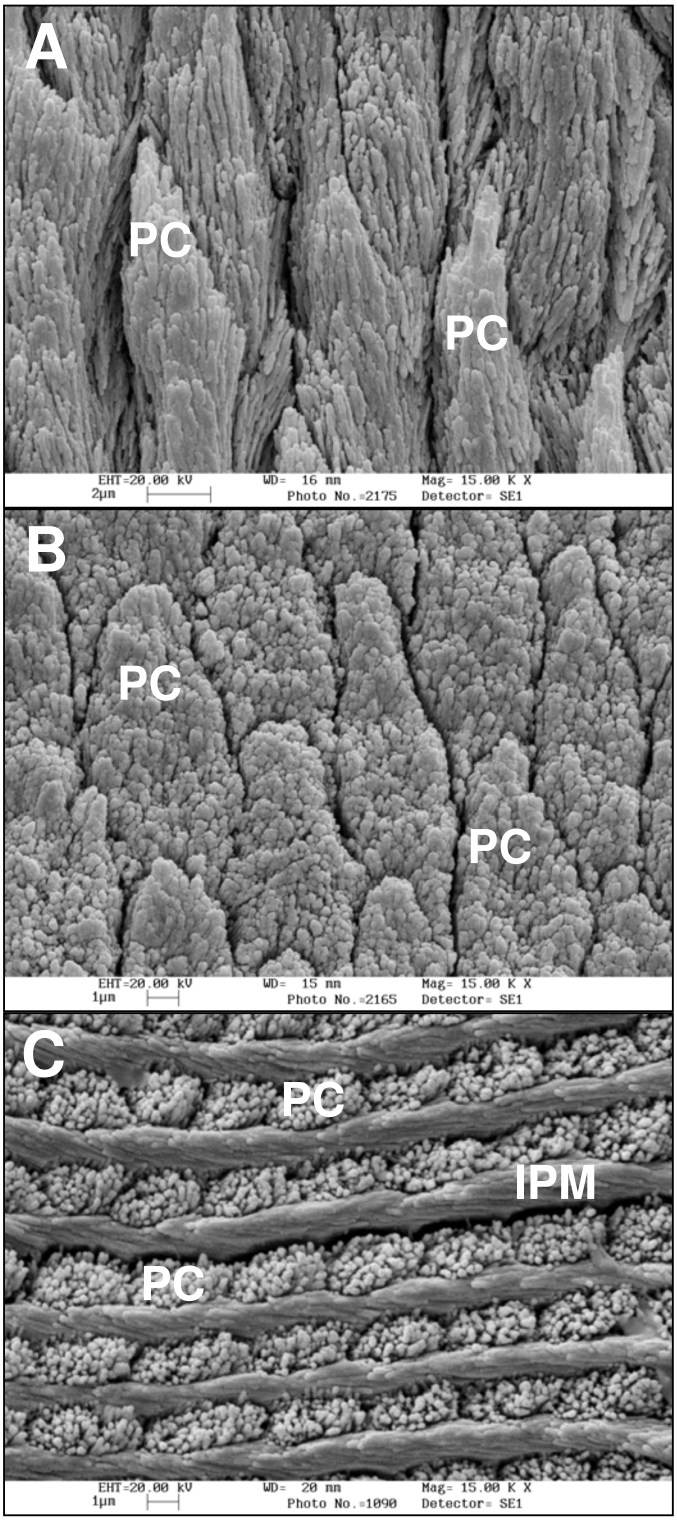


FIGURE 2



CAPITULO 2:

The Effect of Organic Solvents on One-Bottle Adhesives Bond Strength to Enamel and Dentin

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Short title: Bonding of solvent-based adhesive systems

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SUMMARY

The aim of this study was to evaluate the microtensile bond strength (μTBS) of ethanol/water- and acetone-based, one-bottle adhesive systems to enamel (E) and dentin (D), in the presence (P) or absence (A) of their respective solvents. Thirty-two freshly extracted third molars were flattened with 600-grit SiC paper, and restored with either Single Bond (SB) or Prime&Bond 2.1 (PB) according to manufacturers' instructions and after complete solvent elimination. Teeth were divided into 8 test groups (n=4): G1- SB-E-P, G2- SB-E-A, G3- PB-E-P, G4- PB-E-A, G5- SB-D-P, G6- SB-D-A, G7- PB-D-P and G8- PB-D-A. After application of adhesive resins, composite crowns of approximately 8 mm were built up with TPH Spectrum composite. After 24 h water storage, specimens were serially sectioned bucco-lingually direction to obtain 0.8 mm slabs, which were trimmed to an hourglass shape, approximately 0.8 mm^2 at the bonded interface. Specimens were tested in tension using a universal testing machine (0.5 mm/min). Results were statistically analyzed by ANOVA and Tukey test. The frequency of fracture modes was compared using Kruskal-Wallis test. There were no statistically significant differences in mean bond strength among groups restored with or without solvent for enamel. However, results were significantly different for dentin groups (MPa): G5 – 26.2 ± 8.6^a; G7 – 23.6 ± 11.3^{ab}; G6 – 12.8 ± 2.1^{bc}; G8 – 6.2 ± 3.1^c. SEM examination indicated that dentin group failure modes were significantly different from enamel groups. Results suggest that the presence of organic solvents does not influence μTBS to enamel. However, μTBS to dentin was significantly affected by the absence of solvents in the adhesive system.

CLINICAL RELEVANCE

Bonding to enamel is not affected by the absence of organic solvents in the adhesive system; however, their presence is essential for achieving effective bonding to dentin substrate. Efforts should be taken to minimize solvent loss during clinical use by immediately and securely replacing caps and lids of solvent-based dentin bonding systems.

INTRODUCTION

Effective bonding to dental substrates has been one of the major goals in restorative dentistry through the last decades. The findings of Buonocore (1955) combined with the studies of Fusayama & others (1979) and Nakabayashi & others (1982) brought dentistry to the adhesive era. When acid etched, enamel shows predictable and high bond strengths to resin-based restorative materials. However, the development of a strong and durable bond to dentin substrate has been more arduous (Eick & others, 1993; Burke & McCaughey, 1995). The concept of etching dentin and infiltrating a hydrophilic monomer into the demineralized collagen mesh has been a key to achieving durable dentin bonding (Kato & Nakabayashi, 1998).

Kanca (1992) introduced the concept of wet bonding to dentin after acid etching. He demonstrated that bonding to moist dentin improves bond strength. The collagen structure of moist dentin remains open, due to the ability of water to keep interfibrillar spaces from collapsing (Pashley & others, 1993), and facilitates resin infiltration (Jacobsen & Söderholm, 1995). A high-quality hybrid layer requires optimal infiltration of adhesive monomers into the demineralized dentin surface and proper polymerization (Ferrari & others, 1997; Pashley & Carvalho, 1997; Chaves & others, 2002).

All current adhesive systems are designed to be hydrophilic, containing resin monomers dissolved in acetone, ethanol, water, or some combination of these solvents (Swift & Bayne, 1997). Water and organic solvents, also called as “water-chasers”, play an important role in wet bonding. These components are responsible for water displacement from the collagen network and infiltration of resin monomers into these spaces, previously occupied by water (Kanca, 1992). However, solvents are naturally volatile and, consequently, their concentration in one-bottle adhesives may decrease with time, compromising adhesion.

The purpose of this study was to evaluate the effects of the presence of organic solvents on micro-tensile bond strength of one-bottle adhesive systems to enamel and dentin substrates, and analyze the failure modes of fractured specimens. The rate of solvent evaporation was also determined for each adhesive solution at room temperature.

METHODS AND MATERIALS

Thirty-two freshly extracted, intact, third molars (stored in 0.05% thymol) were used in this study. The teeth were obtained by protocols analyzed and approved by the appropriate institutional review board of the Piracicaba School of Dentistry – University of Campinas and with the informed consent of the donors (process # 66/2002). After being cleaned and pumiced, tooth crowns were flattened with 600-grit SiC paper under running water to expose either enamel (E) or superficial dentin (D) surfaces. This process also created a standard smear layer before bonding with the one-bottle ethanol/water-based Single Bond (SB) and the acetone-based Prime&Bond 2.1 (PB) adhesive systems.

Adhesives were applied according to manufacturers' instructions (P – presence of solvents) or after complete solvent elimination (A – absence of solvents). For solvent elimination, several drops of each bonding agent were dispensed in an adhesive dispenser, and stored at 37^{o} C for 8 h in a dark box, to prevent polymerization. Teeth were randomly assigned to eight test groups (n=4): G1- SB-E-P, G2- SB-E-A, G3- SB-D-P, G4- SB-D-A, G5- PB-E-P, G6- PB-E-A, G7- PB-D-P and G8- PB-D-A. After application of the adhesive resins, composite crowns of approximately 8 mm were built up with TPH Spectrum composite resin. A light curing unit (XL2500, 3M Espe) with an output of 650 mW/cm^2 was used to polymerize specimens. Teeth were stored in distilled water for 24 h and were then serially sectioned bucco-lingually to obtain 0.8 mm thick slabs (ISOMET 1000 – Buehler Ltd, Lake Bluff, IL 60044, USA). Three to six slabs were obtained for each tooth. Each slab was trimmed with a diamond bur mounted in a high-speed handpiece to an hourglass shape with a cross-sectional area of approximately 0.8 mm^2 at the bonded interface. Specimens were tested in tension in a universal testing machine (model 4411, Instron Corp, Canton, MA 02021, USA) at a cross-head speed of 0.5 mm/min until failure. After testing, specimens were carefully removed from the grips with a scalpel blade and the cross-sectional area at the site of fracture was measured to the nearest 0.01 mm with a digital caliper (Starret 727-6/150 – Starret Ltd, Itu, SP 13300-000, Brazil) Maximal tensile load was divided by specimen cross-sectional area to express results in units of stress (MPa). Differences in microtensile bond strength were statistically evaluated using a two-way ANOVA and the Tukey post-hoc test at a pre-set significance level of 0.05. All statistical analysis was done using a personal computer program (SAS, version 8.0, SAS Institute, Cary, NC 27513-2414, USA).

Rate of Solvent Evaporation

A drop of each adhesive solution was dispensed on an analytical balance (JEX-200, YMC Co. Ltd., Kyoto 600-8106, Japan). The solution mass was recorded at five-second intervals until reaching equilibrium at room temperature. The rate of solvent evaporation and the amount of solvent in each adhesive solution was then determined using weight changes at each time point.

Additionally, in order to determine how much solvent evaporation does take place if the lid of the adhesive bottle is not immediately replaced, a full bottle of each adhesive solution with the lid removed was placed on the analytical balance and their weight change was monitored over 48 hours at room temperature (22 ^{o}C). The bottles were agitated for 5 seconds before having their lids removed, and were left undisturbed until the analysis had been done.

Scanning Electron Microscopy

For determination of fracture mode, the dentin sides of fractured specimens were mounted on an aluminum stub, gold sputter coated (MED 010, BAL-TEC, Furstentum FL-9496, Liechtenstein) and examined using a scanning electron microscope (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge CB1 3QH, United Kingdom) at 200 X or higher magnification. Failure mode was classified into one of four types: Type 1, adhesive failure between adhesive resin and enamel or dentin; Type 2, partial adhesive failure between enamel or dentin and adhesive resin, and partial cohesive failure in the adhesive resin; Type 3, partial cohesive failure in enamel or dentin; Type 4, cohesive failure in adhesive resin. The frequency of fracture modes was statistically analyzed using the Kruskal-Wallis test.

In order to observe hybrid layer formation, two additional specimens were similarly prepared as in test groups to permit SEM examination. These specimens were sectioned perpendicular to the bonded surfaces using a low speed wheel saw under water. Each interface was finished with 1000-grit SiC paper under water and then polished with 6, 3, 1, and 0.25 µm diamond paste using a polish cloth. Afterwards, specimens were demineralized with 37% H_3PO_4 for 10 s and immersed in a 5% NaOCl solution for 5 min. After each step, specimens were rinsed, and debris was removed ultrasonically 10 min. Each specimen was sputter-coated with gold (MED 010) and examined using an SEM (LEO 435 VP). Representative areas of the interfaces were photographed at 5000 X.

RESULTS

Mean µTBS values are presented in Table 1. Two-way ANOVA revealed that there were statistically significant differences for the factor “substrate” ($p \leq 0.0001$), for the factor “adhesive system” ($p \leq 0.0004$), and identified a significant interaction between factors ($p=0.04$). The Tukey post-hoc test showed significant differences among adhesive systems applied on enamel and dentin ($p<0.05$).

There were no statistically significant differences in µTBS values between groups restored with or without solvent for enamel. However, a significant reduction in bond strength was observed when both one-bottle adhesive systems were applied without solvent to dentin.

Rate of Solvent Evaporation

The rate of adhesive solution mass loss as function of time is depicted in Figure 1. After 5.5 minutes, the Prime&Bond 2.1 adhesive solution had lost 81% of its mass, and for the Single Bond solution, a 31% reduction was verified after 11.5 minutes. These values may represent approximately the total amount of solvent in each adhesive solution.

The adhesive bottles weight presented little or almost no variation with the lids removed. PB lost 0.08% of its mass during the first minute, 0.15% after two hours and 0.22% after 48 hours. SB presented an even smaller weight reduction: 0.01% at the first minute. Two hours later, no weight change was noticed. After 48 hours, a 0.04% mass reduction was recorded.

Scanning Electron Microscopy

SEM observation of failure mode showed considerable variation (Fig. 2). A great number of Type 4 failures was observed in enamel groups, whereas in dentin groups, no specimen presented a cohesive failure in the adhesive resin. For dentin groups restored with solvent-depleted adhesives, most fractures were Type 1 failures, probably due to the week bond promoted by the adhesive systems and absence of hybrid layer formation (Figs. 6 and 8).

Figures 3 to 8 show bonded interfaces resulting from application of the two adhesive systems, with or without their respective solvents, on enamel or dentin substrates. As shown in Figs. 3 and 4, no changes in resin-enamel interface morphology were noted when PB-P and PB-A were applied. The same observations were noted for SB-E-P and SB-E-A specimens (not shown).

When PB-P was applied to dentin surfaces, hybrid layer formation and resin tags were noted (Fig. 5). However, when PB-A was used, no hybrid layer and short resin tags were seen (Fig. 6). In SB-P-D specimens, the presence of long resin tags with lateral, secondary canals infiltrated with adhesive resin (arrows) and hybrid layer formation were evident (Fig. 7). In SB-A-D bonded interfaces, no hybrid layer was noted as well (Fig. 8).

DISCUSSION

The µTBS values show that SB and PB performed similarly on enamel and dentin surfaces when their respective solvents were present. This data suggests that adhesive systems are approaching what Burke & McCaughey (1995) describe as an ideal bonding agent: one that provides a bond strength to dentin similar to that of enamel. In interpreting data of the current study, it is important to note that no attempt was made to judge the long-term performance of these adhesives. Thus, conclusions should be limited to the conditions presented in this work.

The bonding mechanism to enamel is quite different from that of dentin. Bonding to enamel depends on micromechanical retention of resin tags to the etched enamel surface. The retentive ability of etched enamel is a function of the increase in surface area and surface energy of etched enamel (Gwinnett, 1971; Retief, 1973; Miyazaki, Sato & Onose, 2000). When the adhesive systems were tested on enamel, no significant differences in bond strengths were detected and no resin-enamel morphological changes were noted with or without the presence of solvents in the bonding solutions (Figs. 3 and 4). These findings may be attributed to tooth treatment after acid-etching and rinsing: enamel was air-dried for 15 s before being bonded with SB or PB. Thus, no water was present on the bonding substrate. Solvents present in the adhesive solutions had no water to “chase” or drive away. However, their presence provided a less viscous characteristic to the solutions, facilitating contact of bonding resin with the substrate.

Since introduction of the “wet bonding” concept to dentin (Kanca, 1992), dentin desiccation is no longer indicated (Swift & Bayne, 1997). The main reason for this elimination is that spatial alteration of collagen occurs upon drying demineralized dentin that may prevent monomer penetration into the collagen network (Maciel & others, 1996). The moist bonding technique uses a monomer mixture with hydrophilic groups for wetting of and penetrating into exposed dentin collagen fibrils. The resin monomers are dissolved in a water-chasing solvent, such as ethanol or acetone, for exchange of water inside the exposed collagen matrix with monomer. This zone is then capable of forming a well-polymerized cross-linked polymer network (Kanca, 1992; Finger & Fritz, 1996).

High μTBS values have been reported when SB and PB adhesive systems are applied to both enamel and dentin following manufacturers’ instructions (Kanemura, Sano & Tagami, 1999; Nakajima & others, 2000; Giannini & others, 2001). However, in the present study, μTBS values were significantly reduced when SB and PB were applied to moist dentin without their solvents. This reduction probably occurred as a function of two factors: incomplete monomer infiltration and incomplete water displacement. The absence of a water-chaser monomer-carrier resulted in difficult resin infiltration. This absence left unprotected collagen fibers, reducing immediate bond strength and, consequently, possibly elevating susceptibility for hydrolytic degradation in the long-term (Burrow, Satoh & Tagami, 1996). If not completely removed, water will compete with the infiltrating resin for space within the collagen network, preventing hybrid layer formation (Jacobsen & Söderholm, 1995; Tay, Gwinnett & Wei, 1996). In addition, failure to remove water may have resulted in dilution of water-soluble resin components, reducing the degree of conversion and bond strength. It is currently believed that, even when manufacturers' instructions are followed, bonding resin is unable to completely penetrate to the base of decalcified collagen fibers, or to completely surround them (Nakabayashi & Takarada, 1992). This assumption has been demonstrated by the phenomenon of nanoleakage, where silver crystals are deposited in the hybrid layer due to microleakage occurring in extremely small spaces (Sano & others, 1995).

The SB system contains ethanol/water in a HEMA/Bis-GMA solution, whereas PB contains acetone in PENTA/UDMA. During adhesive application, it was noted that the PENTA/UDMA solvent-depleted resin solution (PB-A) presented a notably higher viscosity than did the HEMA/Bis-GMA solution (SB-A), and a smaller resin volume as well. The amount of solvent in each adhesive composition is presented in Fig 1. Solvent content in PB (approximately 80%) is much higher than in SB (approximately 30%). It is speculated that solvent evaporation and viscosity changes can affect bond strength to dentin (Gallo, Burgess & Xu, 2001; Ogata & others, 1999). Figures 6 and 8 depict the non-infiltrated zone formed when PB-A and SB-A resins were applied over demineralized, moist dentin. The area between arrows was deproteinized with NaOCl during microscopy processing, which removed collagen fibrils remains. The same procedures were performed on specimens bonded according to manufacturers' instructions; however, exposed collagen fibrils were enveloped by the bonding resin. Thus, NaOCl had no effect on the fibrils.

A recent study (Gallo & others, 2001) evaluated the effect of a 10-minute delayed application of one-bottle adhesive systems on bond strength to dentin. The authors found that acetone-based adhesives showed a trend towards decreased shear bond strengths when the bonding agents were dispensed 10 minutes prior to their application, even though no statistical difference was detected.

Clinicians must be aware of the volatile characteristics and of the importance of solvents in achieving effective dentin bonding with one-bottle adhesive systems. Thus, if the adhesive solution becomes more viscous, its useful shelf life is limited. A higher vapor pressure results in an easier solvent evaporation. Acetone has a relatively high vapor pressure (184 mm Hg at 20^{o} C) compared to ethanol (43.9) and water (17.5) (Gallo & others, 2001). The viscosity of the adhesive system increases as solvent concentration decreases. Both adhesive systems tested in this study are supplied to the dentist in a smart design bottle that does not allow much solvent evaporation. This characteristic was evidenced by the analysis of the bottles weight over the time. At room temperature (22 ^{o}C) and undisturbed, the evaporation of the solvent is minimum. However, a high frequency of use associated with higher temperatures might accelerate the process of solvent evaporation.

Analysis of bonding failure modes revealed that most dentin failures in groups restored with solvent-depleted adhesives occurred between the adhesive resin and dentin (Fig. 2). In association with the low bond strength values obtained for these groups, it is evident that solvents are essential for the promotion of quality bonding to dentin. For groups restored without solvent, the bonding mechanism was only dependent on the contribution of resin tags because the demineralized dentin region was not infiltrated by monomer solutions (Gwinnett, 1993).

Although enamel bonding was not affected by the absence of solvents, clinically, most preparations include enamel and dentin substrates, which should be bonded with the correct techniques for assuring effective, immediate, and long-term bonding. In this study, solvent elimination was accelerated. Thus, further studies are necessary to determine shelf life of bonding agents according to their frequency of use, because solvent concentration may be decreased when the adhesive bottle is repeatedly opened.

CONCLUSIONS

Complete solvent elimination did not produce a statistical decrease in enamel bond strengths. However, the high-viscosity adhesive solution, which resulted from solvent evaporation, significantly decreased bond strengths to demineralized, moist dentin substrate.

Acknowledgments

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References

Buonocore M (1955) A simple method of increasing adhesion of acrylic filling materials to enamel surfaces *Journal of Dental Research* 34( ) 849-853.

Burke FJT & McCaughey AD (1995) The four generations of dentin bonding *American Journal of Dentistry* 8(2) 88-92.

Burrow MF, Satoh M & Tagami J (1996) Dentin bond durability after three years using a dentin bonding agent with and without priming *Dental Materials* 12(5) 302-307.

Chaves P, Giannini M & Ambrosano GMB (2002) Influence of smear layer pretreatments on bond strength to dentin *Journal of Adhesive Dentistry* 4(3) in press.

Eick JD, Robinson SJ, Chappell RP, Cobb CM & Spencer P (1993) The dentinal surface: its influence on dentinal adhesion. Part III *Quintessence International* 24(8) 571-582.

Ferrari M, Goracci G, Garcia-Godoy F (1997) Bonding mechanism of three "one-bottle" systems to conditioned and unconditioned enamel and dentin *American Journal of Dentistry* 10(5) 224-230.

Finger WJ & Fritz U (1996) Laboratory evaluation of one-component enamel/dentin bonding agents *American Journal of Dentistry* 9(5) 206-210.

Fusayama T, Nakamura M, Kurosaki N & Iwaku M (1979) Non-pressure adhesion of a new restorative system *Journal of Dental Research* 58(4) 1364-1370.

Gallo JR, Burgess JO & Xu X (2001) Effect of delayed application on shear bond strength of four fifth-generation bonding systems *Operative Dentistry* 26(1) 48-51.

Giannini M, Carvalho RM, Martins LRM, Dias CTS, Pashley DH. The influence of tubule density and area of solid dentin on bond strength of two adhesive systems to dentin *The Journal of Adhesive Dentistry* 3(4) 315-324.

Gwinnett AJ (1971) Histologic changes in human enamel following treatment with acidic adhesive conditioning agents *Archives of Oral Biology* 16(7) 731-738.

Gwinnett AJ (1993) Quantitative contribution of resin infiltration/hybridization to dentin bonding *American Journal of Dentistry* 6(1) 7-9.

Jacobsen T & Söderholm KJ (1995) Some effects of water on dentin bonding *Dental Materials* 11(2) 132-136.

Kanca J III (1992) Resin bonding to wet substrate. I. Bonding to dentin *Quintessence International* 23(1) 39-41.

Kanemura N, Sano H & Tagami J (1999) Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. *Journal of Dentistry* 27(7) 523-530.

Kato G & Nakabayashi N (1998) The durability of adhesion to phosphoric acid etched, wet dentin substrates *Dental Materias* 14(5) 347-352.

Maciel KT, Carvalho RM, Ringle RD, Preston CD, Russell CM & Pashley DH (1996) The effects of acetone, ethanol, HEMA, and air on the stiffness of human decalcified dentin matrix *Journal of Dental Research* 75(11) 1851-1858.

Miyazaki M, Sato M & Onose H (2000) Durability of enamel bond strength of simplified bonding systems *Operative Dentistry* 25(1) 75-80.

Nakabayashi N, Kojima K & Masuhara E (1982) The promotion of adhesion by the infiltration of monomers into tooth substrates *Journal of Biomedical Materials Research* 16(3) 265-273.

Nakabayashi N & Takarada K (1992) Effect of HEMA on bonding to dentin. *Dental materials* 8(2) 125-130.

Nakajima M, Kanemura N, Pereira PNR, Tagami J & Pashley DH (2000) Comparative microtensile bond strength and SEM analysis of bonding to wet and dry dentin *American Journal of Dentistry* 13(6) 324-328.

Ogata M, Nakajima M, Sano H & Tagami J (1999) Effect of dentin primer application on regional bond strength to cervical wedge-shaped cavity walls *Operative Dentistry* 24(2) 81-88.

Pashley DH, Ciucchi BC, Sano H & Horner JA (1993) Permeability of dentin to adhesives agents *Quintessence International* 24(9) 618-631.

Pashley DH, Carvalho RM (1997) Dentine permeability and dentine adhesion *Journal of Dentistry* 25(5) 355-372.

Retief DH (1973) Effect of conditioning the enamel surface with phosphoric acid *Journal of Dental Research* 52(2) 333-341.

Sano H, Takatsu T, Ciucchi B, Horner J, Matthews WG & Pashley DH (1995) Nanoleakage: leakage within the hybrid layer *Operative Dentistry* 20(1) 18-25.

Swift Jr EJ & Bayne SC (1997) Shear bond strength of a new one-bottle dentin adhesive *American Journal of Dentistry* 10(4) 184-188.

Tay FR, Gwinnett JA & Wei SHY (1996) Micromorphological spectrum from overdrying to overwetting acid-conditioned dentin in water-free, acetone-based, single-bottle primer/adhesives. *Dental Materials* 12(4) 236-244.

Figure Legends

Figure 1. Prime&Bond 2.1 adhesive solution mass (g) as function of time (s). Note a reduction of 81% of its initial mass. Single Bond adhesive solution mass (g) as function of time (s). Note a reduction of 31% of its initial mass.

Figure 2. Failure modes (%). Same letters indicate no significant difference (p>0.05).

Figure 3. SEM resin-enamel interface formed by Prime&Bond 2.1 as per manufacturer's instructions (PB-E-P). (CR- composite resin, BA- bonding agent, E- enamel, the arrow indicates a resin tag). Original magnification X 5000.

Figure 4. SEM resin-enamel interface formed by solvent-depleted Prime&Bond 2.1 (PB-E-A). (CR- composite resin, BA- bonding agent, E- enamel, the arrow indicates a resin tag). Original magnification X 5000.

Figure 5. SEM resin-dentin interface formed by Prime&Bond 2.1 as per manufacturer's instructions (PB-D-P). (CR- composite resin, BA- bonding agent, HL- hybrid layer, D- dentin, RT- resin tags).

Figure 6. SEM resin-dentin interface formed by solvent-depleted Prime&Bond 2.1 (PB-D-A). Note the incompletely cured appearance of the bonding agent. (CR- composite resin, BA- bonding agent, D- dentin, RT- resin tags, the arrow indicates the area of demineralized dentin that was infiltrated by the resin monomers).

Figure 7. SEM resin-dentin interface formed by Single Bond as per manufacturer's instructions (SB-D-P). (CR- composite resin, BA- bonding agent, HL- hybrid layer, D- dentin, RT- resin tags, the arrows indicate lateral secondary canals infiltrated with resin).

Figure 8. SEM resin-dentin interface formed by solvent-depleted Single Bond (PB-D-A). (CR- composite resin, BA- bonding agent, D- dentin, RT- resin tags, the arrow indicates the area of demineralized dentin that was infiltrated by the resin monomers).

Table 1. Mean bond strengths in MPa (SD) of adhesive systems to enamel and dentin.

	Enamel		Dentin	
SBP	30.19 (3.43)	Aa	26.19 (8.59)	A a
PBP	29.08 (5.76)	Aa	23.64 (11.31)	AB a
SBA	28.08 (7.41)	Aa	12.79 (2.06)	BC b
PBA	22.10 (5.54)	Aa	6.72 (3.06)	C b

Means followed by different letters (upper case letter – column, lower case – line) differ among them by Tukey test at the 0.05 level of significance.

Figure 1.

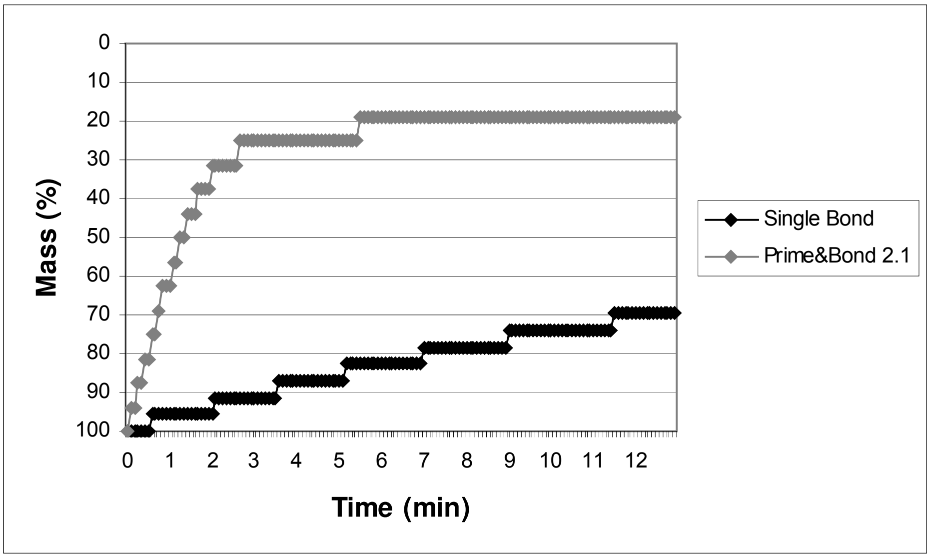


Figure 2.

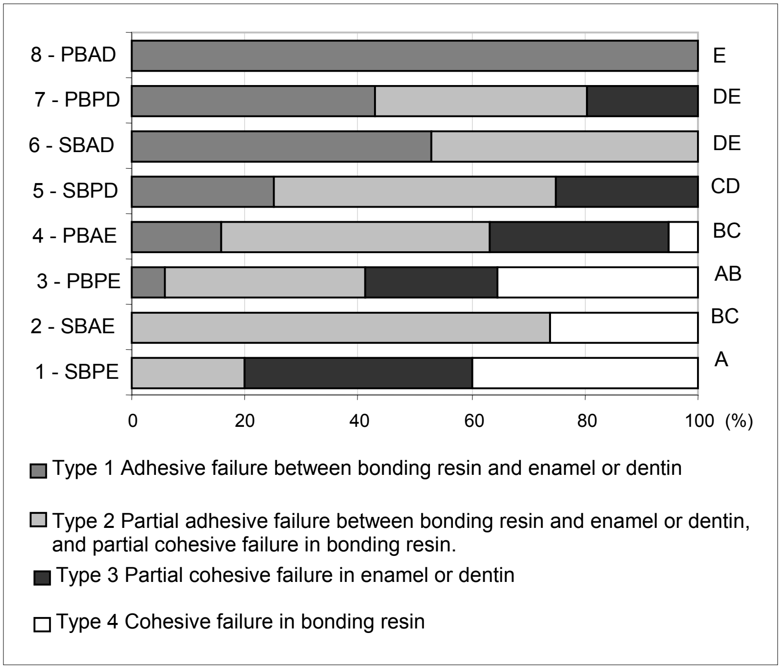


Figure 3.

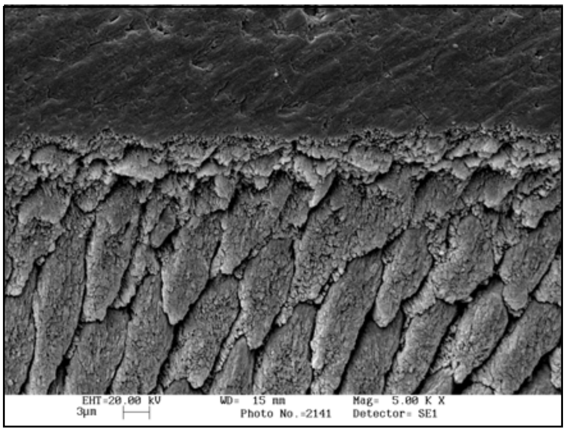
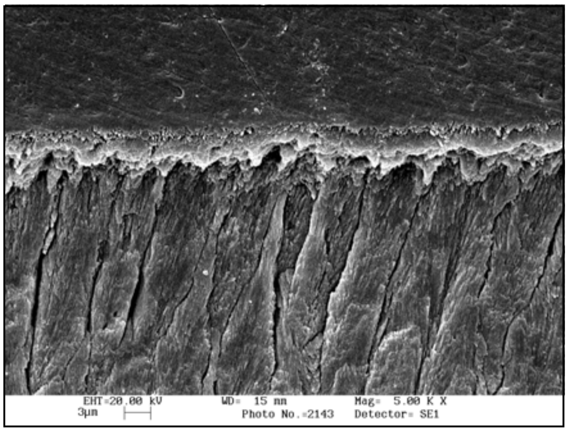


Figure 4.



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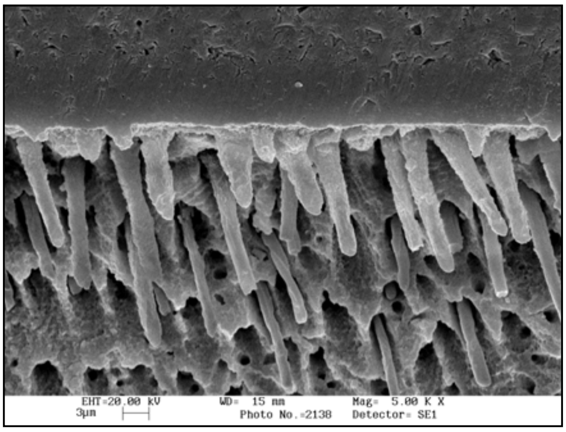


Figure 6.

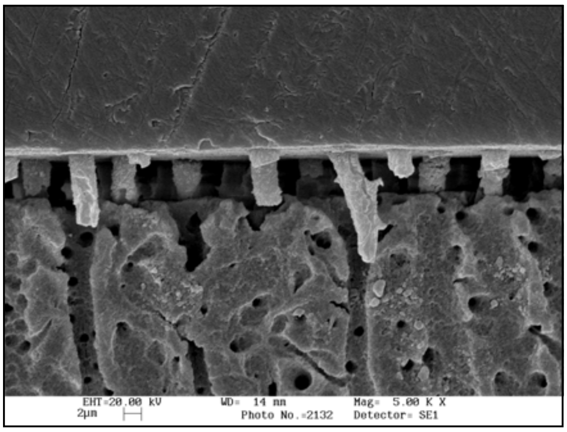


Figure 7.

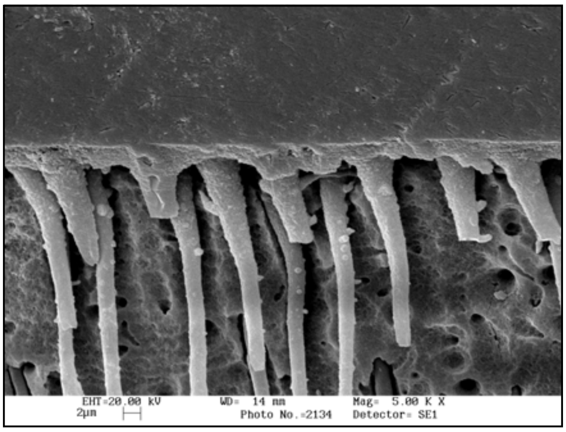
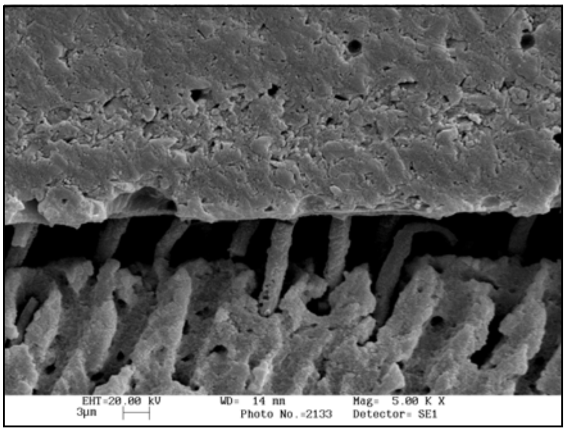


Figure 8.



CAPITULO 3:

Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives. Nanoleakage expression

SHORT TITLE: Ultramorphological analysis of resin-dentin bonds: nanoleakage

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Abstract

This study evaluated the nanoleakage patterns in bonded interfaces using two single-step, self-etch adhesives (Adper Prompt, AD and One-up Bond F, OB), two two-step, self-etch primers (Clearfil SE Bond, CF and Unifil Bond, UB), and one two-step, total-etch adhesive (Single Bond, SB). Dentin surfaces were bonded with the adhesive systems and stored in water at 37°C for 1 week and 6 months. After storage periods, teeth were sectioned into 0.8 mm thick slabs, coated with nail varnish except for the bonded interfaces, and immersed in ammoniacal $AgNO_3$ for 24 hours. After immersion in photodeveloping solution, bonded sections were prepared and observed under a SEM using the backscattered electron mode. Undemineralized, unstained, epoxy resin-embedded sections were prepared for TEM. Nanoleakage patterns were qualitatively compared between periods. Nanoleakage was observed in all bonded specimens at both periods. CF and UB presented silver deposits predominantly restricted to the thin (0.5 μm) hybrid layer (HL) at both periods. While no evident differences were observed in the nanoleakage pattern of UB at 7 days and 6 months, CF presented enlarged areas of silver impregnation after 6 months. SB presented accumulation of silver particles mostly within the HL at 7 days, which was intensified after 6 months. AD and OB presented massive silver accumulation within the HL and the overlying adhesive layer. No evident differences were noticed between storage periods for OB. Silver impregnation increased for all adhesive systems from 7 days to 6 months, except for UB.

Keywords: Dentin bonding, water trees, SEM, TEM, degradation, nanoleakage

1. Introduction

Adhesion to dental hard tissues can be obtained with the use of bonding agents that promote a micromechanical interlocking with both enamel and dentin. While bonding to enamel depends on micromechanical retention of resin tags to the etched enamel surface, bonding to dentin depends on the infiltration of synthetic adhesive monomers into a biological, collagen-rich substrate to form a hybrid layer.[1,2]

Bonding to dentin can be accomplished by two different approaches: total- or self-etching. The first is based on acid etching, which completely removes the smear layer and demineralizes subsurface intact dentin to a depth of 3 to 6 μm.[3] However, this has been considered a sensitive technique, because incomplete resin infiltration might occur if the collagen mesh is excessively dried, or the overwet phenomenon might result from excessive moisture.[4-6] Incomplete resin infiltration and evidence of phase separation in a dentin adhesive and its detrimental effects on the dentin bond has been demonstrated.[7-9] In order to reduce the sensitivity of the technique and simplify bonding procedures, a second approach has been developed, in which self-etching primers composed of acidic monomers are applied on smear layer-covered dentin without further rinsing. Following this etching-priming step, a layer of adhesive resin is then applied.[10] Recently, a more attractive user-friendly one-step self-etching self-priming technique was introduced. The so-called all-in-one adhesives etch, prime and bond in a single application procedure. However, even though these adhesives are marketed as simplified due to a reduced number of steps, they are actually more complex mixtures of acidic resin monomers, solvents, water and additives.[11]

The term nanolekage was first quoted as being the impregnation of silver grains within the porosities of the hybrid layer that were not properly filled with the adhesive resin.[12] More recently, a second mode of nanoleakage has been described to occur in bonded interfaces.[13,14] Silver grains were identified not only in the porosities of the hybrid layer, but also within the adhesive layers.[15] This reticular mode of nanoleakage has been attributed to areas in which water was incompletely removed, and is a morphological manifestation of a “water-treeing” phenomenon.[16] This concept has been previously raised to explain the deterioration of polyethylene coatings used for dielectric cables insulation, and has been recently applied to describe the degradation mechanism of polymer-based dental adhesives and resin-dentin bonds made with such polymers.[13,17]

Adhesive systems are hydrophilic in nature. While such hydrophilicity is desirable for bonding to a moist substrate like dentin, subsequent water sorption within bonded interfaces is thought to contribute to degradation over time.[18,19] The incorporation of increased concentrations of hydrophilic monomers into self-etching adhesives may compromise bond durability, as hydrophilicity and hydrolytic stability are antagonistic properties.[13] One of the major concerns in adhesive dentistry is the durability of bonds to dentin substrate, because bonding is established on a complex hydrated biological composite structure.[17,20,21]

The objectives of this ultrastructural study were to examine, using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), the effects of water storage on the nanoleakage patterns of current simplified adhesive systems. The null hypotheses tested were: (1) there is no difference in the nanoleakage patterns presented by the adhesive systems; and (2) storage in water for 6 months does not affect the nanoleakage patterns of the adhesives examined.

2. Materials and Methods

2.1.Tooth Preparation

Twenty freshly extracted human premolars were used in this study. Teeth were obtained by protocols that were analyzed and approved by the appropriate institutional review board of the Piracicaba Dental School – University of Campinas and with the informed consent of the donors (process # 080/2003). After cleaned, crowns were sectioned with a diamond disc (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling to expose flat, middle dentin surfaces. Before bonding, a standard smear layer was created by polishing dentin surfaces with 600-grit SiC paper in running water.

2.2. Bonding Procedures

Teeth were randomly assigned to ten experimental groups, which were bonded with five adhesive systems divided into three different categories: a two-step total-etch adhesive (Single Bond – SB), two two-step self-etching primers

(Clearfil SE Bond – CF and UniFil Bond – UB), and two single-step self-etching adhesives (Adper Prompt – AD and One Up Bond F – OB). Composition, batch number, application instructions and manufacturers are listed in Table 1.

In order to reduce damage to the diamond knife used for cutting ultrathin sections, a layer of an unfilled bonding resin (Bonding agent of Scotch Bond Multipurpose, 3M ESPE) was applied on top of the bonded surface, instead of the resin composite, and light-cured for 20 s.[11,22] Teeth were then divided into 2 subgroups according to the storage time in distilled water at 37 °C (7 days or 6 months). For the six-month water-storage groups, water was changed weekly, in order to prevent bacterial growth and accelerate the degradation process.[23]

2.3. Nanoleakage Evaluation

After each storage period, specimens were vertically, serially sectioned in the bucco-lingual direction into 0.9 mm thick slabs using a diamond impregnated saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water lubrication. Four slabs were obtained from each tooth, forming a total of 8 specimens per group. Bonded slabs were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. In order to rehydrate specimens, they were immersed in distilled water for ten minutes prior to immersion in the tracer solution for 24 h. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al..[13] Tooth slabs were placed in the ammoniacal silver nitrate in total darkness for 24 h, rinsed thoroughly in distilled water, and immersed in photodeveloping solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface.

2.4. Electron Microscopy

Two out of the four slabs obtained from each tooth were assigned for scanning electron microscopy (SEM) and the other two were prepared for transmission electron microscopy (TEM). All slabs were wet-polished with 600 SiC paper in order to remove the nail varnish. Specimens were examined microscopically to observe silver uptake along the resin-dentin interface, including hybrid and adhesive layers, and compare images obtained after 7 days or 6 months of water-storage.

2.4.1. Scanning Electron Microscopy

Slabs were polished with a 1000-grit SiC paper and 6, 3, 1 and 0.25 μm diamond paste (Buehler Ltd, Lake Bluff, IL) using a polish cloth. They were ultrasonically cleaned, air dried, mounted on stubs and carbon-coated (MED 010, Balzers Union, Balzers, Liechtenstein). Resin-dentin interfaces were observed with a scanning electron microscope (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, United Kingdom) operated in the backscattered electron mode. Electron-dense regions were photographed at 500; 2,000; and 8,000X magnification.

2.4.2. Transmission Electron Microscopy

Undemineralized, epoxy-resin-embedded, ultrathin sections were prepared for TEM. One strip approximately 6 mm wide was sectioned from each slab perpendicular to the flat dentin surface using a diamond disk under copious water supply. Specimens were fixed in Karnovsky’s solution, post-fixed in osmium tetroxide, dehydrated in ascending acetone series (30 to100%) and embedded in epoxy resin. Care was taken to ensure proper orientation of the resin-dentin interface. After initial screening of all semithin sections from each group, representative 90-nm-thick ultrathin sections were prepared with an ultramicrotome (MT-2C, RMC, Florida, USA) using a diamond knife and collected on 100-mesh formvar-coated copper grids. Without additional staining, they were observed in a transmission electron microscope (Zeiss EM 900, Zeiss, Munich, Germany).

3. Results

Observation of resin-dentin interfaces revealed the presence of silver deposits in all bonded interfaces. The amount of silver deposition varied from moderate within the hybrid layer to severe within the hybrid and adhesive layers. Representative leakage patterns at the adhesive-dentin interfaces after 7-day or 6-month water-storage are illustrated for each adhesive system in Figs 1-5. TEM observation revealed nanoleakage patterns that could not be identified with the SEM.

SB presented nanoleakage along most of the extension of the bonded slabs. The hybrid layer formed by this total-etch system was about 3 to 4 μm thick. In some regions, silver deposition was observed at the base of the hybrid layer (Fig. 1A), whereas other regions revealed silver deposition within the entire thickness of the hybrid layer (Figs. 1B-1D). Six-month specimens presented more concentrated silver deposits when compared to 7-day interfaces. TEM also revealed isolated, tiny silver deposits at the base of the adhesive layer (Fig. 1A and 1B).

The two-step self-etching primers presented thin hybrid layers (HL) of approximately 0.5 μm thick. Silver deposits were predominantly restricted to the HL at both periods. No evident differences were observed in the silver deposition patterns of UB at 7 days and 6 months, while CF presented larger areas of silver impregnation after 6 months. TEM observations showed isolated silver grains within the hybrid layer formed by CF, which increased in quantity and size after 6-month water-storage (Fig. 2A and 2B). TEM images of UB demonstrated sparse silver deposits along the interface either after 7 days (Figs. 3A and 3B) or after 6 months (Fig.3C).

The single-step self-etching adhesives AD and OB presented massive silver impregnation, either after 7 days or after 6 months of storage in water. Silver concentration apparently increased for AD after six months, but no evident differences in leakage patterns could be observed in OB images. Figure 4 depicts the leakage patterns for OB. A thin hybrid layer approximately 0.5 μm thick was observed in specimens bonded with this one-step all-in-one adhesive. At 7 days, specimens presented silver deposition along almost the entire extension of the hybrid layer, with water trees protruding approximately 2.5 μm above the hybrid layer to the adhesive layer. AD presented a thicker hybrid layer of approximately 3 to 4 μm that was comparable to the thickness of hybrid layers obtained with the total-etch SB. The leakage patterns for AD are shown in Figure 5. Some water-trees at the adhesive layer and silver deposits within the hybrid layer were observed at 7 days with the SEM (Fig. 5A). Reticular silver deposits oriented to the top of the hybrid layer were observed in the TEM (Fig. 5B). This mode of reticular deposition increased in quantity, and extended to the adhesive layer after six-month water-storage, showing typical water-tree formation (Fig. 5C).

4. Discussion

Ideally, an adhesive system should provide adequate bond strength and sealing of dentin surfaces and be long-lasting. Much information has already been generated, but the mechanisms of resin-dentin interfaces degradation are not yet fully understood. Degradation of the bonds has been attributed to hydrolytic degradation of the resin adhesive and/or dentin collagen.[19,24,25] All adhesives presented a certain degree of nanoleakage with notable differences among patterns of silver deposition, depending on their mode of application and composition. Thus, the first null hypothesis must be rejected. The results support the alternate hypothesis that adhesive systems of different composition and application modes present different nanoleakage patterns.

Potential water-binding domains within hybrid layers and adhesive layers in resin-dentin interfaces are traced by ammoniacal silver nitrate.[13] Differences in hydrophilicity and water content have an important role in nanoleakage patterns presented by adhesive systems. All bonding agents tested in this study present some amount of water and hydrophilic monomers in their composition (Table 1). The presence of water within dentin adhesives composition plays an important role in both total- and self-etching techniques. Water-based adhesives used in total-etch systems have been shown to solvate dried matrices, being able to re-expand dentin collagen.[26] Water is an essential component in self-etching systems, in order to enable ionization of acidic monomers and demineralization of underlying enamel and/or dentin.[11] Besides the presence of water in their composition, the ionizable moieties of acidic monomers are hydrophilic. Thus, different water sorption rates might be expected for the adhesive resins tested in this study.[27] Water is known to be detrimental for the resin-dentin interface. Apparently, as little as 9% water incorporation in adhesive formulas may be detrimental to its mechanical properties.[28] Even if no water is present during polymerization, environmental moisture might enter the polymer bulk,[29,30] trigging the chemical polymer degradation.[31] After aging specimens of adhesive resins in different storage media for six months, Carrilho et al. reported decreased mechanical properties[32] and bond strengths[33] for specimens aged in water, whereas specimens aged in mineral oil presented a remarkable stability over the experiments.

As the pathways for nanoleakage had been attributed to hybrid layers that had not been fully penetrated by resin, this phenomenon would not have been expected to occur with self-etching adhesives that simultaneously etch and infiltrate dentin. However, this concept has been recently refuted.[30,34,35] In addition, our results demonstrated silver grain deposition within interfaces of total-etch and self-etching systems. SEM and TEM images revealed an intensified concentration of silver grains after 6-month water-storage for the total-etch SB, for the two-step self-etching CF and for the one-step self-etching AD. These observations lead us to reject the second null hypothesis, because storage in water for six months did affect the nanoleakage patterns of bonded interfaces.

The total-etch adhesive SB presented a high degree of nanoleakage in the present study. Even though reports have demonstrated high bond strengths for SB, a greater penetration of silver has also been reported for this system when compared to a two-step self-etching system Clearfil Liner Bond 2V.[34,36] Silver deposits were commonly observed along the base of the hybrid layer (Fig. 1A) as seen in previous studies,[12,14] but they were also observed within the entire hybrid layer thickness in some areas (Fig. 1B-D). Silver deposits within hybrid layers promoted by total-etch systems may be attributed to regions of incomplete resin infiltration or incomplete resin polymerization, which represent pathways for fluid penetration. Degradation of bonds has been attributed to fluid penetration through these pathways.[24,37] Our results support this hypothesis, because SB six-month specimens presented increased silver deposition when compared to 7-day interfaces (Fig. 1). De Munck et al.[21] reported decreased bond strengths for SB after four years of storage in water. Interestingly, the reduction in μTBS was higher than that of its three-step precursor Scotchbond Multi-purpose. The application of the adhesive in three steps (etchant, primer and adhesive) favors copolimerization of the primer, and the lower hydrophilicity of the cured resin might result in optimized hybridization and lower sensitivity to water degradation. The benefits of the application of an additional layer of low viscosity bonding resin on dentin surfaces bonded with simplified adhesive systems has been recently demonstrated.[38] Isolated silver grains were also observed at the base of the adhesive layer in the unstained undemineralized TEM sections (Figs. 1A-B). This pattern of silver deposition may be attributed to microvoids formed by water that could not be removed by air-drying after application or that was absorbed into the polymer after polymerization. It has been demonstrated that under wet bonding, the hybridization efficiency decreases as the resin/dentin interface produced by SB is analyzed through its depth by micro-Raman spectroscopy.[39] It was suggested that the adhesive dentin interface is a porous collagen web infiltrated primarily by the low molecular weight, hydrophilic and hydrolytically unstable HEMA.[39] Water molecules that exist in the form of thermodynamically stabilized water clusters may hydrogen bond with hydrophilic functional groups of the adhesive monomers, hampering water evaporation.[15] In addition, as water evaporates from water-HEMA mixtures, the rise in HEMA concentration lowers water vapor pressure, resulting in residual water.[40] Mohsen et al.[29] demonstrated that even if the polymer is completely dry after polymerization, water might be absorbed, interacting with the carbonyl groups and forming tight hydrogen bonds. The amount of water inside the polymer bulk tends to increase with time, forming larger pores.

Two-step self-etching adhesives presented the lowest nanoleakage patterns along the experiment. Pereira et al.[34] reported that although bond strengths were not significantly different, the degree of nanoleakage presented by the self-etching Clearfil Liner Bond 2V, which is the three-bottle version of Clearfil SE Bond, was almost half that presented by the total-etch Single Bond. Both CF and UB adhesives may be considered mildly agressive, based on their depth of demineralization beyond smear layer-covered dentin.[11] They produced thin hybrid layers approximately 0.5 μm thick. For CF, after 7-day water-storage, isolated silver deposits were observed predominantly at the top of the hybrid layer (Fig. 2A). A small amount of isolated silver grains were also observed within or above the hybrid layer, probably in the hydrophilic part of the adhesive layer. The hydrophobic, filled adhesive layer did not take up silver. Even though the primer is not light-cured after application, TEM images suggest that they did not mix with each other during application (Fig. 2). After six months of storage in water, enlarged areas of silver deposition were observed within the hybrid layer (Fig. 2B). This may be an indication of degradation of the resin-dentin interface. If compared with the amount of nanoleakege present in the interfaces of the total-etch SB, which produced hybrid layers ranging from 3 to 4 μm thick, the silver uptake of CF after 6-month water-storage was much lower. However, if a comparison was made per unit volume of hybrid layer, CF aged specimens could have presented similar or more silver uptake than the total-etch SB.[34] A recent modification of this system, which includes the incorporation of an antibacterial monomer into the primer solution and surface-treated NaF into the bonding solution, to act as a fluoride-releasing agent, has shown promising results with respect to long-term bond strength stability.[41] By observing TEM images, it was evident that UB was the adhesive system that presented the least silver accumulation (Fig. 3). When specimens were observed with backscattered SEM, almost no silver was observed along bonded interfaces both after 7 days and 6 months of storage in water. TEM revealed tiny silver grains only at the top of the hybrid layer, which demonstrated a similarity between leakage patterns over the 6-month storage period. The low silver uptake observed for the two-step self-etching systems may be attributed to the thin hybrid layer produced. The thicker the hybrid layer, the greater the potential for nanoleakage of silver ions within demineralized dentin, the hybrid layer or within partially polymerized adhesive resin.[34,35] Another important feature that might have contributed for the low silver uptake is the hydrophobic adhesive layer that is placed over the primer, which might reduce water sorption.

It was observed that both one-step self-etching systems demonstrated great silver deposits within hybrid layers that had been etched and infiltrated simultaneously by the acidic monomers. Nanoleakage within resin-dentin interfaces formed by self-etching adhesives is not necessarily caused by incomplete resin infiltration into demineralized dentin. Conversely, they represent areas within the polymerized resin matrix in which water is incompletely removed, resulting in regions of incomplete polymerization and/or hydrogel formation, or hydrophilic domains of acidic monomers that are more prone to water sorption.[13,30] The ability of single-step adhesives to seal dentin surfaces has been recently questioned, as they function as permeable membranes allowing water movement between the interface and underlying dentin.[42] OB presented a thin hybrid layer of approximately 0.5 μm, and typical water trees of approximately 2.5 μm protruding from the hybrid layer to the adhesive layer after 7 days of water-storage (Fig. 4). Despite the initial high amount of silver deposition, no evident increase in the silver impregnation patterns could be determined after 6 months. Hashimoto et al.[43] have recently demonstrated a drastic decrease in bond strengths and morphological signs of degradation after 6 months of water exposure for the self-etching adhesive OB. The single-step AD is considered an aggressive self-etching system.[11] The thickness of the hybrid layer produced by this system is comparable to that produced by the total etch SB. It is known that increased concentration of acidic resin monomers results in increased hydrophilicity and, consequently, in increased susceptibility to hydrolytic degradation.[11] Among all bonding agents studied, AD presented the highest degree of nanoleakage after 7 days of water-storage, with large silver deposits at the whole extension of the hybrid layer, and water trees protruding to the adhesive layer (Fig. 5). Increased nanoleakage was observed after six months of storage, with an increased number of water trees, indicating that degradation probably occurred at these interfaces.

Resin-dentin interfaces degrade in three stages. First, water is absorbed into the polymer, trigging the chemical degradation.[31] Second, resin is eluted from the hybrid or adhesive layer.[43,44] Third, exposed collagen fibrils might be degraded by matrix metallopropteinases (MMPs) present in dentin, or human saliva.[44,45] Evidence has been shown that collagen is not easily degraded in water.[46] However, the effects of host-derived MMPs on degradation of resin-dentin interfaces have not yet been unmasked.[47] Degradability of synthetic polymer materials is specifically related to the chain architecture,[48] chemical structures, molecular weights, presence of microorganisms and environmental conditions.[49,50] A physical chemical understanding of dental polymers degradation and erosion processes, as well as collagen type I degradation is the key for a better understanding of the problems related to adhesive restorations longevity and probably also for their solution. Another important point that must not be neglected is the potential effects that released degradation products might have on human physiologic functions.[50,51] Combined efforts of molecular biologists and polymer chemists would certainly contribute largely to the promotion of a durable seal of dental hard tissues.[52]

In summary, all adhesives studied presented a certain degree of nanoleakage. Two-step total-etch and single-step self-etching systems were more prone to nanoleakage and, thus, to degradation in the long-term. Two-step self-etching adhesives presented the lowest degree of nanoleakage, and UB presented no signs of degradation over the experiment. Problems commonly associated with the use of dentin adhesives such as premature bond degradation and recurrent caries are still reported, in spite of the great advances attributed to adhesive dentistry during the last decades. To date, the routine use of simplified adhesive systems in combination with resin composites to restored cavities with exposed dentin margins is a questionable recommendation.[53]

Acknowledgments

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References

1. Gwinnett AJ. Histologic changes in human enamel following treatment with acidic adhesive conditioning agents. Arch Oral Biol 1971; 16:731-738.
2. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982; 16:265-273.
3. Perdigão J, Lambrechts P, Van Meerbeek B, Tome AR, Vanherle G, Lopes AB. Morphological field emission-SEM study of the effect of six phosphoric acid etching agents on human dentin. Dent Mater 1996; 12:262-271.
4. Kanca J 3rd. Resin bonding to wet substrate. 1. Bonding to dentin. Quintessence Int 1992; 23:39-41.
5. Tay FR, Gwinnett AJ, Wei SH. The overwet phenomenon: an optical, micromorphological study of surface moisture in the acid-conditioned, resin-dentin interface. Am J Dent 1996; 9:43-48.
6. Ferrari M, Tay FR. Technique sensitivity in bonding to vital, acid-etched dentin. Oper Dent 2003; 28:3-8.
7. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int 1999; 30:501-507.
8. Spencer P, Wang Y, Walker MP, Wieliczka DM, Swafford JR. Interfacial chemistry of the dentin/adhesive bond. J Dent Res 2000; 79:1458-1463.
9. Hashimoto M, Ohno H, Kaga M, Sano H, Endo K, Oguchi H. The extent to which resin can infiltrate dentin by acetone-based adhesives. J Dent Res 2002; 81:74-78.
10. Watanabe I, Nakabayashi N, Pashley DH. Bonding to ground dentin by a Phenyl-P self-etching primer. J Dent Res 1994; 73:1212-1220.

11. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater 2001; 17:296-308.

12. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995; 20:18-25.

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

14. Li H, Burrow MF, Tyas MJ. Nanoleakage patterns of four dentin bonding systems. Dent Mater 2000; 16:48-56.

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

16. Raharimalala V, Poggi y, Filippini JC. Influence of Polymer morphology on water treeing. IEEE Trans Dielect Insul 1994; 1:1094-1103.

17. Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SC, Yiu CK, Cheong C. Aging affects two modes of nanoleakage expression in bonded dentin. J Dent Res 2003; 82:537-541.

18. Tanaka J, Ishikawa K, Yatani H, Yamashita A, Suzuki K.Correlation of dentin bond durability with water absorption of bonding layer.Dent Mater J 1999; 18:11-18.

19. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000; 79:1385-1391.

20. Marshall GW Jr, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent 1997; 25:441-458.

21. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003; 82:136-140.

22. Ferrari M, Mannocci F, Vichi A, Davidson CL. Effect of two etching times on the sealing ability of Clearfil Liner Bond 2 in Class V restorations. Am J Dent 1997; 10:66-70.

23. Kitasako Y, Burrow MF, Nikaido T, Tagami J. The influence of storage solution on dentin bond durability of resin cement. Dent Mater 2000; 16:1-6.

24. Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999;78:906-911.

25. Giannini M, Seixas CAM, Reis AF, Pimenta LAF. Six-month storage-time evaluation of one-bottle adhesive systems to dentin. J Esthet Rest Dent 2003;15:38-43.

26. Pashley DH, Carvalho RM, Tay FR, Agee KA, Lee K-W. Solvation of dried matrix by water and other polar solvents. Am J Dent 2002; 15:97-102.

27. Burrow MF, Inokoshi S, Tagami J. Water sorption of several bonding resins. Am J Dent 1999; 12:295-298.

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

29. Mohsen NM, Craig RG, Filisko FE. The effects of moisture on the dieletric relaxation of urethane dimethacrylate polymer and composites. J Oral Rehab 2001; 28:376-392.

30. Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent 2002; 4:255-269.

31. Göpferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996; 17:103-114.

32. Carrilho MRO, Carvalho RM, Tay FR, Pashley DH. Effects of storage media on mechanical properties of adhesive systems. Am J Dent 2003 (in press)

33. Carrilho MRO, Carvalho RM, Tay FR, Pashley DH. Durability of resin-dentin bonds related to water and oil storage. Am J Dent 2003 (in press)

34. Pereira PNR, Okuda M, Nakajima M, Sano H, Tagami J, Pashley DH. Relationship between bond strengths and nanoleakage: evaluation of a new assessment method. Am J Dent 2001; 14:100-104.

35. Okuda M, Pereira PNR, Nakajima M, Tagami J, Pashley DH. Long-term durability of resin dentin interface: nanoleakage vs microtensile bond strength. Oper Dent 2002; 27:289-296.
36. Okuda M, Pereira PNR, Nakajima M, Tagami J. Relationship between nanoleakage and long-term durability of dentin bonds. Oper Dent 2001; 26:482-490.
37. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. Resin-tooth adhesive interfaces after long-term function. Am J Dent 2001; 14:211-215.
38. Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NRFA, Pashley DH. Adhesive permeability affects coupling of resin cements that utilize self-etching primers to dentine. J Dent 2003; 31: in press.
39. Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding. J Dent Res 2003; 82:141-145.
40. Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, Pashley DH. Effects of HEMA on water evaporation from water-HEMA mixtures. Dent Mater 1998; 14:6-10.
41. Nakajima M, Okuda M, Ogata M, Pereira PN, Tagami J, Pashley DH. The durability of a fluoride-releasing resin adhesive system to dentin. Oper Dent 2003; 28:186-192.
42. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002; 30:371-382.
43. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. Degradation patterns of different adhesives and bonding procedures. J Biomed Mater Res Part B: Appl Biomater 2003; 66B:324-330.
44. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. Biomaterials 2003; 24:3795-3803.
45. Dayan D, Binderman I, Mechanic GL. A preliminary study of activation of collagenase in carious human dentine matrix. Arch Oral Biol 1983;28:185-187.

46. Carvalho RM, Tay F, Sano H, Yoshiyama M, Pashley DH. Long-term mechanical properties of EDTA-demineralized dentin matrix. J Adhes Dent 2000; 2:193-199.

47. Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998; 77:1622-1629.

48. Striegel AM. Influence of chain architecture on the mechanochemical degradation of macromolecules. J Biochem Biophys Methods 2003; 56:117-139.

49. Gu J-D. Microbiological dterioration and degradation of synthetic polymeric materials: recent research advances. International Biodeterioration & Biodegradation 2003; 52:69-91.

50. Yourtee DM, Smith RE, Russo, KA, Burmaster S, Cannon JM, Eick JD, Kostoryz EL. The stability of mathacrylate biomaterials when enzyme challenged: kinetic and systematic evaluations. J Biomed Mater Res 2001; 57:552-531.

51. 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; 12:136-151.

52. Tay FR, Pashley DH. Dental adhesives of the future. J Adhes Dent 2002; 4:91-103.

53. Göhring TN, Schönenberger KA, Lutz F. Potential of restorative systems with simplified adhesives: Quantitative analysis of wear and marginal adaptation in vitro. Am J Dent 2003; 16: 275-282.

LEGENDS TO FIGURES

Figure 1. (A) and (B) are undemineralized, unstained TEM micrographs of the resin-dentin interfaces bonded with the total-etch adhesive Single Bond after 7 days and 6 months of storage in water, respectively. (A) Silver deposits were identified at the base of the hybrid layer (HL) and within all hybrid layer thickness (B). The amount of silver deposits increased within either the hybrid layer (arrowheads) or the adhesive layer (AD) (white arrows). (C) and (D) are backscattered SEM micrographs of the interface bonded with Single Bond after 1 week and 6 months. More concentrated silver deposits were noticed within the hybrid layer after 6-month water-storage. D, dentin.

Figure 2. TEM micrographs denote enlarged areas of silver impregnation within the hybrid layer (HL) of the two-step self-etching adhesive Clearfil SE Bond after 6 months of water-storage. (A) Silver deposits (arrowheads) were observed at the top of the hybrid layer and within the hydrophilic portion of the adhesive layer (AD), next to the filler particles (circle) after 7 days. (B) There was an increase in size and density of silver deposits (black arrows) within the hybrid layer after aging in water for 6 months. D, dentin.

Figure 3. (A) TEM micrographs of the resin-dentin interface bonded with the two-step self-etching adhesive Unifil Bond after 7 days of storage in water. Isolated tiny silver grains (arrowheads) were observed predominantly at the top of the hybrid layer (HL). (B) High magnification view of the interface after 7-day water-storage. (C) A similar silver deposition pattern was observed after aging in water for 6 months. D, dentin.

Figure 4. (A) and (B) are TEM micrographs of the resin-dentin interface bonded with the single-step self-etching adhesive One up Bond F after 7 days of storage in water. Massive silver deposition was observed for this system. Typical water-trees were observed protruding approximately 2.5 μm from the hybrid layer (HL) to the adhesive layer (AD) (arrows). (B) Tiny silver grains (black arrows) were also observed within the adhesive layer, which might coalesce, resulting in new water-trees after aging in water. (C) Backscattered SEM of the resin-dentin interface after 6 months. The zone between arrows (approximately 3 μm thick) represents the impregnation of silver deposits within the hybrid (0.5 μm) and adhesive layers (2.5 μm), as shown in figs 4A-B. D, dentin.

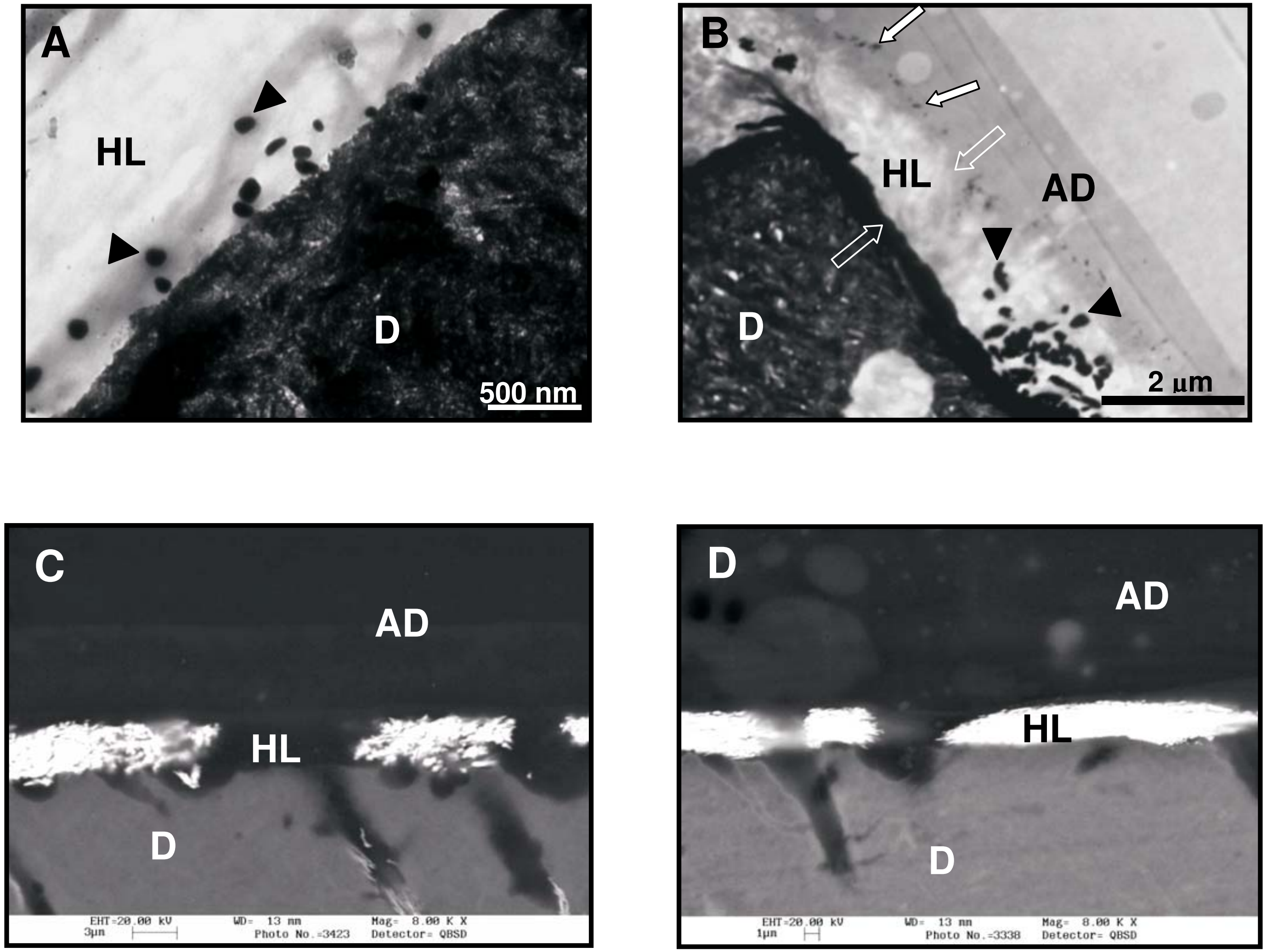
Figure 5. (A) Backscattered SEM micrograph of the resin-dentin interface bonded with the single-step self-etching adhesive Adper Prompt after 7 days of storage in water. Massive accumulation of silver grains can be observed within the hybrid layer (HL) and water-trees can be observed within the adhesive layer (AD). (B) and (C) are TEM micrographs of the resin-dentin interface after 7 days and 6 months of storage in water, respectively. Note silver grains within the hybrid layer (B) and reticular deposits oriented toward the adhesive layer (C) (white arrows). D, dentin.

Table 1 –Materials, Brand (Lot #), composition, application technique and manufacturers of adhesive systems used in this study (Bis-GMA, bisphenol-A diglycidil ether dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; 4-MET, 4-methacryloyloxyethyltrimellitic acid; BAPO, bis-acyl phosphine oxide; UDMA, urethane dimethacrylate; MAC-10, methacryloyloxydecamethlene malonic acid; TEGDMA, triethylene glycol dimethacrylate; MMA, methyl methacrylate):

Material	Brand (Lot #)	Composition	Application technique	Manufacturer
Two-step total-etch adhesive	Single Bond 2KG	*Etchant*: 35% H_3PO_4 *Adhesive*: water, ethanol, Bis-GMA, HEMA, UDMA, Bisphenol A glycerolate, polyalkenoic acid copolymer, dimethacrylate	a (15s); b (15s); c; d; e; i	3M ESPE, St Paul, MN, USA
Two-step Self-etching primers	Clearfil SE Bond 61238	*Primer*: Water, ethanol, MDP, HEMA, dimethacrylate hydrophilic, canphorquinone, N,N-diethanol p-toluidine *Adhesive*: MDP, Bis-GMA, HEMA, dimethacrylate hydrophobic, canphorquinone, N,N-diethanol p-toluidine, silaneited colloidal silica	f (20 s); e; g; i	Kuraray, Osaka, Japan
	Unifil Bond 0203221	*Primer*: Water, ethanol, 4-MET, HEMA, UDMA, photoinitiators *Adhesive*: HEMA, UDMA, TEGDMA, ethanol, silicon dioxide and initiator	f; g; i	GC America Inc., IL, USA
One-step Self-etching adhesives	Adper prompt EXM - 618	*Prompt A*: Methacrylated phosphoric acid esters, fluoride complex, photoinitiator (BAPO), estabilizer, parabenes *Prompt B*: HEMA, Water	h; i	3M ESPE, St Paul, MN, USA
	One-up Bond F 000231E	Water, MMA, HEMA, coumarin dye, metacryloyloxyalkyl acid phosphate, MAC-10, multifuntional methacrilic monomer, fluoraluminosilicate glass, photoinitiator (aryl borate catalyst)	h (leave undisturbed for 20 s); i	Tokuyama Corp., Tokyo, Japan

Application technique – a: acid etch; b: rinse surface; c: dry with cotton-pellet; d: apply one-bottle adhesive; e: gently air dry; f: apply primer; g: apply adhesive; h: apply mixture; i: light cure.

Fig. 1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HL
D
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Mag= 8.00 K X
Detector= QBSD
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Fig. 2

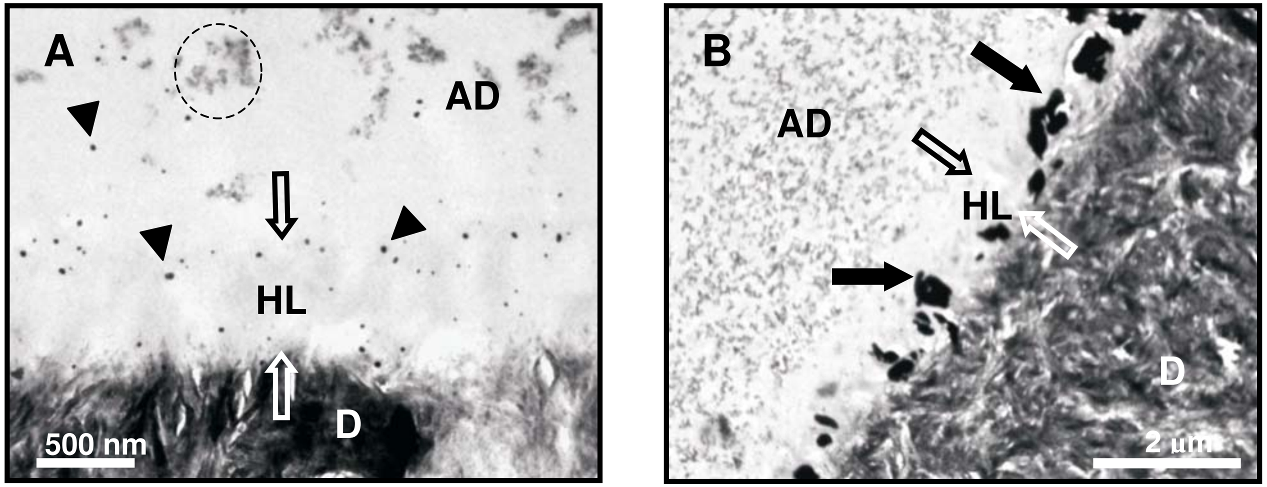
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Fig. 3

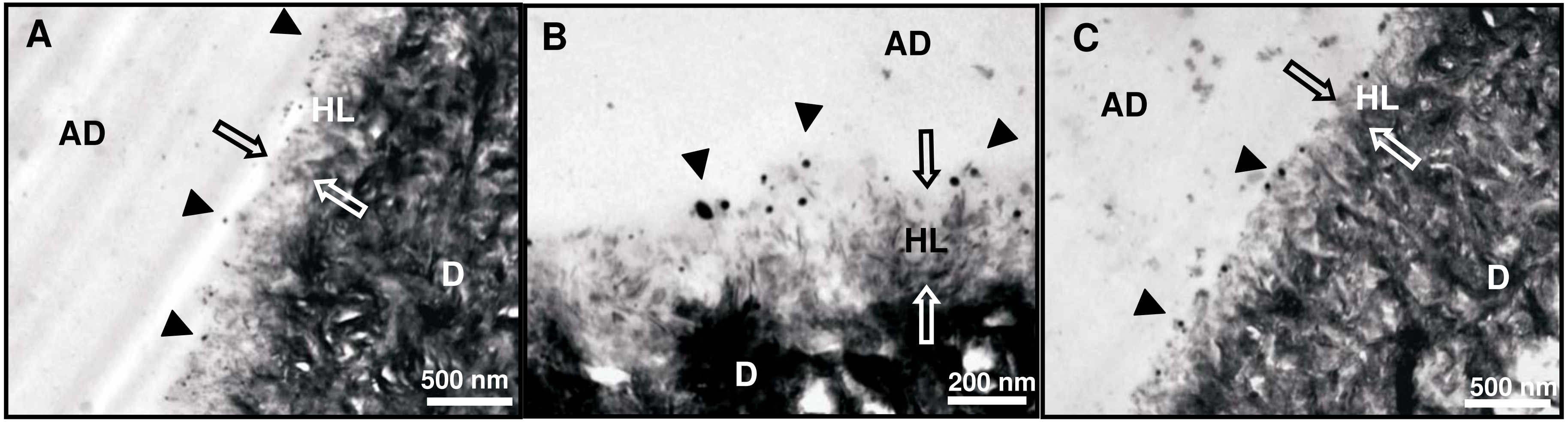
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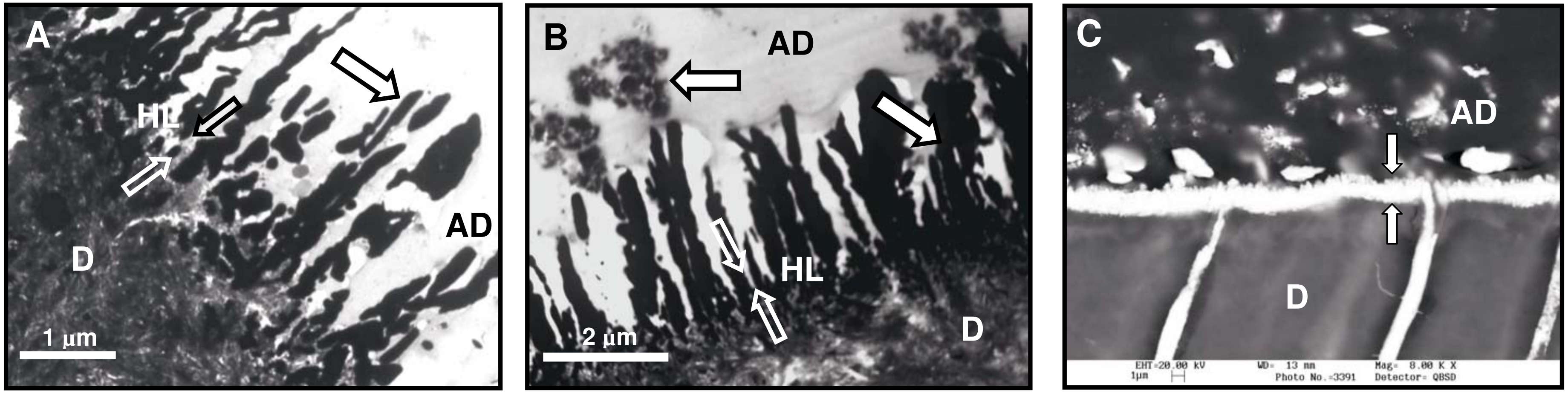
Fig. 4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D
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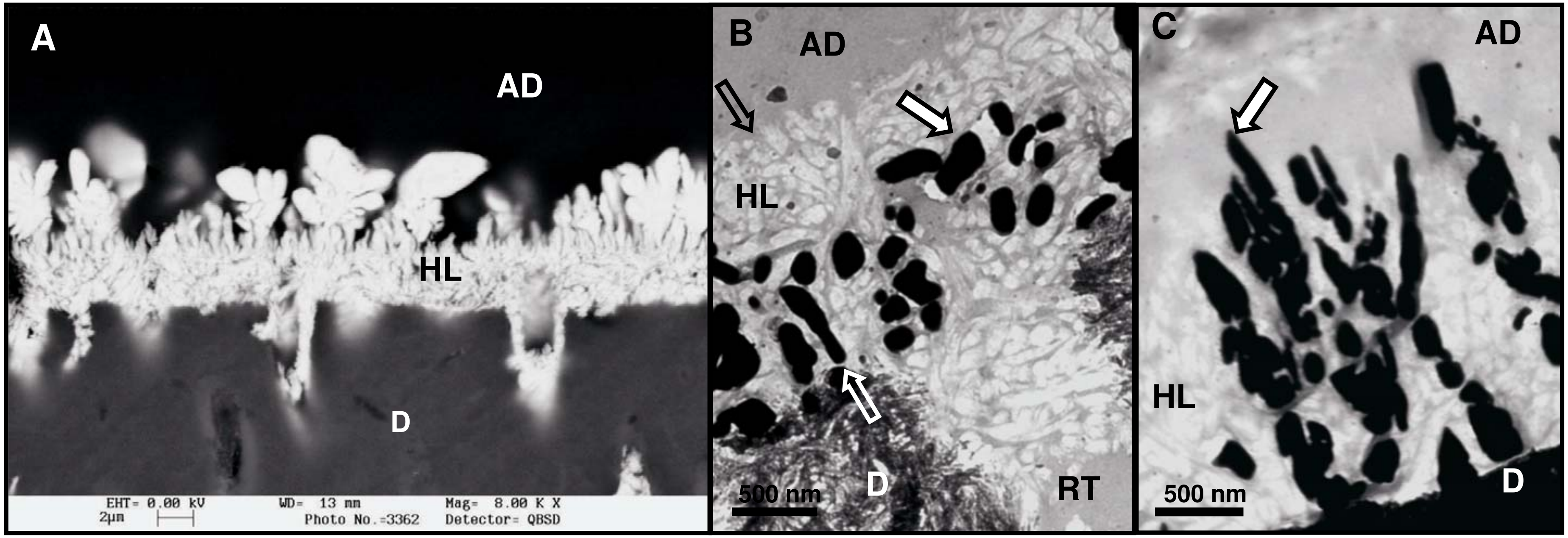
Fig. 5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HL
D
RT
500 nm
C
AD
HL
D
500 nm

CAPÍTULO 4:

Water-Induced Nanoleakage Prevention by Bonding Strategies

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SHORT TITLE: Nanoleakage of resin-dentin interfaces

KEY WORDS: Nanoleakage, adhesion, degradation, aging

Number of words in the abstract: 150

Number of words in the abstract and the text: 2,500

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ABSTRACT

This study evaluated the ability of etch-and-rinse and self-etch adhesives to prevent time- and water-induced nanoleakage in resin-dentin interfaces over a 6-month storage period. Five commercial adhesives were tested: a one-step self-etching adhesive, two two-step self-etching primers, and two etch-and-rinse systems (an ethanol/water-based and an acetone-based). A silver tracer solution was used to show nanometer-sized spaces and degradation signs within resin-dentin interfaces. Characterization of interfaces was performed with the TEM. Two-step self-etching primers presented low silver deposition. Etch-and-rinse adhesives presented silver deposits within the hybrid layer (HL), which was intensified for the ethanol/water-based system after water-storage. The one-step self-etching adhesive presented silver accumulation within the HL and water-trees protruding into the adhesive layer, which increased in size and quantity after water-storage. Different levels of water-induced nanoleakage prevention were observed for the different bonding strategies. The two-step self-etching primers were the most effective in preventing nanoleakage after 6 months of water-storage.

INTRODUCTION

Bonding of resin-based composites to dentin can be accomplished by means of etch-and-rinse or self-etch adhesive systems. The etch-and-rinse technique has been considered sensitive (Kanca, 1992; Tay *et al.*, 1996; Ferrari and Tay, 2003). Incomplete infiltration and evidence of phase separation within resin-dentin interfaces and its detrimental effects have been demonstrated (Spencer *et al.*, 2000; Hashimoto *et al.*, 2002). With the attempt to reduce technique sensitivity, a second approach was developed, in which two-step self-etching primers were applied without further rinsing, followed by application of a hydrophobic resin layer (Watanabe *et al.*, 1994). Recently, a more user-friendly one-step self-etching/self-priming technique was introduced. Even though single-step systems are marketed as simplified, they are actually more complex mixtures of hydrophilic and hydrophobic monomers, solvents, water and additives (Tay and Pashley, 2001; Van Landuyt *et al.*, 2005).

Water sorption within resin-dentin interfaces has been thought to contribute to their degradation over time (Tanaka *et al.*, 1999). Increased concentrations of hydrophilic monomers into contemporary adhesives may compromise bond durability, since hydrophilicity and hydrolytic stability are antagonistic properties (Tay *et al*, 2002a). Adhesive systems should promote an effective, long-lasting seal of tooth structures. However, several papers have demonstrated deterioration signs within resin-dentin interfaces over time. (Hashimoto *et al.*, 2000; Tay *et al.*, 2003; Reis *et al.*, 2004). The nanoleakage technique has been used to trace nanometer-sized spaces within bonded interfaces (Sano et al., 1995a; 1995b).

The objectives of this ultrastructural study were to examine the effectiveness of contemporary adhesives that use different bonding strategies in preventing nanoleakage. The null hypotheses to be tested were: (1) adhesives of different compositions and application modes would present no difference in nanoleakage patterns; (2) different storage media (water or mineral oil) would not result in different silver deposition means; and (3) increased storage time would not affect the stability of resin-dentin bonds.

MATERIALS & METHODS

Bonding Procedures

Twenty freshly extracted third molars were used. Teeth were obtained by protocols that were approved by the review board of the Piracicaba School of Dentistry (#080/2003). After disinfection and removal of soft tissues, flat coronal dentin surfaces were exposed with 600-grit SiC papers under running water to create a standardized smear layer.

Teeth were randomly assigned to five experimental groups, which were bonded with five adhesive systems. These systems comprise three categories: one single-step self-etching adhesive (One-up Bond F (OB), Tokuyama, Tokyo, Japan), two two-step self-etching primers (Clearfil SE Bond (CF) and an antibacterial fluoride-containing system, Clearfil Protect Bond (CP), Kuraray Inc. Tokyo, Japan), and two two-step etch-and-rinse adhesives: a water/ethanol-based (Single Bond (SB), 3M ESPE, St Paul, MN, USA) and an acetone-based filled adhesive (Prime&Bond NT (PB), Dentsply Caulk, Milford, DE, USA). Composition, batch number, application instructions and manufacturers are listed in Table 1 (Web appendix). A thin layer of a low viscosity composite (Protect Liner F, Kuraray) was applied on top of bonded surfaces, and light-cured for 40 s. After 24 hours of water-storage, teeth were sectioned into 0.9 mm thick slabs using a diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA), and divided into 5 subgroups according to storage media and time. Teeth were stored in water or mineral oil at 37^{o}C for 3 and 6 months (Carrilho et al., 2005). Specimens retrieved at 24 hours of water-storage were used as control.

Nanoleakage Evaluation

Four specimens were analyzed per group. Bonded slabs were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay *et al.* (2002a). Tooth slabs were placed in the tracer solution in total darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in photodeveloping solution for 8 hours under a fluorescent light to reduce silver ions into metallic silver grains within voids along the interface.

Transmission Electron Microscopy

Specimens were examined with the TEM to compare silver uptake patterns along resin-dentin interfaces. Specimens were retrieved after 24 hours, 3 or 6 months of storage in water or mineral oil. Undemineralized specimens were fixed in Karnovsky's solution, post-fixed in osmium tetroxide, dehydrated in ascending ethanol series (30 to100%) and embedded in epoxy resin. Propylene oxide was used as a transitional fluid. Representative 90-nm-thick ultrathin sections were prepared with an ultramicrotome (Sorvall Porter-Blum MT-2B, Newtown, CT, USA) and collected on 100-mesh carbon/formvar-coated copper grids. Without additional staining, they were observed in a TEM (Philips CM12, Philips, Eindhoven, The Netherlands) operated at 80KV.

Representative photographs were analyzed using image analysis software (Image J, NIH, Bethesda, MD, USA). The area of silver deposition was calculated and expressed in μm^2. Nanoleakage patterns were compared among adhesives and storage conditions. Data were statistically analyzed by two-way ANOVA and Tukey test at the 0.05 confidence level.

RESULTS

Distributions of silver deposits within resin-dentin interfaces are shown in Figure 1. Two-way ANOVA revealed that there were statistically significant differences for the factor “adhesive system” ($p \leq 0.0001$) and for the factor “storage condition” ($p \leq 0.0001$). In addition, it identified a significant interaction between the two factors ($p \leq 0.0001$). Tukey test showed significant differences among adhesives and stored in water or mineral oil ($p<0.05$). OB presented the highest silver deposition means after 24 hours of water-storage. Increased leakage was observed for SB and OB with increased water-storage time. Reduced or no silver deposition was observed for all groups stored in mineral oil. CP, CF and PB presented no significant increase in silver deposition means during the experiment. After 3 months of water-storage, a small increase was observed for CF and PB, but no significant difference was detected.

The two-step self-etching primers and the one-step self-etching adhesive presented thin hybrid layers (HL) of approximately 0.5 μm (Fig 2). For the self-etching primers, silver deposits were predominantly restricted to the HL or top of HL when specimens were stored in water. No significant differences were detected in the silver deposition patterns of CP and CF at 24 hours, 3 and 6 months of water-storage. However, TEM observations showed isolated silver grains within the HL formed by CF, which increased in quantity and size after storage in water (Figs. 2D,2E). TEM images of CP demonstrated sparse silver deposits along the interface after 24 hours (Fig. 2A), with a small increase after 3 months (Fig.2B). CP and CF presented no silver deposition after storage in oil (Figs. 2C,2F). The single-step OB presented massive silver impregnation after 24 hours in water (Fig. 2G). Water-trees were observed protruding from the hybrid into the adhesive layer, which increased in quantity and size after water-storage (Fig. 2H). Storage in mineral oil resulted in decreased silver deposition after 3 and 6 months (Fig. 2I).

The etch-and-rinse systems presented thick HL of approximately 5 µm. PB and SB presented initial silver deposition values statistically similar to CP and CF. PB presented increased nanoleakage after 3 and 6 months of water-storage (Figs 3A,3B), but no significant difference was detected. Almost no silver deposition was detected for PB and SB when stored in oil (Fig 3C,3F). After 3 and 6 months of water-storage, SB presented significantly increased nanoleakage (Figs 3D,3E). Figure 4 shows the leakage pattern after the 6-month water-storage period. It can be noticed that there is a preferential silver deposition within the polyalkenoic acid copolymer in the adhesive layer (Fig 4B). In a higher magnification (Fig 4C), the degradation pathway can be observed. It can be clearly seen that water comes from an outside source, and is headed to the polymer bulk via self-propagating water channels.

DISCUSSION

The main goal of adhesive dentistry is to promote an effective, durable, seal of tooth hard tissues. Bonding to a vital, wet substrate such as dentin has been proven to be a difficult task. The mineral phase of the substrate needs to be totally or partially removed, and substituted by an adhesive solution, that will permeate this collagen-rich layer and polymerize in situ, forming the hybrid layer (Nakabayashi *et al.*, 1982). All adhesives presented a certain degree of nanoleakage with notable differences among patterns of silver deposition, depending on their application mode and composition. Thus, the first null hypothesis was rejected.

Potential water-binding domains within hybrid and adhesive layers are traced by ammoniacal $AgNO_3$ (Tay *et al.*, 2002a). Differences in hydrophilicity and water content strongly influence the nanoleakage patterns of adhesive systems. Water plays an important role in both etch-and-rinse and self-etching approaches (Pashley *et al.*, 2002). Water is an essential component in self-etching systems, in order to enable ionization of acidic monomers and demineralization of underlying enamel and/or dentin (Tay and Pashley, 2001). Besides the presence of water in their composition, the ionizable moieties of acidic monomers are hydrophilic. Thus, different water sorption rates might be expected for the adhesives tested in this study (Burrow *et al.*, 1999). In addition, environmental water is known to be detrimental for the resin-dentin interface. Even if no water is present during polymerization, environmental moisture might enter the polymer bulk (Mohsen *et al.*, 2001; Tay *et al.*, 2002b), triggering the chemical polymer degradation (Göpferich, 1996). Our study demonstrated that if there is no environmental water to challenge the interfaces, decreased or no silver deposition is observed (Figs. 2C,2F,2I,3C and 3F). This observation leads us to reject the second null hypothesis, because storage in mineral oil did result in decreased or no silver deposition. No significant differences were observed among groups stored in mineral oil for 3 and 6 months.

Our third null hypothesis was also rejected, because increased water-storage time did affect the stability of resin-dentin bonds. Even though significant differences were only detected for OB and SB, storage in water affected the nanoleakage patterns for all groups in a certain degree (Figs. 2 and 3). Of all adhesives tested, the two-step self-etching primer CP presented the smallest increase in silver deposition after 3 months of water-storage. Small, isolated silver grains that were initially observed at the top of the HL after 24 hours, slightly increased in size and quantity after 3 months in water. The incorporation of an antibacterial monomer into the primer and surface-treated NaF into the bonding solution has shown promising results with respect to long-term bond stability both *in vitro* and *in vivo* (Nakajima *et al.*, 2003; Donmez *et al.*, 2005). It has been speculated that fluoride release might inhibit some enzymes activities within the interface. (Donmez *et al.*, 2005). After 6 months of water-storage CF presented enlarged areas of silver within the HL (Fig. 2E), which might be an indication of degradation at the interface. The one-step self-etching OB presented massive silver deposition along the interface, with water-trees protruding into the adhesive layer. Increased silver deposition was observed after 3 and 6 months of water-storage. Nanoleakage within resin-dentin interfaces formed by self-etching adhesives is not solely caused by incomplete resin infiltration into demineralized dentin. They also represent areas within the polymerized resin matrix in which water is incompletely removed, resulting in regions of incomplete polymerization and/or hydrogel formation, or hydrophilic domains of acidic monomers that are more prone to water sorption (Tay *et al.*, 2002a; Tay *et al.*, 2002b). Also, phase separation between adhesive ingredients, might contribute to the lower bonding effectiveness of some one-step adhesives (Van Landuyt *et al.*, 2005). The ability of single-step adhesives to seal dentin surfaces has been questioned, as they function as permeable membranes allowing water movement between the interface and underlying dentin (Tay *et al.*, 2002c; Chersoni *et al.*, 2004). An important feature of two-step self-etching systems that might contribute for the better results when compared to one-step systems, is the hydrophobic resin layer that is placed over the primer, which might reduce water sorption. Another important aspect is the ability of some functional monomers in mild self-etching systems to chemically interact with interfacial hydroxyapatite in partially demineralized interfaces, forming salts with different rates of solubility. This interaction seems to be important for bonding efficacy and stability (Yoshida *et al.*, 2004).

A reduction in the number of steps has also occurred for etch-and-rinse adhesives. However, it has been shown that two-step etch-and-rinse systems are more susceptible to water degradation than three-step systems (De Munck *et al.*, 2003). The application of the adhesive in three steps (etchant, primer and adhesive) favors copolimerization of the primer, and the lower hydrophilicity of the cured resin might result in optimized hybridization and lower susceptibility to water degradation. Silver deposits within hybrid layers promoted by etch-and-rinse systems may be attributed to regions of incomplete resin infiltration or polymerization, which represent pathways for fluid penetration. Degradation of bonds has been attributed to fluid penetration through these pathways (Hashimoto *et al.*, 2001). The two-step acetone-based filled etch-and-rinse adhesive PB presented lower susceptibility to water-degradation than the water/ethanol-based SB (Fig. 3). For PB, silver deposits were mainly observed within the HL. A small increase was observed with increased storage time. On the other hand, SB presented significant increased nanoleakage, after 3 and 6 months of water-storage. Differences in the nanoleakage patterns between PB and SB cannot be attributed only to the different solvents used in each system, because some other important components are also present in the adhesive solution. The resin composition as well as the presence of fillers might play important roles in bonding effectiveness (Tay *et al.*, 2004). For SB, silver deposits were observed not only in the HL, but also in the adhesive layer, with preferential silver deposition within the polyalkenoic acid copolymer component (Fig. 4). After 6 months of water-storage the tracer detected self-propagating water channels linking the outside water source to the polymer bulk (Fig. 4C).

In summary, no adhesive was able to totally prevent water-induced degradation of resin-dentin interfaces. The two-step self-etching approach, specially the fluoride-containing antibacterial system CP, was the most effective in preventing nanoleakage over the 6-month storage period. The simplified single-step adhesive OB presented discouraging results right after 24 hours. The initial effectiveness of etch-and-rinse systems was similar to the self-etching primers; however, SB presented a significant increase in nanoleakage patterns after water-storage.

ACKNOWLEDGMENTS

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REFERENCES

Burrow MF, Inokoshi S, Tagami J (1999). Water sorption of several bonding resins. *Am J Dent* 12:295-298.

Carrilho MRO, Tay FR, Pashley DH, Tjäderhane L, Carvalho RM (2005). Mechanical stability of resin–dentin bond components. *Dent Mater* 21:232-241.

Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, *et al.* (2004). In vivo and in vitro permeability of one-step self-etch adhesives. *J Dent Res* 83:459-464.

De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, *et al.* (2003). Four-year water degradation of total-etch adhesives bonded to dentin. *J Dent Res* 82:136-140.

Donmez N, Belli S, Pashley DH, Tay FR (2005). Ultrastructural correlates of in vivo/in vitro bond degradation in self-etch adhesives. *J Dent Res* 84:355-359.

Ferrari M, Tay FR (2003). Technique sensitivity in bonding to vital, acid-etched dentin. *Oper Dent* 28:3-8.

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

Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H (2000). In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. *J Dent Res* 79:1385-1391.

Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H (2001). Resin-tooth adhesive interfaces after long-term function. *Am J Dent* 14:211-215.

Hashimoto M, Ohno H, Kaga M, Sano H, Endo K, Oguchi H (2002). The extent to which resin can infiltrate dentin by acetone-based adhesives. *J Dent Res* 81:74-78.

Kanca J 3rd. Resin bonding to wet substrate (1992). 1. Bonding to dentin. *Quintessence Int* 23:39-41.

Mohsen NM, Craig RG, Filisko FE (2001). The effects of moisture on the dieletric relaxation of urethane dimethacrylate polymer and composites. *J Oral Rehab* 28:376-392.

Nakabayashi N, Kojima K, Masuhara E (1982). The promotion of adhesion by the infiltration of monomers into tooth substrates. *J Biomed Mater Res* 16:265-273.

Nakajima M, Okuda M, Ogata M, Pereira PN, Tagami J, Pashley DH (2003). The durability of a fluoride-releasing resin adhesive system to dentin. *Oper Dent* 28:186-192.

Pashley DH, Carvalho RM, Tay FR, Agee KA, Lee K-W (2002). Solvation of dried matrix by water and other polar solvents. *Am J Dent* 15:97-102.

Reis AF, Arrais CAG, Novaes PD, Carvalho RM, De Goes MF, Giannini M (2004). Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives: nanoleakage expression. *J Biomed Mater Res* 71B:90-98.

Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH (1995a). Nanoleakage: leakage within the hybrid layer. *Oper Dent* 20:18-25.

Sano H, Yoshiyama M, Ebisu S, Burrow MF, Takatsu T, Ciucchi B, *et al* (1995b). Comparative SEM and TEM observations of nanoleakage within the hybrid layer. *Oper Dent* 20:160-167.

Spencer P, Wang Y, Walker MP, Wieliczka DM, Swafford JR (2000). Interfacial chemistry of the dentin/adhesive bond. *J Dent Res* 79:1458-1463.

Tanaka J, Ishikawa K, Yatani H, Yamashita A, Suzuki K (1999). Correlation of dentin bond durability with water absorption of bonding layer. *Dent Mater J* 18:11-18.

Tay FR, Gwinnett AJ, Wei SH (1996). The overwet phenomenon: an optical, micromorphological study of surface moisture in the acid-conditioned, resin-dentin interface. *Am J Dent* 9:43-48.

Tay FR, Pashley DH (2001). Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent Mater* 17:296-308.

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

Tay FR, King NM, Chan KM, Pashley DH (2002b). How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *J Adhes Dent* 4:255-269.

Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A (2002c). Single-step adhesives are permeable membranes. *J Dent* 30:371-382.

Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SC, Yiu CK, *et al.* (2003). Aging affects two modes of nanoleakage expression in bonded dentin. *J Dent Res* 82:537-541.

Tay FR, Pashley DH, Yiu C, Cheong C, Hashimoto M, Itou K, *et al.* (2004). Nanoleakage types and potential implications: evidence from unfilled and filled adhesives with the same resin composition. *Am J Dent* 17:182-190.

Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, *et al.* (2005). Monomer-solvent phase separation in one-step self-etch adhesives. *J Dent Res* 84:183-188.

Watanabe I, Nakabayashi N, Pashley DH (1994). Bonding to ground dentin by a Phenyl-P self-etching primer. *J Dent Res* 73:1212-1220.

Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, *et al.* (2004). Comparative study on adhesive performance of functional monomers. *J Dent Res* 83:454-458.

Legends

Figure 1. Distribution of silver deposits (μm^2) within resin-dentin interfaces of specimens aged in water or mineral oil for up to 6 months. Groups identified by different letters (upper case letters compares adhesive systems at each storage condition, lower case compares different storage conditions within the same adhesive) differ among them by Tukey test ($p<0.05$). In box plots, the distance between the upper and lower brackets includes 80% of the values. The box includes 50% of the values. The horizontal line in the box represents the median. CP- Clearfil Protect Bond, CF- Clearfil SE Bond, OB- One-up Bond F, PB- Prime&Bond NT, SB- Single Bond.

Figure 2. TEM micrographs of undemineralized, unstained sections denote the different silver deposition patterns along interfaces produced by the self-etching systems after 24 hours (A, D and G), 3 or 6 months of water-storage (B, E and H), and 6 months of storage in mineral oil (C, F and I). A mild increase in silver tracer penetration within the hybrid layer (HL) of the two-step self-etching primer CP (A-C) was observed after 3 months of water-storage. Silver deposits (arrowheads) were initially observed at the top of the HL, but after 3 months they were also observed within the hybrid and adhesive layers. CF (D-F) presented an increase in silver tracer penetration within the HL after 6 months of water-storage (E). Resin-dentin interfaces produced by the one-step self-etching adhesive OB presented massive silver deposition within the HL and water-trees protruding to the adhesive resin layer (AR) right after 24 hours of water-storage (G), which increased after storage in water (H). Storage in mineral oil resulted in no (C and F) or decreased (I) silver deposition along interfaces.

Figure 3. TEM micrographs denote the different silver deposition patterns along interfaces produced by the two-step etch-and-rinse adhesives after 24 hours (A and D) or 6 months of storage in water (B and E), and 6 months of storage in mineral oil (C and F). Images denote a moderate increase in silver deposition for

the acetone-based, filled adhesive PB after 6 months of water-storage (A and B). A significant increase in silver deposition within the HL was observed for the water/ethanol-based SB after 6 months of water-storage (D and E). Almost no leakage was detected for specimens aged in mineral oil (C and F). CR- composite resin, PAA- polyalkenoic acid copolymer, AR- adhesive resin.

Figure 4. TEM micrographs show degradation signs at the resin-dentin interface produced by the etch-and-rinse adhesive SB after 6 months of storage in water. Silver deposits were observed not only in the HL, but also in the adhesive layer (A), with preferential silver deposition within the polyalkenoic acid copolymer (B). In a high magnification, it can be observed that the tracer detected self-propagating water channels linking the outside water source to the polymer bulk (Fig. 4C). (A – 1250X, B – 5600X, C – 25,000X)

Figure 1.

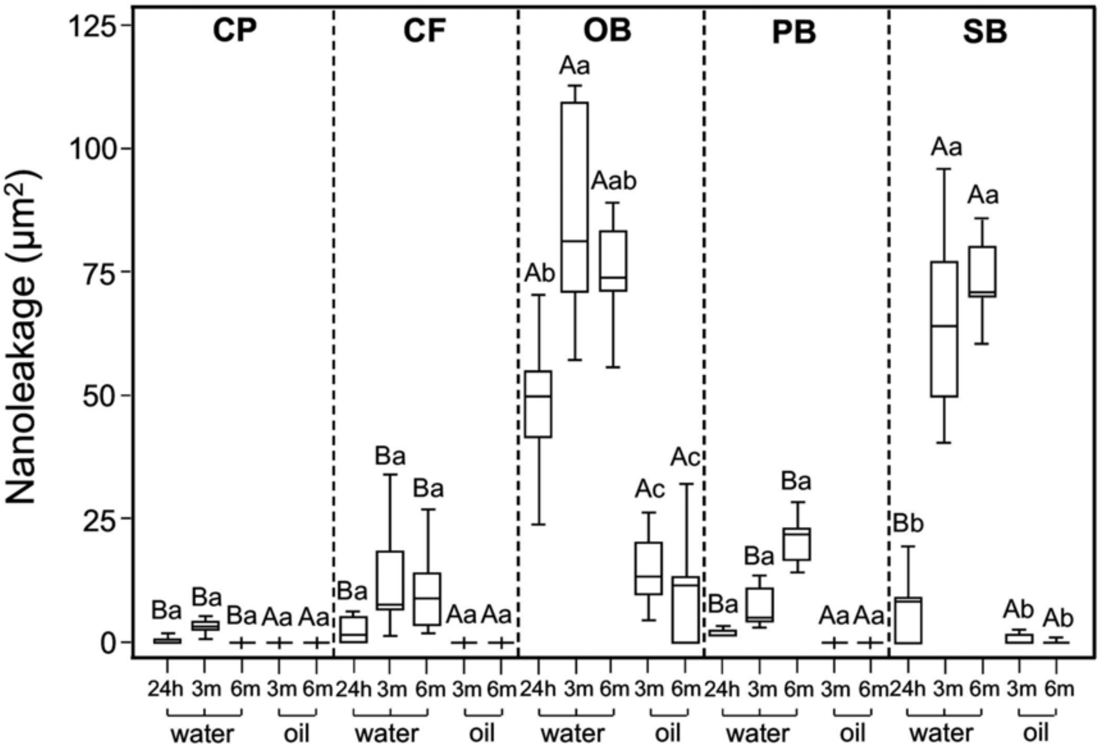

Figure 2.

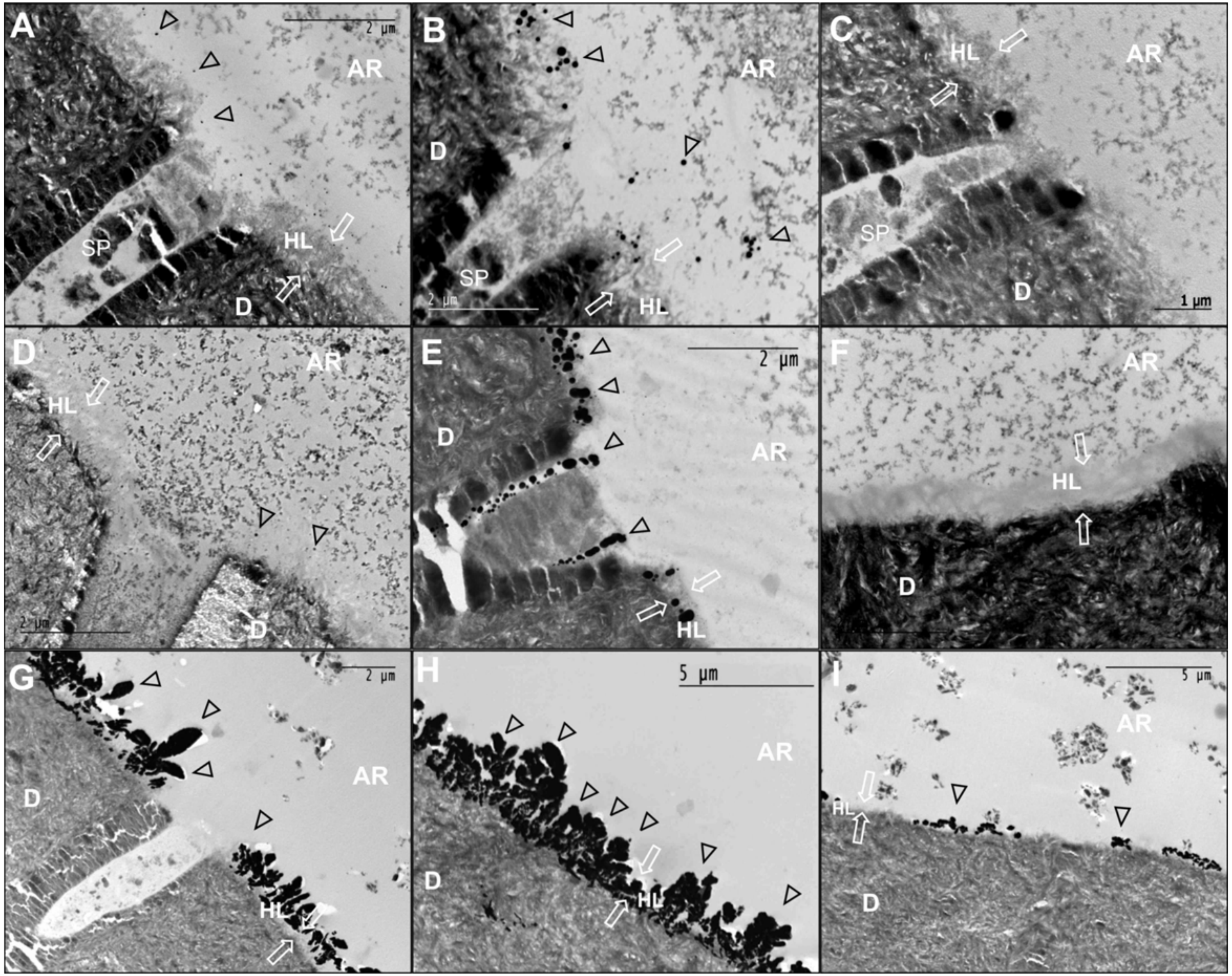
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Figure 3.

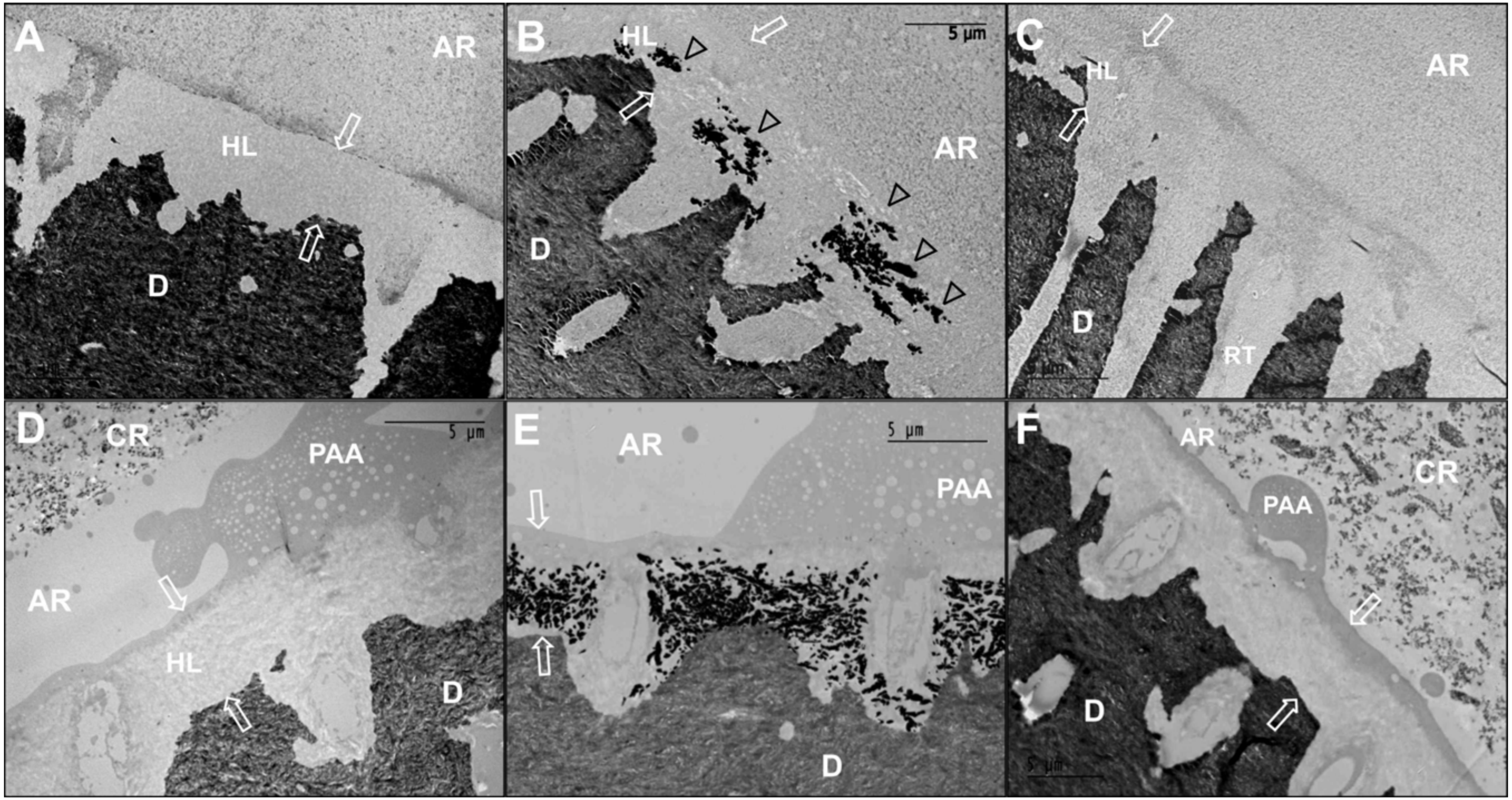
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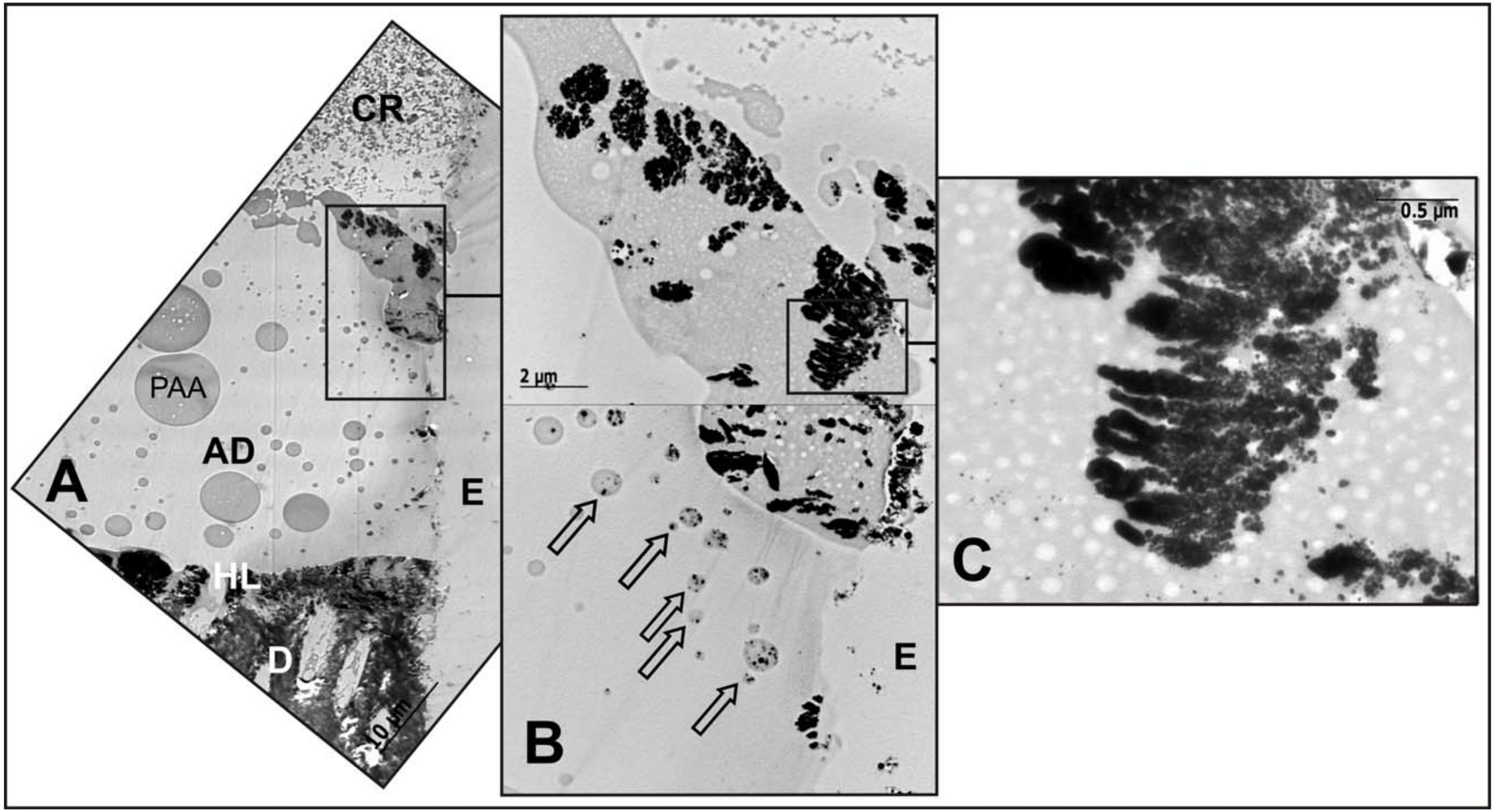
Figure 4.



WEB APPENDIX

Table 1. Materials, Brand (Lot #), pH, composition, application technique and manufacturers of adhesive systems used in this study: (MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; MDPB, 12-methacryloyloxydodecylpyridinium bromide; MMA, methyl methacrylate; MAC-10, methacryloyloxydecamethlene malonic acid; PENTA, dipentaerythritol pentacrylate phosphoric acid ester; UDMA, urethane dimethacrylate; Bis-GMA, bisphenol-A diglycidil ether dimethacrylate.)

Material	Brand (Lot #)	pH	Composition	Application Technique	Manufacturer
Two-step Self-etching primers	Clearfil Protect Bond (primer: 00002A bond: 00004A)	2.0	*Primer*: MDP, HEMA, MDPB, dimethacrilates, photoinitiator, water *Adhesive*: MDP, HEMA, dimethacrylates, photoinitiator, NaF, silanated colloidal silica	f (20 s); e; g; i	Kuraray Inc., Tokyo, Japan
	Clearfil SE Bond (primer:00400A bond:00541A)	2.0	*Primer*: Water, ethanol, MDP, HEMA, dimethacrylate hydrophilic, canphorquinone, N,N-diethanol p-toluidine *Adhesive*: MDP, Bis-GMA, HEMA, dimethacrylate hydrophobic, canphorquinone, N,N-diethanol p-toluidine, silanated colloidal silica	f (20 s); e; g; i	Kuraray Inc., Tokyo, Japan
One-step Self-etching adhesive	One-up Bond F (Bonding A: 084 Bonding B: 578)	1.2	Water, MMA, HEMA, coumarin dye, metacryloyloxyalkyl acid phosphate, MAC-10, multifuntional methacrilic monomer, fluoraluminosilicate glass, photoinitiator (aryl borate catalyst)	h (leave undisturbed 20 s); i	Tokuyama Corp., Tokyo, Japan
Two-step total-etch adhesives	Prime&Bond NT (030822)	0.02	*Etchant*: 35% H3PO4 *Adhesive*: PENTA, UDMA, resin R5-62-1, resin T, resin D, silica nanoparticles, photoinitiators, cetilamine hidrofluoride and acetone	a (15s); b (15s); c; d; e; i	Dentsply Caulk, Milford, DE, USA
	Single Bond (3JL)	0.02	*Etchant*: 35% H3PO4 *Adhesive*: water, ethanol, Bis-GMA, HEMA, UDMA, Bisphenol A glycerolate, polyalkenoic acid copolymer, dimethacrylate, canphorquinone	a (15s); b (15s); c; d; e; i	3M ESPE, St Paul, MN, USA

Application technique – a: acid etch; b: rinse surface; c: dry with cotton-pellet; d: apply one-bottle adhesive; e: gently air dry; f: apply primer; g: apply adhesive; h: apply mixture; i: light cure.

CAPÍTULO 5:

Interfacial Ultramorphology of Single-step Adhesives: Nanoleakage as a Function of Time

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Abstract

Objectives. This study examined the effectiveness of single-step self-etching adhesives in preventing nanoleakage over a 90-day water-storage period, and analyzed the ultramorphological characteristics of resin-dentin interfaces. *Methods.* Three single-step self-etching adhesives were evaluated: Adper Prompt L-Pop – LP (3M ESPE), iBond – iB (Heraeus Kulzer), and Clearfil Tri-S Bond – S3 (Kuraray). Bonded specimens were sectioned into 0.9 mm thick slabs and stored in water for 1, 60 or 90 days. After storage periods, a silver tracer solution was used to evidence nanometer-sized spaces and degradation signs within resin-dentin interfaces. Epoxy resin-embedded sections were prepared, and characterization of interfaces was performed with the TEM. Nanoleakage patterns were compared among adhesives and storage periods using image analysis software. Data were statistically analyzed by two-way ANOVA and Tukey test.

Results. LP presented the lowest silver deposition means at 1 day. However, after 60 and 90 days, the area of silver deposition significantly increased for LP, whereas for S3 they were kept stable. iB presented intense silver deposition after 1 day and presented a small increase after 90 days. S3 presented the lowest silver deposition means after 60 and 90 days of water-storage.

Significance. Nanoleakage was observed in all resin-dentin interfaces produced by the single-step self-etching adhesives. After 90 days of storage in water, S3 presented the lowest silver deposition means.

KEYWORDS: Dentin bonding; self-etching adhesives; nanoleakage; TEM; aging; degradation.

Introduction

Bonding to enamel and dentin is mainly accomplished by micromechanical interlocking between synthetic, naturally degradable polymers, and enamel or dentin collagen fibrils [1]. Effective, long-lasting bonding to dentin has been a challenge to dental clinicians. In order to promote adhesion to dentin, the mineral phase needs to be totally or partially removed, and substituted by an adhesive solution. The resin monomers will permeate this collagen-rich layer and polymerize in situ, forming what has been called the hybrid layer [1-3].

Different approaches, with different numbers of steps and degrees of sensitivity have been used to bond resin-based materials to enamel and dentin [4-7]. Efforts have been directed to reduce the number of steps and technique sensitivity. However, the long-term effectiveness of resin-based restorations has not necessarily improved with the “simplification” of bonding procedures [8-10]. One-step self-etching adhesives present a shorter clinical application time, reduction in technique sensitivity and are user-friendly. Even though they are marketed as simplified, a more complex chemistry is necessary to blend hydrophilic and hydrophobic monomers, solvents, water and additives [6,11]. Several studies have recently shown that despite the simplified approach of all-in-one adhesives, they do not necessarily promote an effective seal of dentin [7-10,12,13]. Nanometer-sized spaces within resin-dentin interfaces can be detected by silver tracer solutions and electron microscopy observation [14,15]. Degradation of bonds has been attributed to fluid penetration through these pathways [3,16]. This phenomenon has been termed nanoleakage and has been reported to occur not only within hybrid but also within adhesive layers [17,18]. The presence of water channels within resin-dentin interfaces might expedite water sorption and disintegration of bonds, especially if under functional stresses [10].

The aim of this study was to examine the effectiveness of one-step self-etching adhesives in preventing nanoleakage, using transmission electron microscopy. Ultramorphological interfacial characteristics were also compared. The tested null hypotheses were: (1) different one-step self-etching adhesives would present no significant differences in the silver deposition means among each other; and (2) water-storage for 90 days would result in no difference in the nanoleakage patterns for the one-step self-etching adhesives.

Materials and Methods

Bonding Procedures

Nine freshly-extracted third molars were used in this study. Three teeth were prepared for nanoleakage evaluation on each group. After disinfection and removal of soft tissues, flat coronal dentin surfaces were exposed with 600-grit SiC papers under running water to create a standardized smear layer.

Three one-step self-etching adhesives were used: Adper Prompt L-Pop – LP (3M ESPE), iBond – iB (Heraeus Kulzer), and Clearfil Tri-S Bond – S3 (Kuraray Inc.). Composition, lot #, application instructions, pH values and manufacturers are listed in Table 1. After applying the self-etching adhesives according to manufacturers' instructions, a thin layer of a low-viscosity composite (Protect Liner F – Kuraray Inc.) was applied on top of the bonded surface, and light-cured for 40 s. After 24 hours of storage in water, teeth were sectioned into 0.9 mm thick slabs using a diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA), and divided into 3 subgroups according to storage periods: 1, 60 or 90 days in distilled water.

Nanoleakage Evaluation

After each storage period, bonded slabs were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. In order to rehydrate specimens, they were immersed in distilled water for twenty minutes prior to immersion in the tracer solution for 24 h. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay *et al.* (2002) [17]. Tooth slabs were placed in the tracer solution in total darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in photodeveloping solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface.

Transmission Electron Microscopy

Specimens were examined with the TEM to compare silver uptake patterns along resin-dentin interfaces. Bonded interfaces were retrieved after 24 hours, 60 or 90 days of storage in water. Undemineralized specimens were fixed in Karnovsky's solution, post-fixed in osmium tetroxide, dehydrated in ascending ethanol series (30 to100%) and embedded in epoxy resin. Propylene oxide was used as a transitional fluid. Representative 90-nm-thick ultrathin sections were prepared with an ultramicrotome (Sorvall Porter-Blum MT-2B, Newtown, CT, USA) and collected on 100-mesh carbon/formvar-coated copper grids. Without additional staining, they were observed in a TEM (Philips CM12, Philips, Eindhoven, The Netherlands) operated at 80KV.

Representative photographs (n=5) were analyzed using image analysis software (Image J, NIH, MD, USA). The area of silver deposition was calculated and expressed in μm^2. Nanoleakage patterns were compared among adhesives and storage periods. Data were statistically analyzed by two-way ANOVA and Tukey test at the 0.05 confidence level.

Ultramorphological analysis

For ultramorphological analysis of resin-dentin interfaces, slabs of each self-etching adhesive were retrieved after 24 hours of storage in water. These specimens were not subjected to the nanoleakage protocol previously described. Instead, they were demineralized in 10% phosphoric acid for 4 hours prior to fixation, resin-embedding and sectioning procedures described above. Ultrathin sections were double-stained with uranyl acetate and Reynold's lead citrate. Grids were observed and representative photographs were taken with a transmission electron microscope operated at 80 KV.

Results

Distributions of silver deposits within resin-dentin interfaces are shown in Figure 1. Two-way ANOVA revealed that there were statistically significant differences for the factor “adhesive” ($p<0.0001$), for the factor “storage time” ($p=0.002$). In addition, it identified a significant interaction between the two factors ($p=0.0005$). Tukey post-hoc test showed significant differences among adhesive systems at the different storage periods in water ($p<0.05$). LP presented the lowest silver deposition means after 24 hours of storage in water, significantly different from iB, which presented the highest means at this period. However, LP presented a significant increase in silver deposition after 60 days of storage in water. After 60 and 90 days of storage in water, S3 presented the lowest silver deposition means. S3 and iB silver deposition means presented no significant increase over the experiment. However, an increase in silver deposition was observed for iB after 90 days of water-storage.

Demineralized, double-stained sections of the resin-dentin interfaces revealed the extent of infiltration of the adhesive solution into dentin surfaces can be clearly observed. A thin hybrid layer approximately 0.5 µm thick was observed in specimens bonded with the one-step all-in-one adhesive S3 (Fig. 2A). The HL produced by iB was about 1 µm (Fig. 2B), whereas LP presented a thicker hybrid layer of approximately 4 to 5 µm (Fig. 2C).

Observation of undemineralized, unstained sections revealed the presence of silver deposits in all bonded interfaces. The amount of silver deposition varied from moderate within the hybrid layer to severe within the hybrid and adhesive layers. Representative leakage patterns at the adhesive-dentin interfaces after 1, 60 or 90 days of water-storage are illustrated for each adhesive system at the different storage periods in Figures 3 to 6. Almost no leakage was observed for LP after 24 hours, but silver concentration notably increased for LP after 60 days (Fig. 3). Initially, iB specimens presented silver deposition along almost the entire extension of the hybrid layer, with water trees protruding approximately 2 µm above the hybrid into the adhesive layer (Fig. 4A). No significant increase in silver deposition means could be detected for iB over the experiment. However, an increase in silver deposition in the form of isolated silver grains occurred in the adhesive layer of iB after 90 days of water-storage (Fig. 4C). S3 produced a thin hydroxyapatite crystals-containing, 0.5 µm hybrid layer (Fig. 5A). Lower amounts of silver deposits were traced for this system, and a similar pattern of silver deposition was observed after 60 and 90 days of storage in water (Fig 5 A-C). Figure 6 simplifies comparison of silver deposition patterns among the three one-step self-etching adhesives after 90 days of storage in water. It was observed that the nanoleakage patterns of the three adhesives are very different. After 90 days, S3 presented the lowest silver deposition values (Fig. 6A).

Discussion

Adhesive dentistry has been facing a trend towards simplification of bonding procedures [7]. One-step self-etching adhesives are intended to produce resin-dentin interfaces with the same characteristics of their multiple-steps precursors, with the advantages of saving time and being less technique sensitive. These attributes instantly gained sympathy with dental clinicians. Thus, in order to keep up with the market needs, manufacturers have come up with their simplified versions of dental bonding agents.

Self-etching adhesives are supposed to simultaneously etch and infiltrate the underlying substrate [19]. However, this concept has been recently challenged because discrepancy between the depth of demineralization and infiltration might also occur for some self-etching adhesives [20-22]. The self-etching ability of contemporary adhesives is commonly achieved by the incorporation of polymerizable, methacrylate-based resin monomers that contain carboxylic/phosphoric acid moieties or their esters or by incorporating mineral or organic acids as additives to non acidic hydrophilic resin monomers [6,23]. The presence of water is also an essential component, in order to enable ionization of acidic monomers and demineralization of underlying enamel and/or dentin [6]. Besides the presence of water in their composition, the ionizable moieties of acidic monomers are hydrophilic. Thus, different water sorption rates might be expected for the adhesive resins tested in this study [24,25]. Our first null hypothesis was rejected, because different nanoleakage patterns were observed for the single-step adhesives tested in this study.

Demineralized, double-stained sections of the resin-dentin interfaces revealed that all self-etching adhesives produced authentic hybrid layers on intact dentin surfaces (Fig. 2). TEM micrographs indicate that a micromechanical interlocking was obtained with the three single-step adhesives tested. Dentin surfaces were totally or partially demineralized, depending basically on their acidity. The single-step adhesives tested in this study fall into different categories, based on their ability to etch and infiltrate intact dentin. S3 (Fig. 2A) is the mildest adhesive (pH~2.5, hybrid layer – 0.5 μm); iB (Fig. 2B) can also be considered mild (pH~2.0, hybrid layer – 1 μm), whereas LP (Fig. 2C) is considered an aggressive system (pH~0.8, hybrid layer – 4.5 μm) [6]. In order to increase the acidity of the adhesive solution, the contents of acidic monomers and water is elevated, resulting in increased hydrophilicity, which will lead to increased water sorption, and consequently, decreased hydrolytic stability [6,17,26,27]. The second null hypothesis was also rejected, because increased water-storage time resulted in a significant increase in silver deposition for LP (Fig. 3).

Among the self-etching adhesives studied, LP presented the smallest silver deposition means after 24 hours of storage in water. Even though only a small amount of silver deposits was detected at 24 hours (Fig. 3A), high-magnification TEM micrograph revealed isolated silver grains within the hybrid layer (Fig 3B), which might be related to the drastic increase observed after 60 days of water-storage (Fig 3C). Large amounts of silver deposits were observed mainly at the hybrid layer and in the form of water-trees in some regions of the adhesive layer, indicating that degradation probably occurred at these interfaces (Fig. 3). A significant decrease in the quality of the resin-dentin interface produced by LP after 4 weeks of water-storage has been recently reported and is in agreement with our findings [21]. Wang and Spencer reported that the degree of conversion of resin tags was quite reduced compared to the hybrid layer [21]. It was suggested that fluid within dentin tubules could inhibit monomer conversion. Therefore, unpolymerized acidic monomers could continue to etch dentin leading to a detrimental impact on the bond [21].

Massive silver deposition was observed for iB after 24 hours of water-storage (Fig. 4A). It seems that water was entrapped, and could not be removed by air-drying. Even though no significant difference was detected, a slight increase in silver deposition was observed after 90 days (Fig. 4C). Silver grains were observed not only as water-trees close to the hybrid layer, but also as isolated silver grains in the adhesive layer, which are probably the ionizable moieties of the acidic monomers, which are hydrophilic [19]. A tendency of increased degradation susceptibility was observed, as the pH values of the adhesives decreased. The amount of silver deposits observed within resin-dentin interfaces produced by iB were notably higher than S3 in all periods tested. Evidence of phase separation has been reported for iB [11], and might explain the poor results observed right after 24 hours of storage in water. The manufacturer of S3 reported that this system presents a Molecular Dispersion Technology, which enables the 2-phase liquids of hydrophilic and hydrophobic components to be maintained in a homogeneous state even when the solvent is evaporated, improving bond quality. S3 presents a mild self-etching ability, as a result dentin is only partially demineralized and hydroxyapatite crystals are also enveloped by the adhesive resin (Fig. 5A). Sparse, isolated silver grains were observed within the hybrid and adhesive layer in some areas after 24 hours of water-storage. This might be attributed to residual water that could not be removed. High-pressure air-drying is recommended for this system; however, the high viscosity of the densely filled adhesive solution might have impaired solvent evaporation. A similar silver deposition pattern was observed throughout the experiment for this system (Fig. 5A-D).

Nanoleakage within interfaces formed by self-etching adhesives is not solely caused by incomplete resin infiltration into demineralized dentin. They also represent areas within the adhesive layer in which water is incompletely removed, resulting in regions of incomplete polymerization and/or hydrogel formation, or hydrophilic domains of acidic monomers that are more prone to water sorption [17,19]. Also, phase separation of adhesive ingredients, might contribute to a lower bonding effectiveness [11]. The ability of single-step adhesives to seal dentin surfaces has been questioned, as they function as permeable membranes allowing water and fluids movement between the interface and underlying dentin [8,12,13]. An important feature of two-step self-etching systems that might contribute for the better results when compared to one-step systems, is the hydrophobic resin layer that is placed over the primer, which might reduce water sorption [9,28].

The resin composition as well as the presence of fillers might play important roles in bonding effectiveness [29]. Each self-etch adhesive contains its specific functional monomer that, to a large extent, determines its actual adhesive performance [30]. The specific molecular formula of the functional monomer and the dissolution rate of its calcium salt are thought to influence bonding efficacy and stability. The potential to chemically interact with interfacial hydroxyapatite might be helpful to achieve bond durability. This interaction occurs only with mild self-etching adhesives that partially demineralize the dentin surface. It has been shown that 10-MDP (functional monomer of S3) is effective in bonding to hydroxyapatite, and seems to be very stable. On the other hand, the bonding potential of 4-MET (functional monomer of iB) has been reported to be substantially lower [30]. These observations are in agreement with our results, because significant differences in the nanoleakage patterns were found among the self-etching adhesives.

Resin-dentin interfaces degrade in three stages. First, water is absorbed into the polymer, trigging the chemical degradation [31]. Second, resin is eluted from the hybrid and/or adhesive layer [32,33]. Third, exposed collagen fibrils might be degraded by host-derived matrix metallopropteinases (MMPs) present in dentin, or human saliva [34]. Further research is necessary for the development of improved simplified self-etching adhesives that provide a long-lasting seal of dentin surfaces.

5. Conclusions

As seen in the present investigation, no one-step self-etching adhesive system was able to prevent nanoleakage. Significantly different nanoleakage patterns were observed for the three one-step self-etching adhesives. Differences in silver deposition patterns and in bond stability are mainly attributed to the acidity and homogeneity of the adhesive solution as well as to the bonding efficacy of the functional monomer. Increased silver deposition was observed for LP after 60 and

90 days of storage in water, whereas S3 presented the lowest silver deposition means and stability over the 90-day storage period.

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References

[1] Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982;16:265-273.

[2] Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000;79:1385-1391.

[3] Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999;78:906-911.

[4] Kanca J 3rd. Resin bonding to wet substrate. 1. Bonding to dentin. Quintessence Int 1992;23:39-41.

[5] De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003;82:136-140.

[6] Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater 2001;17:296-308.

[7] 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;84:118-132.

[8] Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002;30:371-382.

[9] Reis AF, Arrais CAG, Novaes PD, Carvalho RM, De Goes MF, Giannini M. Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives: nanoleakage expression. J Biomed Mater Res 2004;71B:90-98.

[10] Frankenberger R, Tay FR. Self-etch vs etch-and-rinse adhesives: effect of thermo-mechanical fatigue loading on marginal quality of bonded resin composite restorations. Dent Mater 2005;21:397-412.

[11] 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.. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res 2005;84:183-188.

[12] 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. J Dent Res 2004;83:459-464.

[13] Carvalho RM, Tay FR, Giannini M, Pashley DH. Effects of pre- and post-bonding hydration on bond strength to dentin. J Adhes Dent 2004;6:13-17.

[14] Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20:18-25.

[15] Sano H, Yoshiyama M, Ebisu S, Burrow MF, Takatsu T, Ciucchi B, Carvalho R, Pashley DH. Comparative SEM and TEM observations of nanoleakage within the hybrid layer. Oper Dent 1995;20:160-167.

[16] Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. Resin-tooth adhesive interfaces after long-term function. Am J Dent 2001;14:211-215.

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

[18] Tay FR, Pashley DH. Water treeing - a potential mechanism for degradation of dentin adhesives. Am J Dent 2003;16:6-12.

[19] Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent 2002;4:255-269.

[20] Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, Tay FR. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomaterials 2005;26:1035-1042.

[21] Wang Y, Spencer P. Continuing etching of an all-in-one adhesive in wet dentin tubules. J Dent Res 2005;84:350-354

[22] Oliveira SS, Marshall SJ, Habelitz S, Gansky SA, Wilson RS, Marshall GW Jr. The effect of a self-etching primer on the continuous demineralization of dentin. Eur J Oral Sci 2004;112:376-383.

[23] Hasegawa T, Manabe A, Itoh K, Wakumoto S. Investigation of selfetching dentin primers. Dent Mater 1989;5:408-410.

[24] Burrow MF, Inokoshi S, Tagami J. Water sorption of several bonding resins. Am J Dent 1999;12:295-298.

[25] Mortier E, Gerdolle DA, Jacquot B, Panighi MM. Importance of water sorption and solubility studies for couple bonding agent/resin-based filling material. Oper Dent 2004;29:669-676.

[26] Tay FR, Pashley DH. Dentine adhesives: have they become too hydrophilic. J Can Dent Assoc 2003;69:724-731.

[27] Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. Biomaterials 2004;25:5789-5796.

[28] Reis AF, Pereira PNR, Giannini M. Water-induced nanoleakage prevention by bonding strategies. J Dent Res (submitted)

[29] 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. Am J Dent 2004;17:182-190.

[30] Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, Van Meerbeek B. Comparative study on adhesive performance of functional monomers. J Dent Res 2004;83:454-458.

[31] Göpferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996;17:103-114.

[32] Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. Degradation patterns of different adhesives and bonding procedures. J Biomed Mater Res 2003;66B:324-330.
[33] Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. Biomaterials 2003;24:3795-3803.
[34] Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83:216-221.

Figure captions

Figure 1. Distribution of silver deposits (μm^2) within resin-dentin interfaces of specimens aged in water for 1, 60 or 90 days (1 d, 60 d or 90 d, respectively). Groups identified by different letters (upper case letters compares adhesive systems at each storage period, lower case compares different storage periods and conditions within the same adhesive) differ among them by Tukey test ($p<0.05$). In box plots, the distance between the upper and lower brackets includes 80% of the values. The box includes 50% of the values. The horizontal line in the box represents the median and the cross marks represent mean values.

Figure 2. TEM micrographs of demineralized, double-stained sections of the resin-dentin interfaces revealing the extent of infiltration of the adhesive solution into dentin surfaces can be clearly observed. (A) A thin hybrid layer (HL) approximately 0.5 μm thick was observed in specimens bonded with the one-step all-in-one adhesive S3. The HL produced by iB was about 1 μm (B), whereas LP presented a thicker hybrid layer of approximately 4 to 5 μm (C). D – lab demineralized dentin; AD – adhesive. (Magnification: A – 11,500X; B – 5,600X; C – 3,400X).

Figure 3. TEM micrographs of undemineralized, unstained sections denote the different silver deposition patterns along resin-dentin interfaces bonded with the single-step self-etching adhesive LP after 24 hours (A and B), 60 (C) or 90 days (D) of water-storage. (A) Almost no silver deposition was observed after 1 day of storage in water. However, high-magnification TEM (B) revealed a distribution of isolated silver grains within the hybrid layer (HL), which might be related to the high silver deposition, observed after 60 (C) and 90 days (D) of water-storage. Water-trees (arrowheads) could also be observed in the adhesive layer (D). AD-adhesive, D- dentin. (Magnification: A, C and D – 2,650X; B – 53,000X).

Figure 4. TEM micrographs of undemineralized, unstained sections denote massive silver deposition along the resin-dentin interface produced by iB right after 24 hours of storage in water (A). The electron-dense regions (arrowheads) are water-trees protruding from the hybrid (HL) into the adhesive layer (AD). After 90 days (C), isolated silver grains were also observed in the adhesive layer. (D) It could also be observed, that the seal was not so effective, because silver nitrate traced pathways of water originating from the tubule orifice towards the adhesive layer (arrowheads). CR- composite resin, D- dentin. (Magnification: A-C – 5,600X; D – 3,400X).

Figure 5. TEM micrographs of undemineralized, unstained sections denote the silver deposition patterns in resin-dentin interfaces produced by S3. (A) A thin, hydroxyapatite crystals-containing, 0.5 μm thick, authentic hybrid layer was observed for this system. (B) Lower amounts of silver deposits were traced for this system, and a similar pattern of silver deposition within the hybrid (HL) and adhesive layer (AD) was observed after 24 hours (A and B), 60 (C) and 90 days (D) of storage in water. It can also be observed that the adhesive is densely filled. (Magnification: A – 19,500X; B and C – 5,600X; D – 15,000X).

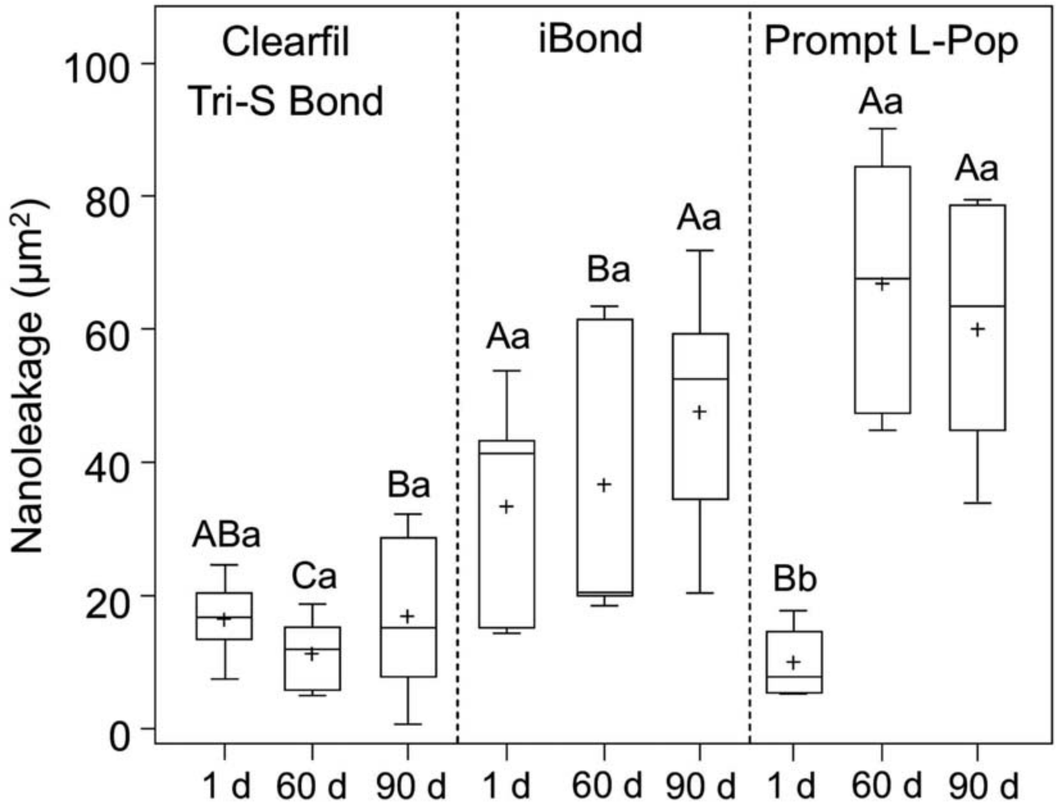
Figure 6. TEM micrographs of undemineralized, unstained sections compare the silver deposition patterns among the three one-step self-etching adhesives after 90 days of storage in water. It was observed that the nanoleakage patterns of the three adhesives are very different. (A) S3 presented the lowest silver deposition values, followed by iB (B) and LP (C). HL- hybrid layer, AD- adhesive, D- dentin. (Magnification: A-C – 5,600X).

Table 1. Materials (lot #), pH values, composition, application technique and manufacturers of the single-step self-etching adhesive systems used in this study:

Single-step Adhesives	pH	Composition	Application technique	Manufacturer
Clearfil Tri-S Bond (040219)	~2.5	MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-canphorquinone, ethanol, water, silanated colloidal silica	Apply adhesive (20s), high-pressure air (5s); light-cure (10s)	Kuraray Medical Inc., Kurashiki, Japan
iBond (010055)	~2	UDMA, 4-MET, glutaraldehyde, acetone, water, photo-initiator, stabilizer	Apply adhesive 3 times (30s); gentle air stream (30s); light-cure (20s)	Hereaus-Kulzer, Hanau, Germany
Adper Prompt L-Pop (174701)	~0.8	*Liquid 1 (red blister)*: Methacrylated phosphoric esters, Bis-GMA, initiators based on canphorquinone, stabilizers *Liquid 2 (yellow blister)*: Water, HEMA, polyalkenoic acid copolymer, stabilizers	Apply mixture (15s); blow until it becomes a thin film; light-cure (10s)	3M ESPE, St Paul, MN, USA

(Bis-GMA, bisphenol-A diglycidil ether dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; 4-MET, 4-methacryloyloxyethyl trimellitic acid; UDMA, urethane dimethacrylate)

Figure 1.



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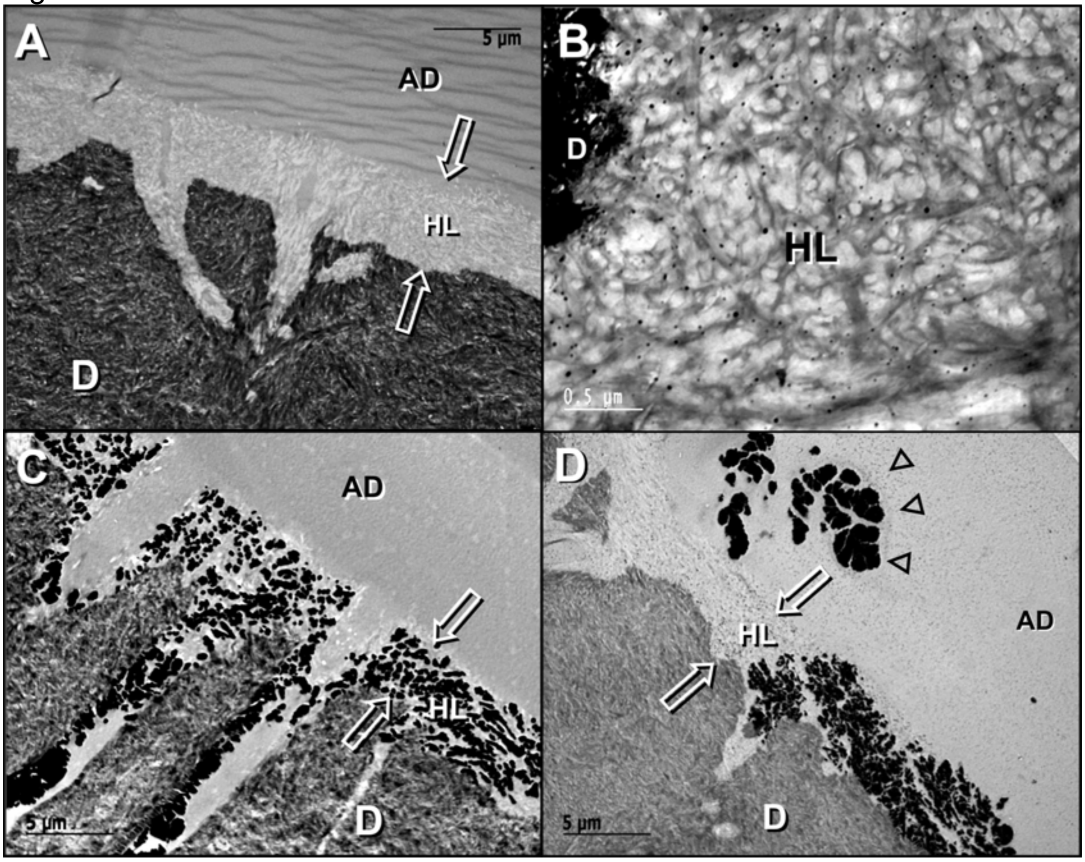

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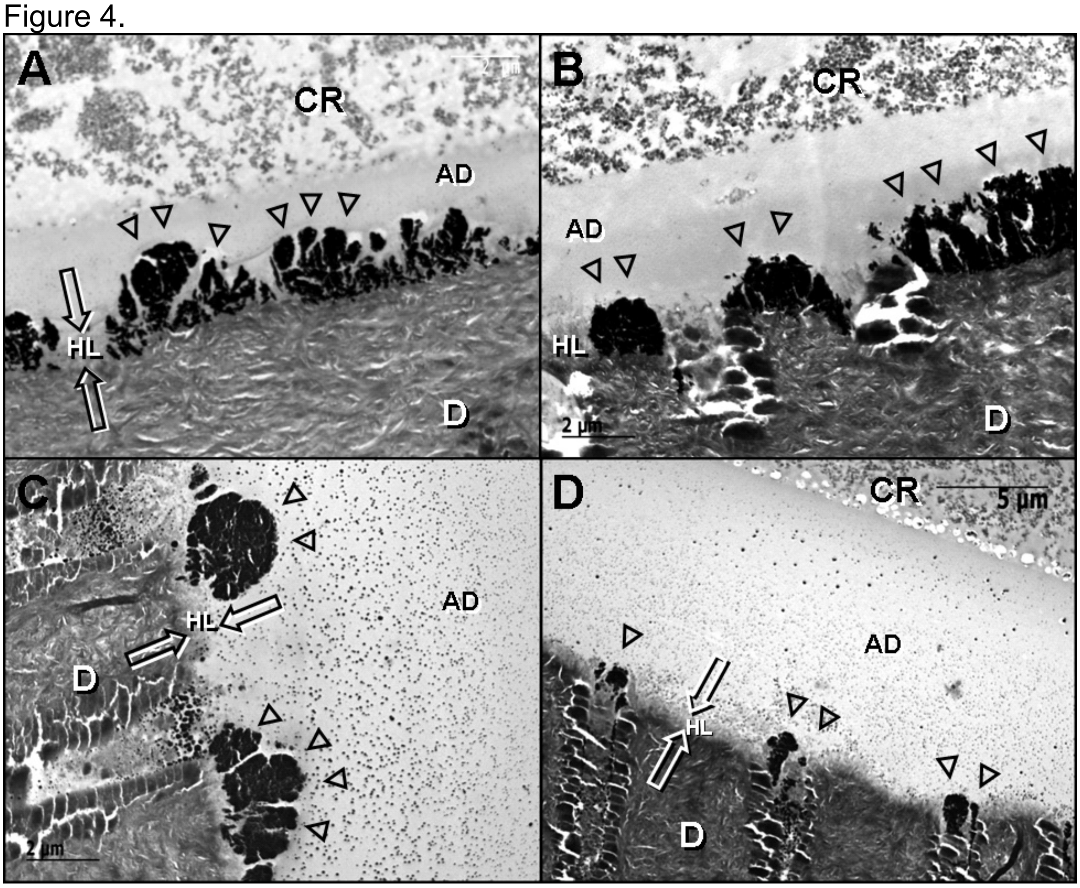

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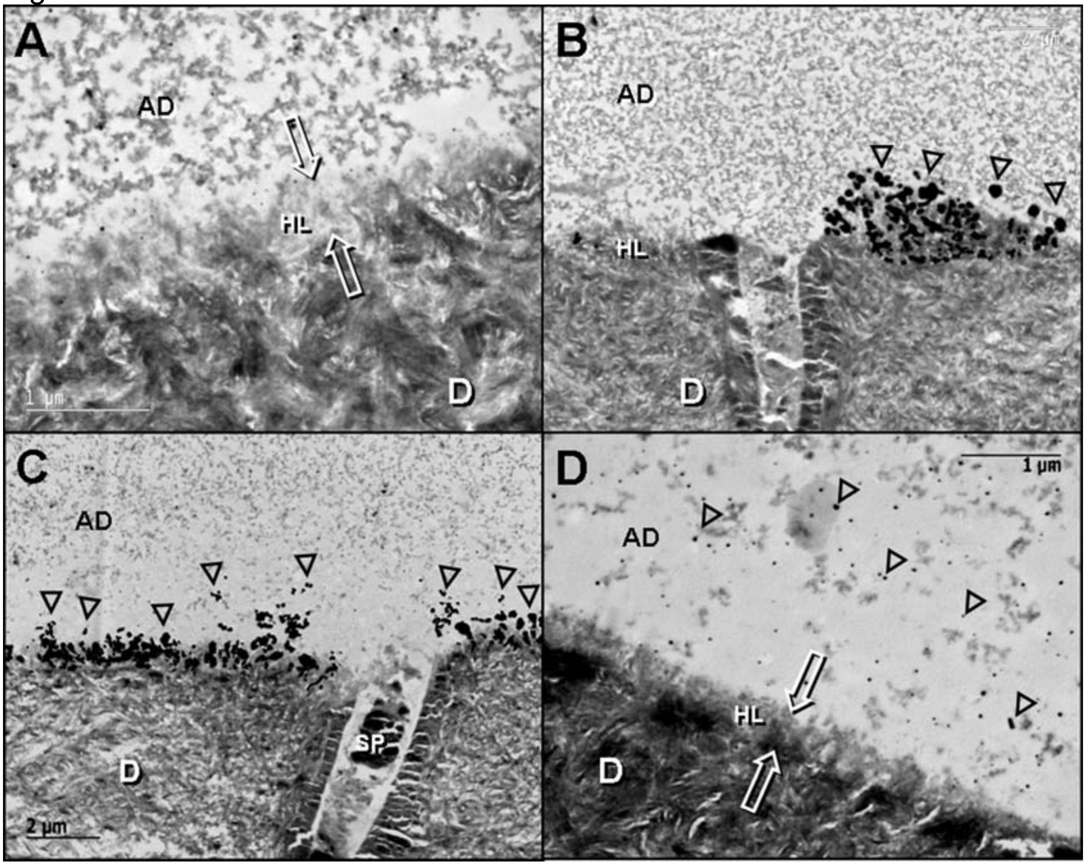

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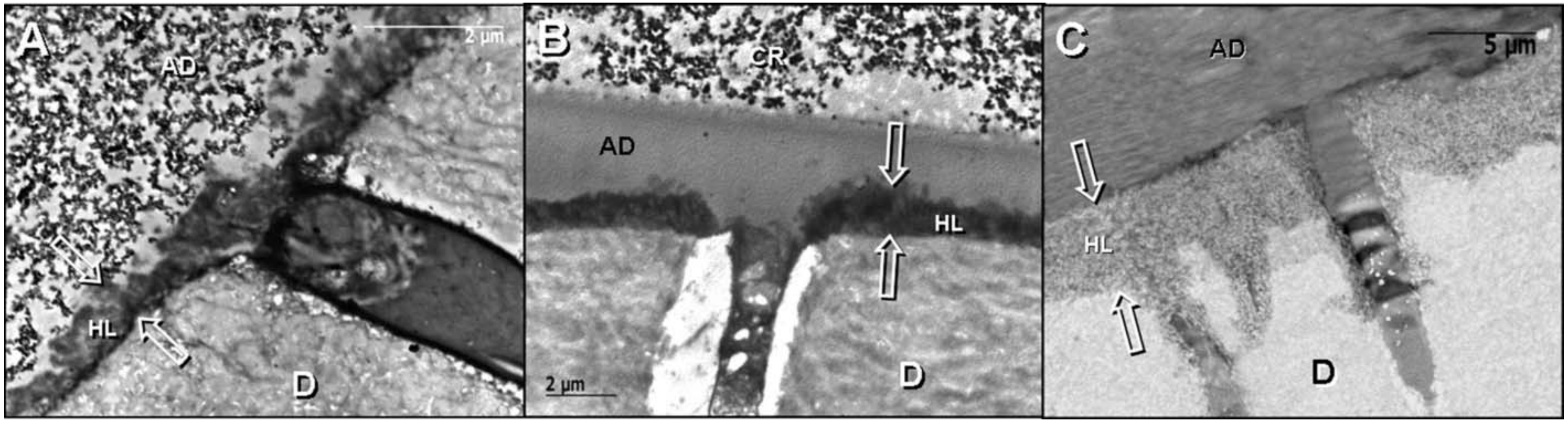
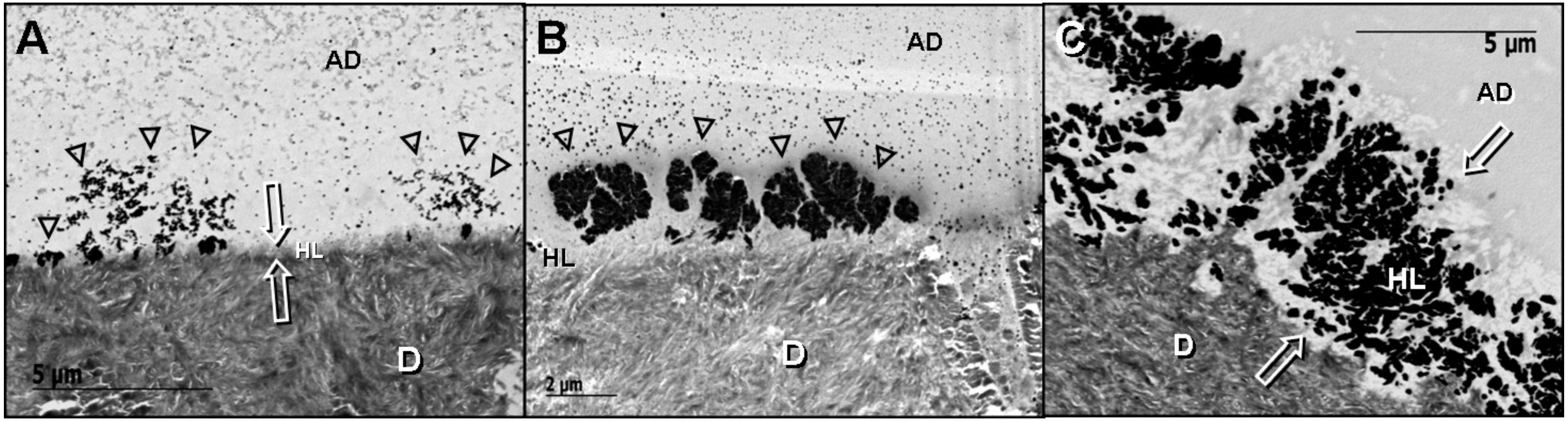


Figure 6.



CAPITULO 6:

Influence of water-storage time on the sorption and solubility behavior of current adhesives and primer/adhesive mixtures

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Running Title: Water sorption and solubility of adhesive systems

Key words: Water sorption, solubility, adhesives, aging.

Clinical Relevance

The simplified one-step self-etching adhesive and the primer/adhesive blends presented high water sorption values, which increased with increased water-storage time. High water sorption and solubility values might be related to marginal discoloration and degradation of the bond.

SUMMARY

The aim of this study was to evaluate the effects of water-storage on the water sorption and solubility behavior of five commercially available dental adhesive systems and two primer/adhesive mixtures. The adhesives comprised three different approaches to bonding to tooth hard tissues: a one-step self-etching adhesive (One-up Bond F), two two-step self-etching primers (Clearfil SE Bond and Clearfil Protect Bond), and two etch-and-rinse systems: a water/ethanol-based (Single Bond) and an acetone-based filled adhesive (Prime&Bond NT). The bonding agents and primers of the two-step self-etching systems were mixed in a 1:1 volume ratio. Water sorption and solubility values were determined after 1, 7, 30, 90 and 180 days. The results showed that except for SB, all adhesives presented increased water sorption with increased storage time. The one-step self-etching adhesive and the self-etching primer/adhesive mixtures presented the highest water sorption and solubility values. Equilibrium in the water sorption values was observed for all adhesives after 90 days of water-storage. However, solubility values continued to increase for some materials until 180 days. The sorption and solubility behavior of the materials tested seem to be related to the hydrophilicity of the adhesive resin solution and might influence the long-term performance of resin-based composite restorations.

INTRODUCTION

One of the major concerns in adhesive dentistry is the durability of bonds to dentin substrate, because bonding is established on a complex hydrated biological composite structure.[1,2] Adhesive systems are hydrophilic in nature, because hydrophilicity is desirable for bonding to a moist substrate like dentin. However, subsequent water sorption within bonded interfaces is thought to contribute to degradation over time.[3,4]

Adhesive dentistry has been facing a trend towards simplification of bonding procedures. Different approaches, with different numbers of steps and degrees of sensitivity have been used to bond resin-based materials to enamel and dentin.[5-7]

However, simplification of bonding procedures has resulted in increased hydrophilicity and consequently, in decreased long-term bonding effectiveness.[8] One-bottle self-priming etch-and-rinse systems, as well as single-step self-etching adhesives are more hydrophilic versions of their multiple-step precursors.[7,8] The incorporation of increased concentrations of hydrophilic monomers into adhesive systems may compromise bond durability, as hydrophilicity and hydrolytic stability are antagonistic properties.[9]

It is well known that the bond strength and quality of the seal produced by bonding agents decrease with time both *in vitro* and *in vivo*.[4,10-12] Water sorption within resin-dentin interfaces has been quoted as one of the dominant factors involved in the adhesion degradation.[3] However, while numerous studies have examined the water sorption and solubility behavior of restorative composite resins, data on the water sorption and solubility characteristics of adhesive systems currently available in the market and their influence in bond longevity is scarce.[13,14] The aim of this study was to evaluate the water sorption and solubility characteristics of five adhesive systems and two primer/adhesive mixtures over a 180-day water-storage period. The null hypotheses tested were: (1) different adhesive solutions present no significant difference in the water sorption and solubility behavior, and (2) increased water-storage time does not affect the water sorption and solubility behavior of the materials tested.

METHODS & MATERIALS

Specimen preparation

Five adhesive systems were used in this study. These systems comprise three categories: one single-step self-etching adhesive (One-up Bond F (OB), Tokuyama, Tokyo, Japan), two two-step self-etching primer systems (Clearfil SE Bond (CF) and an antibacterial fluoride-containing system, Clearfil Protect Bond (CP), Kuraray Medical Inc., Tokyo, Japan), and two two-step etch-and-rinse adhesives: a water/ethanol-based (Single Bond (SB), 3M ESPE, St Paul, MN, USA) and an acetone-based filled adhesive (Prime&Bond NT (PB), Dentsply/Caulk, Milford, DE, USA). In addition, two primer adhesive mixtures were tested. The self-etching primers of Clearfil SE Bond and Clearfil Protect Bond were mixed with their respective hydrophobic bonding agents in a 1:1 volume ratio (CF+P and CP+P). Composition, batch number and manufacturers for each adhesive system are listed in Table 1.

All adhesive systems tested present a certain amount of solvents and water in their composition, which could impair polymerization of specimens. Thus, we tried to eliminate or at least reduce their content in the adhesive solutions of SB, PB, CP and CF primers. No attempt to reduce the presence of solvent in OB was done, because manufacturer's instructions do not recommend air drying after application. For solvent elimination, several drops of each adhesive or primer were dispensed in an adhesive dispenser and the solution mass was recorded on an analytical balance (JEX-200, YMC Co. Ltd., Kyoto 600-8106, Japan) until reaching equilibrium at room temperature.[15] The amount of time necessary for each adhesive system to reach a constant mass was determined. Solvent evaporation was done in a dark box to prevent early polymerization of the adhesives. Afterwards, glass pipettes were used to fill a hollow cylinder of approximately 12 mm that was cut from micro bore tygon tubing (TYG-030, Small Parts Inc., Miami Lakes, FL) with an internal diameter of approximately 0.73 mm. A light curing unit (Astralis 5, Ivoclar Vivadent, Schaan, Liechtenstein) with an output of 650 mW/cm^2 was used to photo-activate specimens for 120 s. Afterwards, a razor blade was used to section the cylinders into 2-mm high cylinders. The specimens were carefully removed from the tygon tubing and randomly assigned to each of the storage periods in water (n=5): 1, 7, 30, 90 and 180 days.

Sorption and solubility analysis

A micro-balance (Sartorius 142P, Goettingen, Germany) with a precision of 0.001 mg was used for weighing specimens. The water sorption/solubility test was performed according to ISO 4049 (1988) for resin-based filling materials with the exceptions that the specimens were weighed shortly after preparation (m_1) and the dimensions of the specimens were reduced in relation to the original standard.

Specimens were immersed individually in an eppendorf container with 1.5 ml of distilled water at 37 ^{o}C for each storage period. After completed storage periods, specimens were removed from water, any visible moisture was removed with a paper towel and 1 min after removal from water mass m_2 was recorded. After this weighing, specimens were stored in a desiccator containing freshly dried silica gel for 24 hours and a constant mass (m_3) was obtained. The diameter and the thickness of the specimens were measured at three different points with a digital caliper with a precision of 0.01 mm (Mitutoyo, Tokyo, Japan) and the volume (V) was calculated in cubic millimeters. The values of water sorption (W_{sp}) and solubility (W_{sl}) were measured and calculated using the following formulae:

$$W_{sp} = (m_2 - m_3)/V \qquad W_{sl} = (m_1 - m_3)/V$$

where:

m_1 is the mass of the specimen in micrograms, before immersion in water;

m_2 is the mass of the specimen in micrograms, after immersion in water;

m_3 is the mass of the specimen in micrograms, after desiccation, and

V is the specimen volume in cubic millimeters.

Statistical analysis

Differences in water sorption and in solubility values were statistically evaluated using two-way ANOVA (adhesive vs. storage time) and the Tukey post-hoc test at a pre-set significance level of 0.05. Statistical analyses were done using SAS for Windows (V8, SAS Institute, Cary, NC, USA).

RESULTS

Results for water sorption and solubility tests are presented in Tables 2 and 3 respectively. Figure 1 graphically demonstrates water sorption and solubility behavior for the adhesive systems and primer/adhesive mixtures after storage in water. Two-way ANOVA revealed that there were statistically significant differences for the factor “adhesive” ($p \leq 0.0001$), for the factor “storage time” ($p \leq 0.0001$), and identified a significant interaction between factors ($p \leq 0.0001$). The above information is valid for both analysis (sorption and solubility). Tukey post-hoc test showed significant differences among adhesive systems at the different storage times ($p<0.05$).

Water sorption analysis revealed that tested groups present significant different water sorption patterns and a different behavior after storage in water (Table 2). PB presented the lowest sorption values, followed respectively by CF, CP, SB, CF+P; and CP+P and OB. Except for CP+P and OB, significant differences were observed among adhesives at all periods tested. Similar values were also observed between CF+P and CP+P after 180 days of storage in water. Except for SB, all groups presented a significant increase in the water sorption values with increased water-storage time. Stability in water sorption values was only observed after 90 days of immersion in water for the other groups. The primer/adhesive mixtures (CF+P and CP+P) and the single-step self-etching adhesive OB absorbed considerably more water than the other groups.

A tendency of increased solubility was observed with increased storage time for all groups (Table 2, Fig. 1). The primer-adhesive mixture CF+P and the single-step adhesive OB presented the highest solubility values. On the other hand, CP presented the lowest values over the experiment. Solubility increased significantly for all materials tested. After 180 days, the solubility values of all materials were at least twice higher than values observed after 1 day of storage in water.

DISCUSSION

Bonding to a vital, wet substrate such as dentin has been proven to be a difficult task. The mineral phase of the substrate needs to be totally or partially removed, and substituted by an adhesive solution, that will permeate this collagen-rich layer and polymerize in situ, forming the hybrid layer.[16] Adhesive systems are largely exposed to dentinal fluids and to a lower extent, to salivary fluids in the oral environment. The water sorption and solubility characteristics of adhesive materials are important in determining the longevity and marginal quality of a restoration. High water sorption values might contribute for marginal staining around composite restorations.[13] Water plays an important role in the chemical degradation process of polymer materials.[17,18] Thus, more hydrophobic materials tend to take up lower quantities of water and consequently, present a lower hydrolytic degradation velocity.

Two different theories, which are believed to occur simultaneously, have been proposed for the diffusion of water molecules into polymer matrices. In the “free volume theory”, water molecules diffuse through nanopores or micromorphological defects of the material without mutual relationship to the polar sites of the material. In the “interaction theory”, water molecules diffuses through the material binding successively to the hydrophilic groups.[19] There are several factors involved in the polymer water sorption and solubility characteristics, such as: pH of the storage media;[20,21] degree of conversion;[22] polarity of the molecular structure; presence of pendant hydroxyl groups capable of forming hydrogen bonds with water; degree of crosslinking;[23] presence of residual water; and presence and type of filler particles.[14,24,25] After entering the polymer matrix, water triggers the chemical degradation, resulting in the formation of oligomers and monomers.[17] The microstructure might be changed due to progressive degradation through formation of pores. Residual monomers, oligomers and degradation products might be released via these pores.[17,26,27] In addition to the degradation process within the polymer, debonding between the polymer and filler particles might take place, resulting in leakage of filler particles and ions. The degradation and erosion process leads to a mass loss of the adhesive material measured as solubility.

The adhesive materials tested in this experiment present different amounts of hydrophilic and hydrophobic monomers in their composition. Water and solvents are also present in the adhesive blend. In order to bond to intrinsically wet dentin surface, adhesive systems incorporate resin molecules with both hydrophilic and hydrophobic moieties, or hydrophilic resins such as 2-hydroxyethylmethacrylate (HEMA). The self-etching ability of contemporary adhesives is commonly achieved by the incorporation of polymerizable, methacrylate-based resin monomers that contain carboxylic/phosphoric acid moieties or their esters or by incorporating mineral or organic acids as additives to non acidic hydrophilic resin monomers.[6,28] The presence of water is also an essential component, in order to enable ionization of acidic monomers and demineralization of underlying enamel and/or dentin.[6] Besides the presence of water in their composition, the ionizable moieties of acidic monomers are hydrophilic. These observations might explain the highest water sorption rates recorded for the self-etching adhesive OB and the self-etching primer/adhesive blends (CF+P and CP+P) tested in this study. High solubility values were also observed for these groups. The primer/adhesive volume ratio (1:1) was certainly higher than it would be in actual clinical conditions. It has been demonstrated that mixtures containing higher amounts of primer present a lower degree of conversion and inferior mechanical properties when compared with mixtures containing a lower proportion of primer.[29] Both null hypotheses were rejected, because different water sorption and solubility rates were observed for the groups tested in this study, and tended to increase with increased water-storage time.

Except for OB, all filled adhesives (PB, CF and CP) presented lower water sorption rates than the unfilled adhesives. The presence of filler might provide adhesives with improved mechanical properties, and decreased water sorption.[24,30] The bonding agents of the two-step self-etching systems proved to be more hydrophobic resins. Low water sorption rates, as well as decreased solubility in water were observed for CP and CF. Even though CF presented lower water sorption means than CP, CP presented the lowest solubility rates over the experiment. The same trend was observed for the primer/adhesive mixtures. CP+P presented higher water sorption rates, but lower solubility in water than did CF+P. Moreover, CP also showed a mass increase after 1 and 7 days of storage in water. A possible explanation for the increased mass of CP and the lower water solubility of CP and CP+P is chemical reactions with water within the adhesive and production of reaction products.[31] An important feature of two-step self-etching systems that might contribute for the better results when compared to one-step systems, is the hydrophobic resin layer that is placed over the primer, which might reduce water sorption.[12]

The solvent depleted UDMA/PENTA filled solution of PB presented the lowest water sorption values. However, the solubility values were higher than those observed for SB, CF and CP; and comparable to those recorded for CP+P. The low water sorption values are probably due to the presence of nanofillers within the bonding solution. However, even though the filler themselves are relatively inert inorganic materials, the coupling agents associated with them are themselves prone to hydrolysis via ester linkages within the molecules or siloxane links that are formed with the filler particle.[32] Elution of filler particles might have occurred for PB after storage in water.

The materials components are inherently prone to hydrolysis, due to the presence of unprotected ester linkages in the monomers.[17] The presence of monomers with different properties such as molecular weight, hydrophilicity (hydrophobicity), and degradation behavior, might induce preferential degradation of one phase in a polymer system.[33] This could lead to the formation of pores within the polymer matrix. Yiu et al.[34] recently reported that increased hydrophilicity of resin blends such as those employed in dentin adhesives resulted in decreased mechanical strength after long-term water storage. Increased water-storage time resulted in increased solubility values for all adhesives tested in the present investigation. Stabilization in the solubility values was observed after 7 days for SB; 30 days for CP+P; 90 days for CF+P, CF and PB. Increased solubility was still observed for CP and OB after 180 days of storage in water. Elution of degradation products might have occurred over the water-storage period. The leaching of monomers has a potential impact on the structural stability and biocompatibility of the material.[35] The reduction of some mechanical properties of composite resins (such as modulus of elasticity, strength and hardness) has been attributed to the plasticizing effect of water.[23] The same plasticization effect can be expected to occur in adhesive systems.[36]

The water sorption and solubility values of bonding agents have been reported to be much higher than composite filling materials.[14] High water sorption and solubility values could lead not only to marginal discoloration, but to decreased mechanical properties of the resin-dentin interface, and possibly, to compromised restoration longevity. Simplification of clinical application procedures has resulted in a loss of bonding effectiveness.[8,37] High water sorption and solubility rates were observed for the one-step self-etching adhesive OB, and for the primer/adhesive blends CP+P and CF+P. Further research is necessary for the promotion of simplified adhesives able to bond to a moist substrate like dentin, but with more hydrophobic characteristics after polymerization and less susceptibility to water solubility.

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References

1. Marshall GW Jr, Marshall SJ, Kinney JH & Balooch M (1997) The dentin substrate: structure and properties related to bonding *Journal of Dentistry* 25(6) 441-458.

2. Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SC, Yiu CK & Cheong C (2003) Aging affects two modes of nanoleakage expression in bonded dentin *Journal of Dental Research* 82(7) 537-541.

3. Tanaka J, Ishikawa K, Yatani H, Yamashita A & Suzuki K (1999) Correlation of dentin bond durability with water absorption of bonding layer *Dental Materials Journal* 18(1) 11-18.

4. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H & Oguchi H (2000) In vivo degradation of resin-dentin bonds in humans over 1 to 3 years *Journal of Dental Research* 79(6) 1385-1391.

5. Kanca J 3rd (1992) Resin bonding to wet substrate. 1. Bonding to dentin *Quintessence International* 23(1) 39-41.

6. Tay FR & Pashley DH (2001) Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers *Dental Materials* 17(4) 296-308.

7. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P & Vanherle G (2003) Four-year water degradation of total-etch adhesives bonded to dentin *Journal of Dental Research* 82(2) 136-140.

8. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M & Van Meerbeek B (2005) A critical review of the durability of adhesion to tooth tissue: methods and results *Journal of Dental Research* 84(2) 118-132.

9. Tay FR, Pashley DH & Yoshiyama M (2002) Two modes of nanoleakage expression in single-step adhesives *Journal of Dental Research* 81(7) 472-476.

10. Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J & Pashley DH (1999) Long-term durability of dentin bonds made with a self-etching primer, in vivo *Journal of Dental Research* 78(4) 906-911.

11. Giannini M, Seixas CAM, Reis AF & Pimenta LAF (2003) Six-month storage-time evaluation of one-bottle adhesive systems to dentin *Journal of Esthetic and Restorative Dentistry* 15(1) 43-48.

12. Reis AF, Arrais CAG, Novaes PD, Carvalho RM, De Goes MF & Giannini M (2004) Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives: nanoleakage expression *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 71(1) 90-98.

13. Burrow MF, Inokoshi S & Tagami J (1999) Water sorption of several bonding resins *American Journal of Dentistry* 12(6) 295-298.

14. Mortier E, Gerdolle DA, Jacquot B & Panighi MM (2004) Importance of water sorption and solubility studies for couple bonding agent/resin-based filling material *Operative Dentistry* 29(6) 669-676.

15. Reis AF, Oliveira MT, Giannini M, De Goes MF & Rueggeberg FA (2003) The effect of organic solvents on one-bottle adhesives bond strength to enamel and dentin *Operative Dentistry* 28(6) 700-706.

16. Nakabayashi N, Kojima K & Masuhara E (1982) The promotion of adhesion by the infiltration of monomers into tooth substrates *Journal of Biomedical Materials Research* 16(3) 265-273.

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

18. Reis AF, Pereira PNR & Giannini M. Water-induced nanoleakage prevention by bonding strategies. (submitted)

19. Bellenger V, Verdu J & Morel E (1989) Structure-properties relationships for densely cross-linked epoxy-amine systems based on epoxide or amine mixtures *Journal of Materials Science* 24(1) 63-68.

20. Ortengren U, Andersson F, Elgh U, Terselius B & Karlsson S (2001) Influence of pH and storage time on the sorption and solubility behaviour of three composite resin materials *Journal of Dentistry* 29(1) 35-41.

21. Prakki A, Cilli R, Mondelli RF, Kalachandra S & Pereira JC (2005) Influence of pH environment on polymer based dental material properties *Journal of Dentistry* 33(2) 91-8.

22. Pearson GJ & Longman CM (1989) Water sorption and solubility of resin-based materials following inadequate polymerization by a visible-light curing system *Journal of Oral Rehabilitation* 16(1) 57-61.

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

24. Tay FR, Pashley DH, Yiu C, Cheong C, Hashimoto M, Itou K, Yoshiyama M & King NM (2004) Nanoleakage types and potential implications: evidence from

unfilled and filled adhesives with the same resin composition *American Journal of Dentistry* 17(3) 182-190.

25. Marcovich NE, Reboredo MM & Aranguren MI (1999) Moisture diffusion in polyester-woodflour composites *Polymer* 40 7313-7320.

26. Geurtsen W (1998) Substances released from dental resin composites and glass ionomer cements *European Journal of Oral Sciences* 106(2) 687-695.

27. Ortengren U (2000) On composite resin materials. Degradation, erosion and possible adverse effects in dentists *Swedish Dental Journal* (Supplement 141) 1-61.

28. Hasegawa T, Manabe A, Itoh K & Wakumoto S (1989) Investigation of self-etching dentin primers *Dental Materials* 5(6) 408-410.

29. Hotta M, Kondoh K & Kamemizu H (1998) Effect of primers on bonding agent polymerization *Journal of Oral Rehabilitation* 25(10) 792-799.

30. Tay FR, Moulding KM & Pashley DH (1999) Distribution of nanofillers from a simplified-step adhesive in acid-conditioned dentin *Journal of Adhesive Dentistry* 1(2) 103-17.

31. Ortengren U, Wellendorf H, Karlsson S & Ruyter IE (2001) Water sorption and solubility of dental composites and identification of monomers released in an aqueous environment *Journal of Oral Rehabilitation* 28(12) 1106-1115.

32. Santerre JP, Shajii L & Leung BW (2001) Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products *Critical Reviews in Oral Biology and Medicine* 12(2) 136-151.

33. Shastri VP, Hildgen P & Langer R (2003) In situ pore formation in a polymer matrix by differential polymer degradation *Biomaterials* 24(18) 3133-3137.

34. 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.

35. Costa CA, Giro EM, do Nascimento AB, Teixeira HM & Hebling J (2003) Short-term evaluation of the pulpo-dentin complex response to a resin-modified glass-

ionomer cement and a bonding agent applied in deep cavities *Dental Materials* 19(8) 739-746.

36. Carrilho MRO, Carvalho RM, Tay FR & Pashley DH (2004) Effects of storage media on mechanical properties of adhesive systems *American Journal of Dentistry* 17(2) 104-108.

37. Frankenberger R & Tay FR (2005) Self-etch vs etch-and-rinse adhesives: effect of thermo-mechanical fatigue loading on marginal quality of bonded resin composite restorations *Dental Materials* 21(5) 397-412.

Table 1. Materials, Brand (Lot #), composition, application technique and manufacturers of adhesive systems used in this study:

Material	Brand (Lot #)	Code	Composition	Manufacturer
Two-step Self-etching primers	Clearfil Protect Bond (primer: 00002A bond: 00004A)	CP	*Primer*: MDP, HEMA, MDPB, dimethacrilates, photoinitiator, water *Adhesive*: MDP, HEMA, dimethacrylates, photoinitiator, NaF, silanated colloidal silica	Kuraray Medical Inc., Tokyo, Japan
	Clearfil SE Bond (primer:00400A bond:00541A)	CF	*Primer*: Water, ethanol, MDP, HEMA, dimethacrylate hydrophilic, canphorquinone, N,N-diethanol p-toluidine *Adhesive*: MDP, Bis-GMA, HEMA, dimethacrylate hydrophobic, canphorquinone, N,N-diethanol p-toluidine, silanated colloidal silica	Kuraray Medical Inc., Tokyo, Japan
One-step Self-etching adhesive	One-up Bond F (Bonding A: 084 Bonding B: 578)	OB	Water, MMA, HEMA, coumarin dye, metacryloyloxyalkyl acid phosphate, MAC-10, multifuntional methacrilic monomer, fluoraluminosilicate glass, photoinitiator (aryl borate catalyst)	Tokuyama Corp., Tokyo, Japan
Two-step total-etch adhesives	Prime&Bond NT (030822)	PB	*Etchant*: 35% H3PO4 *Adhesive*: PENTA, UDMA, resin R5-62-1, resin T, resin D, silica nanoparticles, photoinitiators, cetilamine hidrofluoride and acetone	Dentsply Caulk, Milford, DE, USA
	Single Bond (3JL)	SB	*Etchant*: 35% H3PO4 *Adhesive*: water, ethanol, Bis-GMA, HEMA, UDMA, Bisphenol A glycerolate, polyalkenoic acid copolymer, dimethacrylate, canphorquinone	3M ESPE, St Paul, MN, USA

(MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; MDPB, 12-methacryloyloxydodecylpyridinium bromide; MMA, methyl methacrylate; MAC-10, methacryloyloxydecamethlene malonic acid; PENTA, dipentaerythritol pentacrylate phosphoric acid ester; UDMA, urethane dimethacrylate; Bis-GMA, bisphenol-A diglycidil ether dimethacrylate.)

Table 2. Mean values and standard deviation for water sorption (µm/mm^3) of the adhesive materials tested after 1, 7, 30, 90 and 180 days of water storage.

	1 day		7 days		30 days		90 days		180 days	
Prime&Bond NT	48.07 ± 3.25	Fc	55.12 ± 2.39	Fb	55.33 ± 2.26	Fb	63.81 ± 0.73	Fa	66.51 ± 2.02	Fa
Single Bond	92.32 ± 4.15	Ca	94.82 ± 3.19	Ca	96.89 ± 2.89	Ca	98.01 ± 1.25	Ca	93.12 ± 2.77	Ca
C. SE Bond (bonding resin)	67.33 ± 1.45	Eb	70.33 ± 6.22	Eab	70.99 ± 2.26	Eab	75.35 ± 1.60	Ea	74.73 ± 2.94	Ea
C. Protect Bond (bonding resin)	77.01 ± 2.27	Dc	80.02 ± 3.40	Dbc	82.21 ± 2.01	Dabc	87.89 ± 3.10	Da	84.20 ± 2.85	Dab
C. SE Bond + primer	139.56 ± 2.82	Bab	138.99 ± 10.74	Bab	132.59 ± 8.84	Bb	148.15 ± 1.15	Ba	143.58 ± 3.38	Ba
C. Protect Bond + primer	155.23 ± 2.03	Aab	154.89 ± 9.82	Aab	151.49 ± 14.67	Ab	163.30 ± 2.21	Aa	154.69 ± 3.87	Bab
One-up Bond F	151.35 ± 4.05	Ab	155.11 ± 3.44	Ab	149.51 ± 2.88	Ab	172.98 ± 2.94	Aa	172.95 ± 3.88	Aa

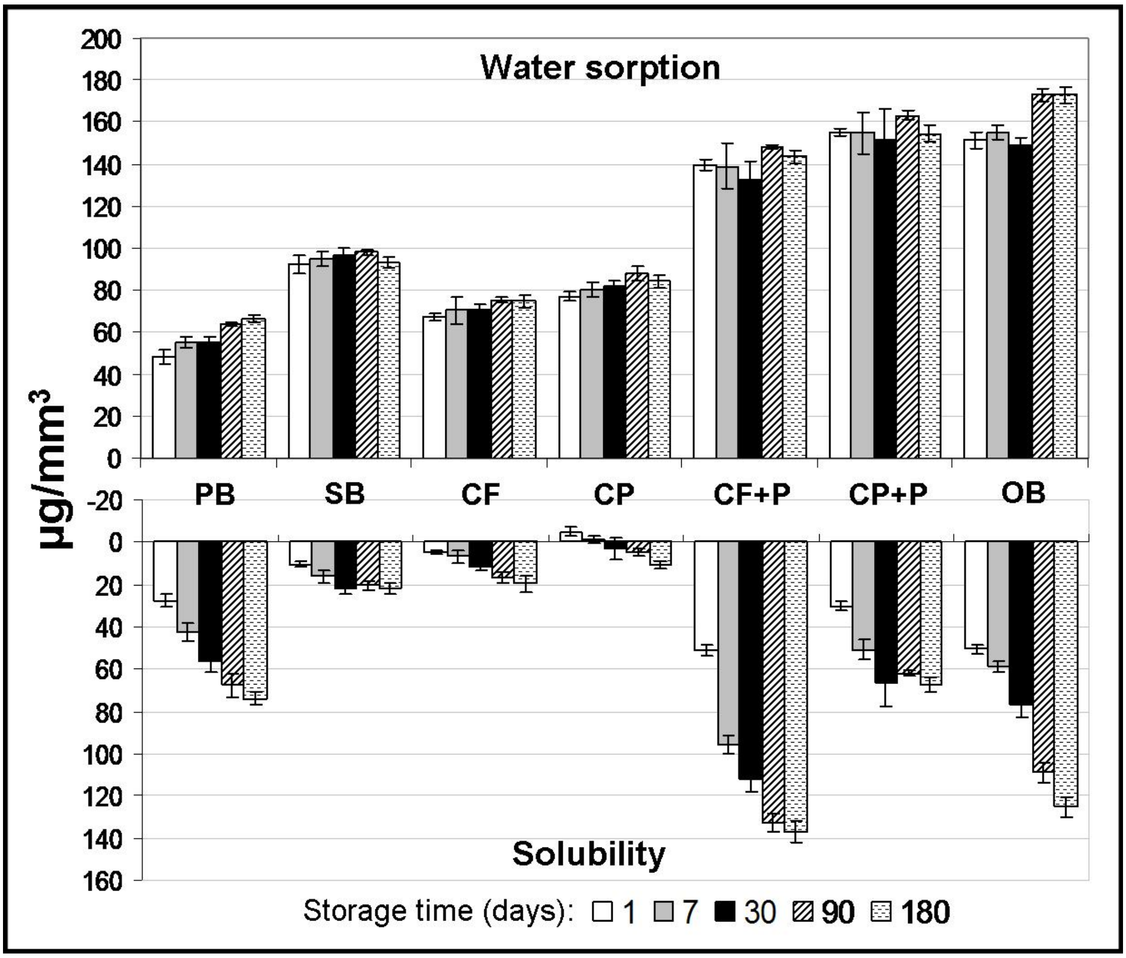
Means followed by different letters (upper case – column, lower case – row) differ among them by Tukey test at the 0.05 confidence level.

Table 3. Mean values and standard deviation for water solubility (µm/mm^3) of the adhesive materials tested after 1, 7, 30, 90 and 180 days of water storage.

	1 day		7 days		30 days		90 days		180 days	
Prime&Bond NT	27.77 ± 2.97	Bd	42.40 ± 4.28	Dc	56.07 ± 5.51	Db	67.82 ± 5.53	Ca	74.25 ± 3.00	Ca
Single Bond	10.58 ± 1.49	Cb	16.31 ± 3.10	Eab	22.01 ± 2.51a	Ea	20.59 ± 2.19	Da	22.01 ± 2.45	Da
C. SE Bond (bonding resin)	4.83 ± 1.03	Cc	6.58 ± 3.01	Fbc	11.30 ± 2.25	Fbc	16.53 ± 2.56	Dab	19.76 ± 3.79	DEa
C. Protect Bond (bonding resin)	-4.95 ± 2.09	Dc	-1.23 ± 1.60	Gbc	3.07 ± 5.42	Gb	4.71 ± 1.85	Eb	12.26 ± 1.90	Ea
C. SE Bond + primer	51.40 ± 2.49	Ad	95.40 ± 4.22	Ac	112.27 ± 6.06	Ab	132.29 ± 4.18	Aa	137.16 ± 5.04	Aa
C. Protect Bond + primer	30.21 ± 2.33	Bc	50.83 ± 4.50	Cb	66.30 ± 11.40	Ca	62.15 ± 1.42	Ca	67.35 ± 3.28	Ca
One-up Bond F	50.56 ± 2.32	Ae	58.62 ± 2.48	Bd	76.72 ± 6.13	Bc	108.92 ± 4.41	Bb	125.18 ± 4.64	Ba

Means followed by different letters (upper case – column, lower case – row) differ among them by Tukey test at the 0.05 confidence level.

Figure 1. Mean values and standard deviation for water sorption and water solubility (µg/mm^3) of the adhesive materials tested after 1, 7, 30, 90 and 180 days of water storage.



CAPITULO 7:

Effects of water-storage on the mechanical and ultramorphological characteristics of current adhesives and primer/adhesive mixtures

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SHORT TITLE: Long-term mechanical properties of adhesives

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KEY WORDS: adhesives, ultimate tensile strength, elastic modulus, aging, TEM

Summary

Objectives. The aims of this study were: (1) to evaluate the change in the ultimate tensile strength (UTS) and elastic modulus (*E*) of five adhesive systems, and two primer/adhesive mixtures after aging for 6 months in water or mineral oil; and (2) compare the silver uptake patterns with the TEM.

Methods. The adhesives comprised three different approaches to bonding to tooth hard tissues: a one-step self-etching adhesive (One-up Bond F - OB), two two-step self-etching primers (Clearfil SE Bond - CF and Clearfil Protect Bond - CP), and two etch-and-rinse systems (Single Bond - SB and Prime&Bond NT - PB). The bonding agents and primers of the two-step self-etching systems were mixed in a 1:1 volume ratio (CF+P and CP+P). Polymerized resin cylinders beams were prepared for each adhesive or primer/adhesive mixtures, and randomly assigned to three storage conditions: 24 hours in water, 6 months in water or 6 months in oil. After storage periods, specimens were retrieved for ultimate tensile strength (UTS) and elastic modulus (*E*) evaluation. Results were statistically analyzed by two-way ANOVA and Tukey test. Additional specimens were prepared and immersed in ammoniacal $AgNO_3$. After developing, epoxy resin-embedded sections were prepared for TEM.

Results. Except for CF+P and OB, all adhesives presented a decrease in the UTS after storage in water. Similar or increased UTS values were observed after oil storage. Except for SB, *E* values did not change after 6-month water-storage, but they did increase after storage in oil. OB, CP+P and CF+P presented notably more silver uptake than the other groups.

Conclusion. The effects of storage in water for 6 months were material-dependent, and significantly affected the mechanical properties and silver uptake patterns for some adhesive resin beams.

Introduction

Different approaches, with different numbers of steps and degrees of sensitivity have been used to bond resin-based materials to enamel and dentin [1-3]. However, simplification of bonding procedures has resulted in increased hydrophilicity and consequently, in decreased long-term bonding effectiveness [4]. One-bottle self-priming etch-and-rinse systems, as well as single-step self-etching adhesives are more hydrophilic versions of their multiple-step precursors [3,4]. The incorporation of increased concentrations of hydrophilic monomers into adhesive systems may compromise bond durability, as hydrophilicity and hydrolytic stability are antagonistic properties [5].

It is well known that the bond strength and quality of the seal produced by bonding agents decrease with time both *in vitro* and *in vivo* [6-9]. Water sorption within resin-dentin interfaces has been quoted as one of the dominant factors involved in the adhesion degradation [10]. It has been recently reported that increased hydrophilicity of resin blends such as those employed in dentin adhesives resulted in decreased mechanical strength after long-term water storage [11]. Dentin bond strengths shown by some adhesive systems have been correlated with the mechanical properties of the adhesive resins [12]. Adhesion to dental hard tissues is dependent on all parts composing the resin-tooth interface (enamel, dentin, adhesive system and composite resin) [13]. However, adhesive systems play a central role in this process, and their stability in the oral environment might be a determinant factor in the restoration longevity.

The aims of this study were: (1) to evaluate the changes in ultimate tensile strength (UTS) and elastic modulus (*E*) of polymerized adhesive resins and two primer/adhesive mixtures after aging in water or mineral oil; (2) to examine, with the use of transmission electron microscopy (TEM) the patterns of silver nitrate tracer penetration in polymerized adhesives after storage in water or mineral oil. The null hypotheses tested were that water-storage for 6 months has no effect in the mechanical properties of the adhesive resins and primer-adhesive mixtures;

and there is no difference in the patterns of silver tracer penetration of the adhesives tested after storage in water for 6 months.

Material and Methods

Specimen preparation

Five adhesive systems were used in this study. These systems comprise three different approaches of bonding to dental hard tissues: one single-step self-etching adhesive (One-up Bond F (OB), Tokuyama, Tokyo, Japan), two two-step self-etching primer systems (Clearfil SE Bond (CF) and an antibacterial fluoride-containing system, Clearfil Protect Bond (CP), Kuraray Medical Inc. Tokyo, Japan), and two two-step etch-and-rinse adhesives: a water/ethanol-based (Single Bond (SB), 3M ESPE, St Paul, MN, USA) and an acetone-based filled adhesive (Prime&Bond NT (PB), Dentsply/Caulk, Milford, DE, USA). In addition, two primer adhesive mixtures were tested. The self-etching primers of Clearfil SE Bond and Clearfil Protect Bond were mixed with their respective hydrophobic bonding agents in a 1:1 volume ratio (CF+P and CP+P). Composition, batch number and manufacturers for each adhesive system are listed in Table 1.

All adhesive systems tested present a certain amount of solvent and water in their composition, which could impair polymerization of specimens. Thus, we tried to eliminate or at least reduce their content in the adhesive solutions of SB, PB, CP and CF primers. No attempt to reduce the presence of solvent in OB was done, because manufacturer’s instructions do not recommend air drying after application. For solvent elimination, several drops of each adhesive or primer were placed in an adhesive dispenser and the solution mass was recorded on an analytical balance (JEX-200, YMC Co. Ltd., Kyoto 600-8106, Japan) until reaching equilibrium at room temperature [14]. The amount of time necessary for each adhesive system to reach a constant mass was determined. Solvent evaporation was done in a dark box to prevent early polymerization of the adhesives. Glass pipettes were then used to fill a hollow cylinder of approximately 12 mm height that was cut from micro bore tygon tubing (TYG-030, Small Parts Inc., Miami Lakes, FL) with an internal diameter of approximately 0.73 mm. A light curing unit (Astralis 5, Ivoclar Vivadent, Schaan, Liechtenstein) with an output of 650 mW/cm^2 was used to polymerize specimens for 120 s. The specimens were carefully removed from the tygon tubing and the cylindrical beams were randomly assigned to each of the following storage conditions (n=10): 24 hours in water, 6 months in water and 6 months in mineral oil.

Specimen testing

At completion of each storage period, specimens were removed from the water or oil and thoroughly washed with distilled water. Before testing, specimens had their cross-sectional diameters measured with a digital caliper to the nearest 0.01 mm. Test specimens had a diameter of 0.71-0.73 mm and a gauge length of approximately 4 mm was used. Each specimen was fixed to the grips of a universal testing machine (EZ Test, Shimazu Co., Kyoto, Japan) using a cyanoacrylate adhesive (Zapit, DVA, Corona, CA, USA) [15]. Cross-section diameter and gauge length data were entered in a computer software (WinAGS Lite) connected to the testing machine. Specimens were tested in tension at a crosshead speed of 1mm/min until fracture. Load-displacement data were converted to stress-strain curves. The maximum load sustained by the specimen during the test divided by the original specimen cross-sectional area was recorded as the ultimate tensile strength. The elastic modulus was measured by the slope of the elastic region of the stress-strain graph. All values were expressed in MPa. UTS and E values were statistically analyzed by two-way ANOVA and Tukey test at a pre-set confidence level of 0.05.

Transmission Electron Microscopy

Two additional specimens were similarly prepared as in test groups. After aging in water or mineral oil, specimens were immersed in a 50wt% ammoniacal silver nitrate solution used according to the diamine silver impregnation protocol reported by Tay et al. [5]. Resin specimens were placed in the tracer solution in total darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in a photodeveloping solution for 8 hours under a fluorescent light to reduce silver ions into metallic silver particles. The silver impregnated center of the cylindrical beams were cut and embedded in epoxy resin for support. Representative 90-nm-thick ultrathin sections were prepared with an ultramicrotome (Sorvall Porter-Blum MT-2B, Newtown, CT, USA) and collected on 100-mesh carbon/formvar-coated copper grids. The specimens were not further stained and were observed in a TEM (Philips CM12, Philips, Eindhoven, The Netherlands) operated at 80KV.

Results

Mechanical Properties

Mean UTS and *E* values are presented in Tables 2 and 3 respectively. Two-way ANOVA revealed that there were statistically significant differences for the factor “adhesive” ($p<0.0001$), for the factor “storage condition” ($p<0.0001$), and identified a significant interaction between factors ($p<0.0001$). The above information is valid for both analysis (UTS and *E*). Tukey post-hoc test showed significant differences among adhesive systems at the different storage conditions ($p<0.05$).

The primer/adhesive mixtures CF+P and CP+P presented the lowest UTS values after 24 hours in water. Except for OB and CF+P, a significant reduction in UTS values was observed for all adhesives after 6 months of water-storage: SB (-26.9%), PB (-38.1%), CF (-20%), CP (-20.9%), CP+P (-24%). After 6 months of storage in oil, except for the primer/adhesive blends (CF+P and CP+P) which presented an increase in UTS values, no significant change in strength was observed for the other groups. The lowest initial *E* values were observed for the primer/adhesive blends (CF+P and CP+P). *E* values were not altered after 6 months of storage in water for all groups, except for SB, which presented a significant drop in *E*. After 6 months of storage in oil, all materials became stiffer, presenting increased *E* values: SB (+22.6%), PB (+21%), CF (+27.4%), CP (+33.5%), OB (+60.9%), CF+P (+83.4%), CP+P (+125.1%).

Transmission Electron Microscopy

Figures 1 to 7 depict the silver uptake patterns for the different groups. Notable differences in silver tracer penetration were observed among the different adhesives and storage conditions. Figure 1 depicts the distribution of silver particles in resin beams produced with the self-priming adhesive SB. A band of spotted silver grains can be observed along the junction between epoxy resin and the adhesive (Fig 1A). In a higher magnification, there is a preferential silver deposition within the polyalkenoic acid copolymer component of the adhesive (Fig 1B). After 6 months of storage in water, the regions that were initially filled with small silver particles became more densely filled with silver deposits (Fig 1C). No evident differences were observed after storage in oil (Fig 1D). The silver uptake patterns of the self-priming adhesive PB are presented in Figure 2. The silver deposits apparently increased in quantity after 6 months of water-storage (Fig. 2A and C). Fig. 2B and D depict the increase in silver deposition 100 µm away from the surface of the beam after aging in water for 6 months. After aging in oil, the silver deposition patterns were similar to those observed for the control specimens.

A gradient of diffusion was initially observed for CF (Fig. 3A). Silver deposits were initially more concentrated close to the beam surface. After 6 months of water-storage, it seemed that the silver deposits were oriented towards the center of the beam (Fig. 3B). Small silver deposits were also observed for CF specimens aged in mineral oil. Resin beams of CP presented the lowest silver uptake during the experiment with a small increase after 6 months of storage in water (Fig. 4A and B). Empty spaces within the CP beams were observed along the experiment, which could represent spaces left behind following the elution of fluoride ions from the NaF crystals (Fig. 4C). These spaces were normally observed close to the beam surface. 500 µm away from the beam surface, intact NaF crystals could be observed at high magnification.

The primer/adhesive mixtures CF+P and CP+P presented high amounts of silver deposits within the resin beams for all storage conditions (Figs. 5 and 6). The single-step self-etching adhesive OB presented massive silver deposition along the junction between the epoxy resin and adhesive in all storage conditions (Fig 7). No evident differences were observed between specimens stored in water for 6 months and control specimens.

Discussion

Water sorption within resin-tooth interfaces has been quoted as one of the dominant factors involved in the adhesion degradation [10]. Adhesive systems are largely exposed to dentinal fluids and to a lower extent, to salivary fluids in the oral environment. Water plays an important role in the chemical degradation process of polymer materials [16,17]. Thus, more hydrophobic materials tend to take up lower quantities of water and consequently, present a lower degradation rate [16]. Water-storage for 6 months significantly affected the mechanical properties and silver uptake patterns of some resin beams prepared with the different adhesive resins.

The diffusion of water molecules into polymer matrices is controlled by the Fickian diffusion-type mechanism [11,18]. The patterns of water uptake are governed by two theories which are believed to occur simultaneously: the “free volume theory”, in which water molecules diffuse through pores or morphological defects of the material without mutual relationship to the polar sites of the material; and the “interaction theory”, in which water molecules diffuses through the material binding successively to the hydrophilic groups [19]. There are several factors involved in the polymer water sorption and degradation behavior, such as: pH of the storage media [20,21]; degree of conversion [22]; polarity of the molecular structure; presence of pendant hydroxyl groups capable of forming hydrogen bonds with water; degree of crosslinking [23]; presence of residual water; and presence and type of filler particles [24-26]. After entering the polymer matrix, water triggers the chemical degradation, resulting in the formation of oligomers and monomers. The materials compositions are inherently prone to hydrolysis, due to the presence of unprotected ester linkages in the monomers [16].

Except for CF+P (which presented the lowest UTS values at 24 h) and OB, all other groups presented a significant reduction in UTS after aging in water for 6 months, ranging from 20% to 38% of the values obtained by the 24 h controls. These observations demonstrate a negative impact of water-storage in the tensile strength of the adhesives and are in accordance with previous investigations [11,13,27]. On the other hand, except for SB, *E* values were not altered after 6-month water-storage. High UTS values are especially important during composite curing in a cavity, because polymerization shrinkage of composite resins might stress the resin/tooth interface and damage the bond. However, if this initial stress is supported, the elasticity of the material would be extremely important to withstand deformations that occur during mastication and thermal changes in the oral environment. Nevertheless, it cannot be predicted for how long the elastic modulus of these systems would be kept unaffected. Unaltered mechanical properties have been reported for an adhesive system for up to 6 months of storage in water [27]. However, a decrease in the modulus of elasticity of the same material was observed after 1 year of water-storage [13]. It has been speculated that hydrolytic degradation might have taken place compromising the structure of the polymer matrix [13].

The water/ethanol-based one-bottle adhesive SB presented decreased UTS and E values after storage in water for 6 months. Observation of silver uptake patterns in resin beams produced with SB revealed preferential silver deposition within the polyalkenoic acid copolymer component (Fig1. A-B). The presence of monomers with different properties such as molecular weight, hydrophilicity (hydrophobicity), and degradation behavior, might induce preferential degradation of one phase in a polymer system [28]. This could lead to the formation of pores within the polymer matrix as seen in Figure 1C. Regions that were initially filled with isolated silver grains were more densely filled with silver. These pores might have acted as crack initiation/propagation sites during tensile testing, which might have accounted for the decreased mechanical properties after 6 months of storage in water. The solvent depleted UDMA/PENTA filled solution of PB also presented decreased UTS values after water-storage. However, no difference in *E* values was detected. TEM observation revealed an increased number of isolated silver grains within the polymer matrix after immersion in water for 6 months (Fig. 2), which might be related to the lowered UTS values observed at this period.

It was observed that the primer/adhesive mixtures CF+P and CP+P presented significantly lower mechanical properties and notably more silver uptake than their respective bonding resins cured with no primer addition, CF and CP (Figs. 3-6). These findings are in agreement with previous studies which demonstrated that primer addition inhibited free-radical polymerization [29,30] and adversely affected mechanical properties [29]. CF and CP presented similar mechanical properties over the experiment. Their composition is very similar with the exception that surface treated NaF is added to CP [31]. Empty spaces within CP beams were observed along the experiment (Fig. 4), which could possibly represent spaces left behind following the elution of fluoride ions from the NaF crystals [32], however, further research is necessary to confirm this speculation. Fluoride release might be important in the prevention and control of secondary caries around restorations [33,34].

The single-step self-etching adhesive OB presented no significant changes in the mechanical properties after storage in water for 6 months. However, TEM images revealed massive silver deposition along the junction between the epoxy resin and OB resin (Fig. 7). The pattern of silver deposition was very similar to the nanoleakage patterns in resin-dentin interfaces produced by this adhesive reported in previous investigations [9,17,35], with water-trees protruding into the resin beam. The silver deposits might represent areas within the polymerized resin matrix in which water was incompletely removed, resulting in regions of incomplete polymerization and/or hydrogel formation, or hydrophilic domains of acidic monomers that are more prone to water sorption [5,32]. The ability of single-step adhesives to seal dentin surfaces has been questioned, as they function as permeable membranes allowing water movement between the interface and underlying dentin [36,37].

Except for the primer/adhesive mixtures CF+P and CP+P, which presented increased UTS values, aging resin beams in oil for six months produced no significant changes in the tensile strength. However, *E* values significantly increased for all materials. These observations support the degrading effects of water storage. The stability of mechanical properties of resin specimens aged in oil has been attributed to the absence of plasticizing effect of oil on the polymers [11,13]. The higher *E* values observed after 6 months of immersion in oil may not be a result of improved mechanical properties in this medium, rather, the control values are lower because the specimens may have suffered the immediate effects of water immersion after curing [13].

Since storage in water for 6 months caused a reduction in the mechanical properties and silver uptake patterns of the adhesive resin specimens tested in this study, our null hypotheses must be rejected. Even though our study conditions are far removed from real clinical situations, a tendency to decrease the mechanical properties and increase the silver uptake was observed after immersion in water for six months. In real clinical situations, other factors such as enzymatic challenges, pH changes, occlusal load, dentin fluid and saliva could contribute to the physical/chemical degradation of the bond. Further research is necessary for the development of simplified adhesives with more hydrophobic characteristics after polymerization and able to promote a durable bond to a moist substrate like dentin.

Conclusion

A tendency to decrease the mechanical properties and increase the silver uptake of adhesive resins was observed after immersion in water for six months. Unaltered or increased mechanical properties were observed after aging specimens in a non-aqueous storage media.

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References

[1] Kanca J 3rd. Resin bonding to wet substrate. 1. Bonding to dentin. *Quintessence International* 1992; **23**:39-41.

[2] Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dental Materials* 2001; **17**:296-308.

[3] De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. Four-year water degradation of total-etch adhesives bonded to dentin. *Journal of Dental Research* 2003; **82**:136-40.

[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. *Journal of Dental Research* 2005; **84**:118-32.

[5] Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. *Journal of Dental Research* 2002; **81**: 472-6.

[6] Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. *Journal of Dental Research* 2000; **79**:1385-91.

[7] Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. *Journal of Dental Research* 1999; **78**:906-11.

[8] Giannini M, Seixas CAM, Reis AF, Pimenta LAF. Six-month storage-time evaluation of one-bottle adhesive systems to dentin. *Journal of Esthetic and Restorative Dentistry* 2003; **15**:43-8.

[9] Reis AF, Arrais CAG, Novaes PD, Carvalho RM, De Goes MF, Giannini M. Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives: nanoleakage expression. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2004; **71**:90-8.

[10] Tanaka J, Ishikawa K, Yatani H, Yamashita A, Suzuki K. Correlation of dentin bond durability with water absorption of bonding layer. *Dental Materials Journal* 1999; **18**:11-8.

[11] Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. *Biomaterials* 2004; **25**:5789-96.

[12] Takahashi A, Sato Y, Uno S, Pereira PN, Sano H. Effects of mechanical properties of adhesives on bond strength to dentin. *Dental Materials* 2002; **18**:263-8.

[13] Carrilho MRO, Tay FR, Pashley DH, Tjäderhane L, Carvalho RM. Mechanical stability of resin–dentin bond components. *Dental Materials* 2005; **21**:232-41.

[14] Reis AF, Oliveira MT, Giannini M, De Goes MF, Rueggeberg FA. The effect of organic solvents on one-bottle adhesives bond strength to enamel and dentin. *Operative Dentistry* 2003; **28**:700-6.

[15] 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**:209-14.

[16] Göpferich A. Mechanisms of polymer degradation and erosion. *Biomaterials* 1996; **17**:103-14.

[17] Reis AF, Pereira PNR, Giannini M. Water-induced nanoleakage prevention by bonding strategies. (submitted)

[18] Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SC, Yiu CK, *et al.* Aging affects two modes of nanoleakage expression in bonded dentin. *J Dent Res* 2003; **82**:537-41.

[19] Bellenger V, Verdu J, Morel E. Structure-properties relationships for densely cross-linked epoxy-amine systems based on epoxide or amine mixtures. *Journal of Materials Science* 1989; **24**:63-8.

[20] Ortengren U, Andersson F, Elgh U, Terselius B, Karlsson S. Influence of pH and storage time on the sorption and solubility behaviour of three composite resin materials. *Journal of Dentistry* 2001;**29**:35-41.

[21] Prakki A, Cilli R, Mondelli RF, Kalachandra S, Pereira JC. Influence of pH environment on polymer based dental material properties. *Journal of Dentistry* 2005; **33**:91-8.

[22] Pearson GJ, Longman CM. Water sorption and solubility of resin-based materials following inadequate polymerization by a visible-light curing system. *Journal of Oral Rehabilitation* 1989; **16**(1) 57-61.

[23] Beatty MW, Swartz ML, Moore BK, Phillips RW, Roberts TA. Effect of crosslinking agent content, monomer functionality, and repeat unit chemistry on properties of unfilled resins. *Journal of Biomedical Materials Research* 1993; **27**:403-13.

[24] Mortier E, Gerdolle DA, Jacquot B, Panighi MM. Importance of water sorption and solubility studies for couple bonding agent/resin-based filling material. *Operative Dentistry* 2004;**29**:669-76.

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

[26] Marcovich NE, Reboredo MM, Aranguren MI. Moisture diffusion in polyester-woodflour composites. *Polymer* 1999; **40**:7313-20.

[27] Carrilho MRO, Carvalho RM, Tay FR, Pashley DH. Effects of storage media on mechanical properties of adhesive systems. *American Journal of Dentistry* 2004; **17**:104-8.

[28] Shastri VP, Hildgen P, Langer R. In situ pore formation in a polymer matrix by differential polymer degradation. *Biomaterials* 2003; **24**:3133-7.

[29] Hotta M, Kondoh K, Kamemizu H. Effect of primers on bonding agent polymerization. *Journal of Oral Rehabilitation* 1998; **25**:792-9.

[30] Yamada M, Miyazaki M, Moore BK. Influence of interchanging adhesive resins and self-etching primers on the mechanical properties of adhesive resins. *Operative Dentistry* 2004; **29**:532-7.

[31] Nakajima M, Okuda M, Ogata M, Pereira PN, Tagami J, Pashley DH. The durability of a fluoride-releasing resin adhesive system to dentin. *Operative Dentistry* 2003; **28**:186-92.

[32] Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *Journal of Adhesive Dentistry* 2002; **4**:255-69.

[33] Tsuchiya S, Nikaido T, Sonoda H, Foxton RM, Tagami J. Ultrastructure of the dentin-adhesive interface after acid-base challenge. *Journal of Adhesive Dentistry* 2004; **6**:183-90.

[34] Itota T, Torii Y, Nakabo S, Tashiro Y, Konishi N, Nagamine M, Yoshiyama M. Effect of fluoride-releasing adhesive system on decalcified dentin. *Journal of Oral Rehabilitation* 2003; **30**:178-83.

[35] Carvalho RM, Tay FR, Giannini M, Pashley DH. Effects of pre- and post-bonding hydration on bond strength to dentin. *Journal of Adhesive Dentistry* 2004; **6**:13-7.

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

[37] 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. *Journal of Dental Research* 2004; **83**:459-64.

Table 1. Materials, Brand (Lot #), composition, application technique and manufacturers of adhesive systems used in this study:

Material	Brand (Lot #)	Code	Composition	Manufacturer
Two-step Self-etching primers	Clearfil Protect Bond (primer: 00002A bond: 00004A)	CP	*Primer*: MDP, HEMA, MDPB, dimethacrilates, photoinitiator, water *Adhesive*: MDP, HEMA, dimethacrylates, photoinitiator, NaF, silanated colloidal silica	Kuraray Medical Inc., Tokyo, Japan
	Clearfil SE Bond (primer:00400A bond:00541A)	CF	*Primer*: Water, ethanol, MDP, HEMA, dimethacrylate hydrophilic, canphorquinone, N,N-diethanol p-toluidine *Adhesive*: MDP, Bis-GMA, HEMA, dimethacrylate hydrophobic, canphorquinone, N,N-diethanol p-toluidine, silanated colloidal silica	Kuraray Medical Inc., Tokyo, Japan
One-step Self-etching adhesive	One-up Bond F (Bonding A: 084 Bonding B: 578)	OB	Water, MMA, HEMA, coumarin dye, metacryloyloxyalkyl acid phosphate, MAC-10, multifuntional methacrilic monomer, fluoraluminosilicate glass, photoinitiator (aryl borate catalyst)	Tokuyama Corp., Tokyo, Japan
Two-step total-etch adhesives	Prime&Bond NT (030822)	PB	*Etchant*: 35% H3PO4 *Adhesive*: PENTA, UDMA, resin R5-62-1, resin T, resin D, silica nanoparticles, photoinitiators, cetilamine hidrofluoride and acetone	Dentsply Caulk, Milford, DE, USA
	Single Bond (3JL)	SB	*Etchant*: 35% H3PO4 *Adhesive*: water, ethanol, Bis-GMA, HEMA, UDMA, Bisphenol A glycerolate, polyalkenoic acid copolymer, dimethacrylate, canphorquinone	3M ESPE, St Paul, MN, USA

(MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; MDPB, 12-methacryloyloxydodecylpyridinium bromide; MMA, methyl methacrylate; MAC-10, methacryloyloxydecamethlene malonic acid; PENTA, dipentaerythritol pentacrylate phosphoric acid ester; UDMA, urethane dimethacrylate; Bis-GMA, bisphenol-A diglycidil ether dimethacrylate.)

Table 2. Mean values (MPa) and standard deviation of the ultimate tensile strength of the different adhesive resins tested after different storage conditions.

	24 hours H_2O		6 months H_2O		6 months oil	
Single Bond	83.62 ± 9.13	Aa	61.11 ± 12.36	Ab	93.27 ± 17.74	Aa
Prime&Bond NT	60.60 ± 12.65	BCa	37.49 ± 12.61	Bb	54.49 ± 13.02	Da
C. SE Bond (bonding resin)	79.97 ± 12.64	Aa	63.95 ± 9.83	Ab	81.90 ± 16.96	ABa
C. Protect Bond (bonding resin)	77.56 ± 6.47	ABa	61.38 ± 18.81	Ab	75.60 ± 13.21	ABCa
One-up Bond F	67.78 ± 6.25	ABa	65.32 ± 9.64	Aa	65.54 ± 16.96	BCDa
C. SE Bond + primer	32.56 ± 5.80	Db	35.17 ± 6.21	Bb	59.46 ± 13.67	CDa
C. Protect Bond + primer	47.89 ± 5.93	Cb	36.38 ± 11.19	Bc	68.16 ± 17.61	BCDa

Means followed by different letters (upper case – column, lower case – row) differ among them by Tukey test at the 0.05 confidence level.

Table 3. Mean values (MPa) and standard deviation of the Elastic Modulus of the different adhesive resins tested after different storage conditions.

	24 hours H_2O		6 months H_2O		6 months oil	
Single Bond	1028.20 ± 233.13	Aa	841.19 ± 76.02	Bb	1260.44 ± 133.16	ABa
Prime&Bond NT	877.52 ± 201.12	Bab	847.91 ± 103.90	Bb	1062.02 ± 78.51	Ba
C. SE Bond (bonding resin)	995.98 ± 73.10	ABb	1188.14 ± 124.01	Aab	1269.26 ± 139.22	ABa
C. Protect Bond (bonding resin)	957.33 ± 185.19	ABb	959.35 ± 112.52	ABb	1278.14 ± 160.98	ABa
One-up Bond F	769.07 ± 165.52	BCb	821.41 ± 159.12	BCb	1237.85 ± 63.39	ABa
C. SE Bond + primer	594.16 ± 120.94	Cb	765.13 ± 248.29	BCb	1089.74 ± 111.30	ABa
C. Protect Bond + primer	573.99 ± 185.19	Cb	568.64 ± 123.37	Cb	1292.04 ± 168.41	Aa

Means followed by different letters (upper case – column, lower case – row) differ among them by Tukey test at the 0.05 confidence level.

Legends to Illustrations

Figure 1. TEM micrographs of specimens produced with the self-priming adhesive Single Bond (SB) after 24 hours (A and B) and 6 months (C) of storage in water, and 6 months of storage in oil (D). (A) At 24 h, a band of spotted silver grains can be observed along the junction between epoxy resin (ER) and the adhesive (AD). A diffusion gradient of the diamine silver ions into the resin beam can be observed. (B) In a higher magnification, it can be noticed that there is a preferential silver deposition within the polyalkenoic acid copolymer component of the adhesive. (C) After 6 months of storage in water, the regions that were initially filled with small silver particles became more densely filled with silver deposits. (D) No evident differences from control specimens were observed after storage in oil. (Magnification: A, C and D – 5,600X; B – 25,000).

Figure 2. TEM micrographs of specimens produced with the self-priming adhesive Prime&Bond NT (PB) after 24 hours (A and B) and 6 months (C and D) of storage in water. The silver deposits apparently increased in quantity after 6 months of water-storage. Fig. 2B and D depict the increase in silver deposition 100 µm away from the surface of the beam after aging in water for 6 months. AD – adhesive; ER – epoxy resin. (Magnification: A and C – 5,600X; C and D – 66,000X).

Figure 3. TEM micrographs of a specimen produced with the bonding resin of Clearfil SE Bond (CF) after 24 hours (A) and 6 months (B) of storage in water, and after 6 months of storage in oil (C). (A) A gradient of diffusion was initially observed. Silver deposits were initially more concentrated close to the beam surface. (B) After 6 months of water-storage, it seemed that the silver deposits were oriented towards the center of the beam. (C) Small silver deposits were also observed for CF specimens aged in mineral oil. AD – adhesive; ER – epoxy resin. (Magnification: A, B and C – 5,600X).

Figure 4. TEM micrographs of a specimen produced with the bonding resin of Clearfil Protect Bond (CP) after 24 hours (A) and 6 months (B and C) of storage in

water, and after 6 months of storage in oil (D). (A) Resin beams of CP presented the lowest silver uptake at 24 h with a small increase after 6 months of storage in water (B). Empty spaces within the CP beams were observed along the experiment (arrowheads), which could possibly represent spaces left behind following the elution of fluoride ions from the NaF crystals. These spaces were normally observed close to the beam surface. (C) 500 µm away from the beam surface, some intact NaF crystals could be observed at high magnification. AD – adhesive; ER – epoxy resin. (Magnification: A, B and D – 5,600X; C – 25,000X).

Figure 5. TEM micrographs of a specimen produced with the primer/adhesive mixture of the two-step self-etching adhesive Clearfil Se Bond (CF+P) after 24 hours (A) and 6 months (B) of storage in water, and after 6 months of storage in oil (C). (A) High amounts of silver deposits were observed within the resin beams, probably due to the polymerization inhibition by the hydrophilic primer. AD – adhesive; ER – epoxy resin. (Magnification: A, B and C – 5,600X).

Figure 6. TEM micrographs of a specimen produced with the primer/adhesive mixture of the two-step self-etching adhesive Clearfil Protect Bond (CP+P) after 24 hours (A) and 6 months (B) of storage in water, and after 6 months of storage in oil (C). (A) High amounts of silver deposits were observed within the resin beams, probably due to the polymerization inhibition by the hydrophilic primer. AD – adhesive; ER – epoxy resin. (Magnification: A, B and C – 5,600X).

Figure 7. TEM micrographs of a specimen produced with the single-step self-etching adhesive One-up Bond F (OB) after 24 hours (A) and 6 months (B) of storage in water, and after 6 months of storage in oil (C). Massive silver deposition along the junction between the epoxy resin (ER) and adhesive (AD) was observed in all storage conditions. Typical water-trees (arrowheads) were observed protruding into the resin beam. (Magnification: A, B and C – 5,600X).

Figure 1. Single Bond

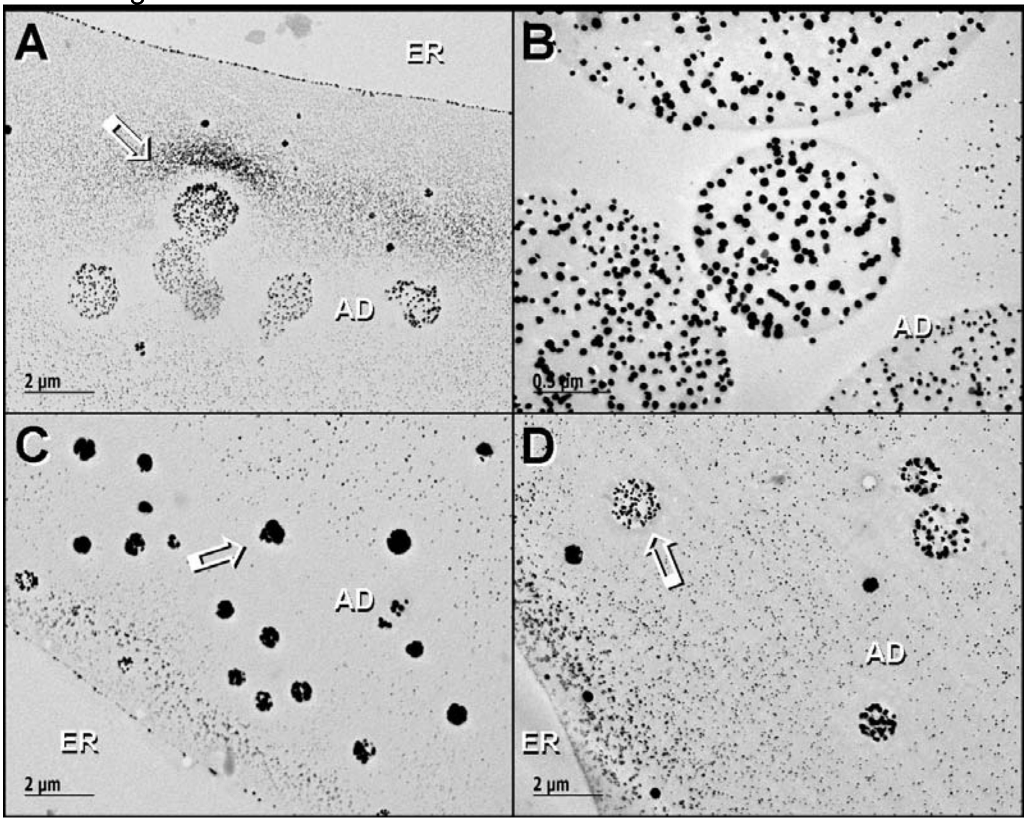


Figure 2. Prime & Bond NT

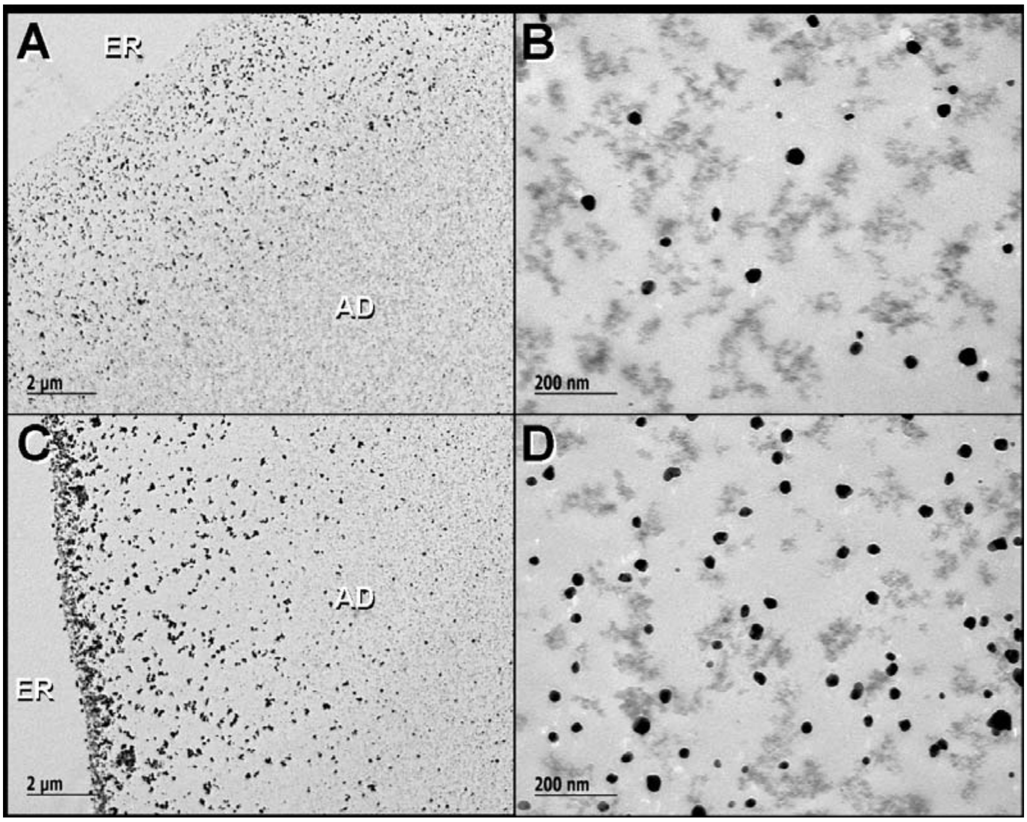


Figure 3. Clearfil SE Bond

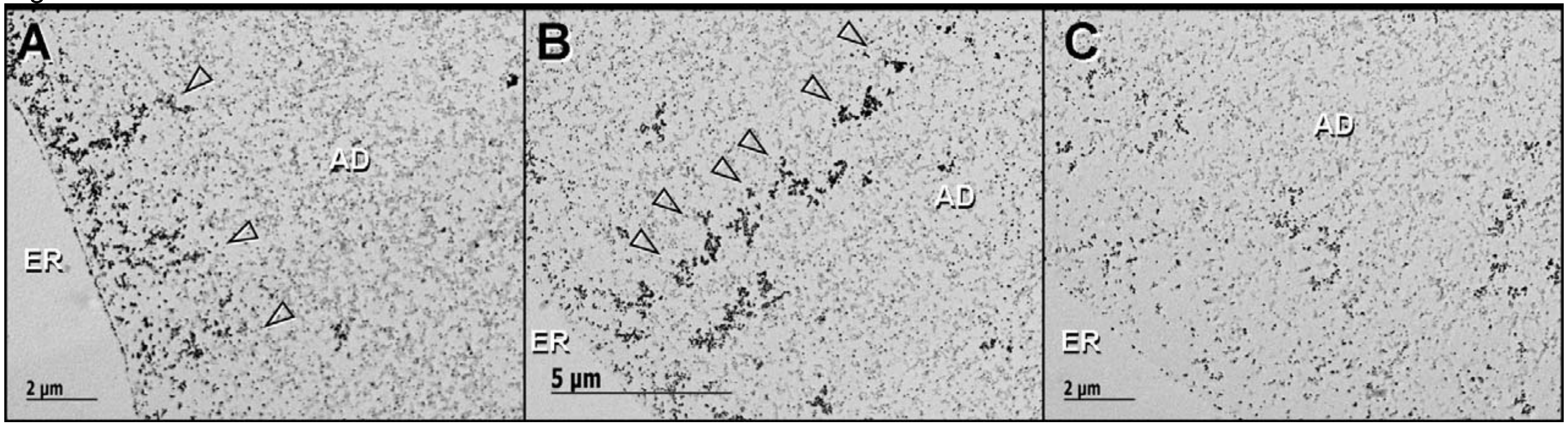


Figure 4. Clearfil Protect Bond

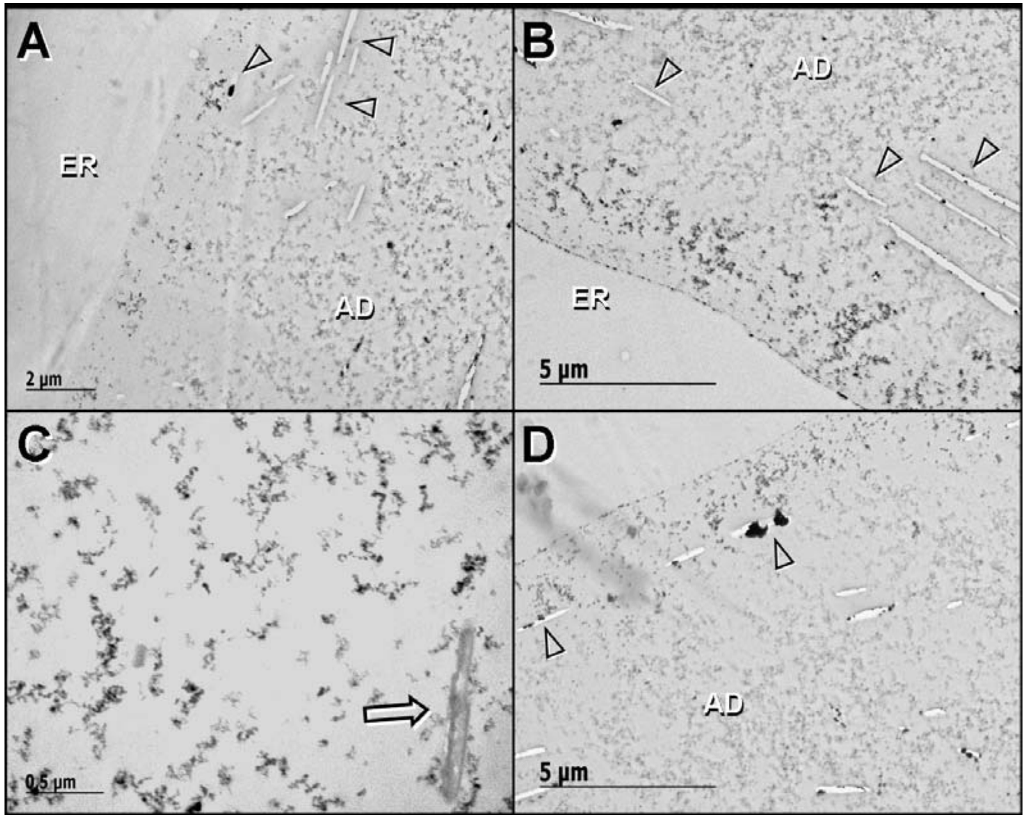


Figure 5. Clearfil SE Bond + Primer

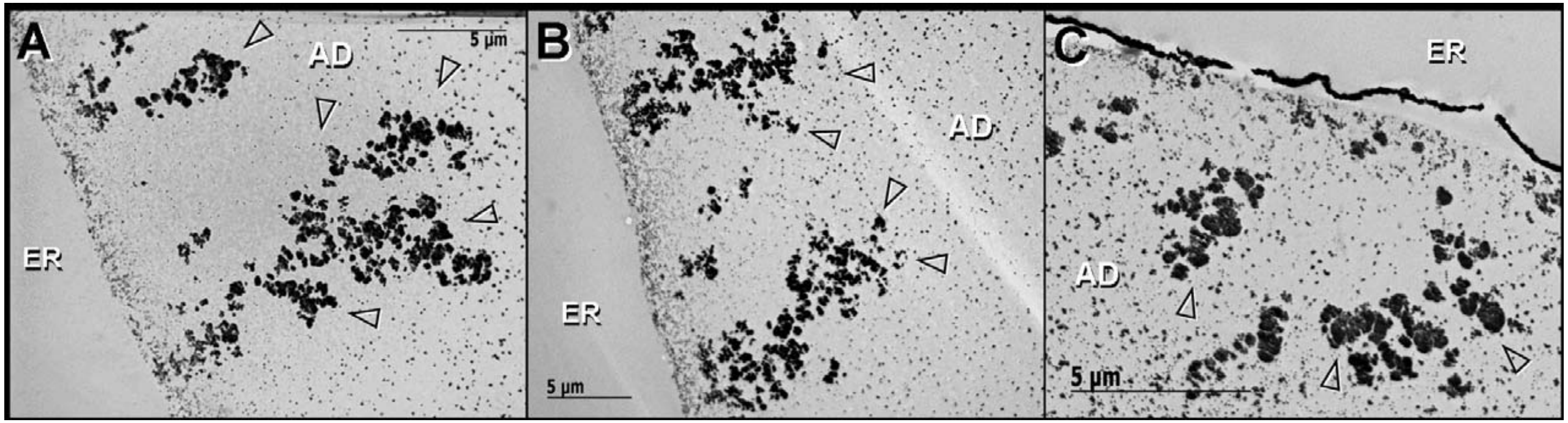


Figure 6. Clearfil Protect Bond + Primer

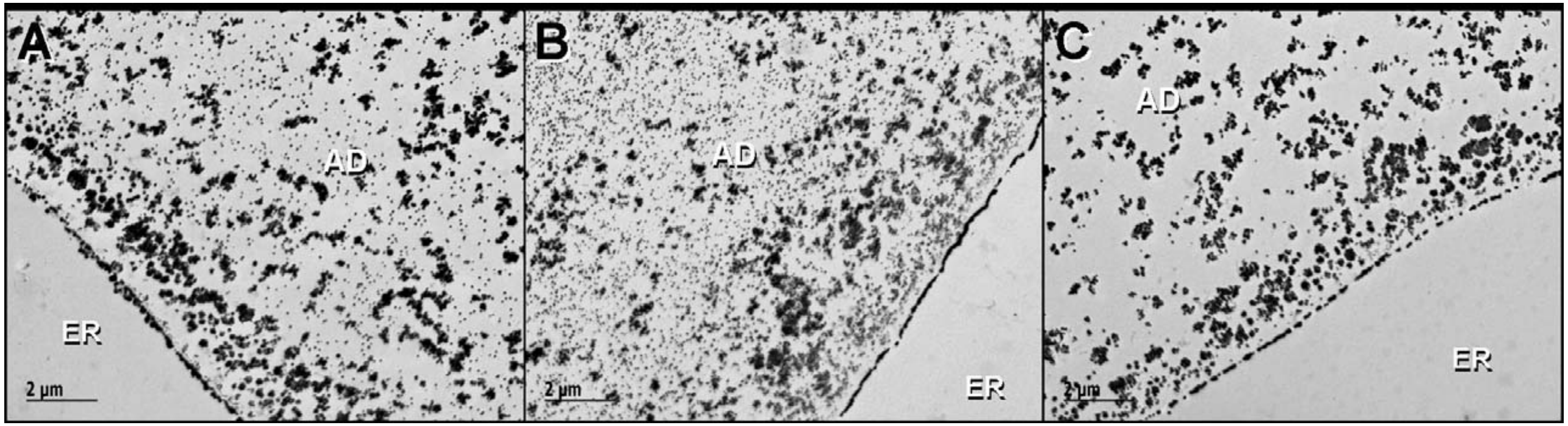
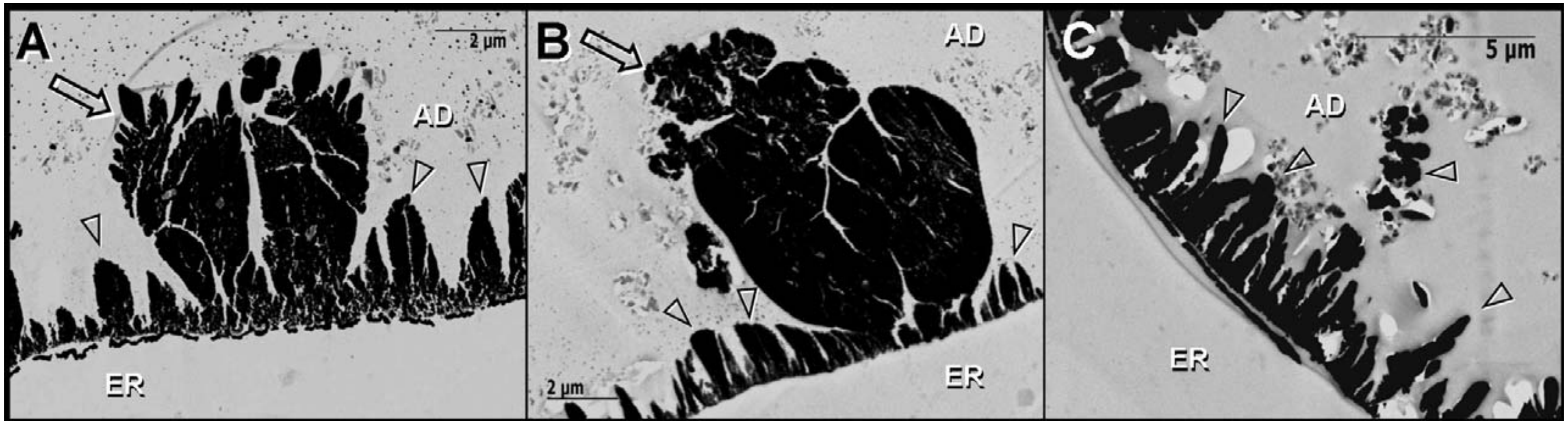


Figure 7. One-up Bond F



CAPITULO 8:

Effects of water-exposure on the long-term effectiveness of different bonding procedures

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SHORT TITLE: Long-term dentin bonding effectiveness

Abstract: This study evaluated the effects of water exposure on the microtensile bond strength (µTBS) to dentin of etch-and-rinse and self-etching adhesives over a one-year storage period. Five adhesive systems were used: a one-step self-etching adhesive (One-up Bond F - OB), two two-step self-etching primers (Clearfil SE Bond - CF and Clearfil Protect Bond - CP), and two etch-and-rinse systems (Single Bond - SB and Prime&Bond NT - PB). Dentin surfaces were bonded, restored and randomly assigned to 4 subgroups, according to the degree of water exposure: 24 h of indirect water exposure (24h-IE), and 1 year of indirect exposure (1Yr-IE), direct exposure (1Yr-DE) or no water exposure (1Yr-NoE). The presence of a composite-enamel bond adjacent to the restoration determined if the water exposure was indirect or direct. Mineral oil was used as the storage media for groups not subjected to water exposure. Elapsed storage periods, specimens were sectioned into 1-mm thick slabs, trimmed to an hourglass shape with a cross-sectional area of 1 mm^2 at the bonded interface and tested in tension (1 mm/min). Failure modes were examined with an SEM. Results were analyzed by two-way ANOVA and Duncan test. No difference between 24h-IE and 1Yr-IE was observed for SB and OB, however, their µTBS values significantly dropped after 1Yr-DE. CF and CP presented decreased µTBS after 1-year water-storage, but no differences between 1Yr-IE and 1Yr-DE. µTBS decreased significantly for PB after 1Yr-IE and 1Yr-DE. 1Yr-NoE resulted in similar or increased µTBS values. The percentage of interfacial failures increased after 1 year of storage in water. Water-storage for 1 year significantly decreased µTBS for all adhesives. However, the presence of a composite-enamel bond seemed to slow down the degradation velocity in resin-dentin interfaces for the etch-and-rinse adhesives SB and PB, and for the single-step adhesive OB.

Keywords: Dentin bonding; adhesives; bond strength; SEM; aging; degradation.

INTRODUCTION

Bonding to enamel and dentin is mainly accomplished by micromechanical interlocking between synthetic polymers and enamel or dentin collagen fibrils.[1] Effective, long-lasting bonding to dentin has been a challenge to dental clinicians, because in order to promote adhesion to dentin, the mineral phase needs to be totally or partially removed, and substituted by an adhesive solution, that will permeate this collagen-rich layer, and polymerize in situ, forming what has been called the hybrid layer.[1-3]

Different approaches, with different numbers of steps and degrees of sensitivity have been used to bond resin-based materials to enamel and dentin.[4-6] However, simplification of bonding procedures has resulted in increased hydrophilicity and consequently, in decreased long-term bonding effectiveness.[7] One-bottle self-priming etch-and-rinse systems, as well as single-step self-etching adhesives are more hydrophilic versions of their multiple-step precursors.[6,7] The incorporation of increased concentrations of hydrophilic monomers into adhesive systems can compromise bond durability, as hydrophilicity and hydrolytic stability are antagonistic properties.[8]

It is well known that the bond strength and quality of the seal produced by bonding agents decrease with time both *in vitro* and *in vivo*.[9-12] Water sorption within resin-dentin interfaces has been quoted as one of the dominant factors involved in the adhesion degradation.[13] It has been demonstrated that a composite-enamel bond adjacent to a composite-dentin bond might help protect the resin-dentin interface against degradation for etch-and-rinse systems.[6] The aim of this study was to evaluate the changes in microtensile bond strength (µTBS) of etch-and-rinse and self-etching adhesives after aging specimens in water or mineral oil for 1 year. The null hypotheses tested were that the different conditions of water-exposure have no effect in the µTBS values; and there is no difference in the µTBS values produced by the different materials after the different storage conditions.

MATERIALS AND METHODS

Sixty freshly extracted third molars were used. Teeth were obtained by protocols that were approved by the review board of the Piracicaba School of Dentistry (#080/2003). After disinfection and removal of soft tissues, flat coronal dentin surfaces were exposed with 600-grit SiC papers under running water to create a standardized smear layer.

Teeth were randomly assigned to five experimental groups, which were bonded with five adhesive systems. These systems comprise three categories: one single-step self-etching adhesive (One-up Bond F (OB), Tokuyama, Tokyo, Japan), two two-step self-etching primers (Clearfil SE Bond (CF) and an antibacterial fluoride-containing system, Clearfil Protect Bond (CP), Kuraray Medical, Kurashiki, Japan), and two two-step etch-and-rinse adhesives: a water/ethanol-based (Single Bond (SB), 3M ESPE, St Paul, MN, USA) and an acetone-based filled adhesive (Prime&Bond NT (PB), Dentsply Caulk, Milford, DE, USA). Composition, batch number, application instructions and manufacturers are listed in Table 1.

After application of the adhesive resins according to manufacturers' instructions, composite crowns of approximately 6 mm were incrementally built up with Clearfil APX composite resin (Kuraray Medical, Tokyo, Japan). A light curing unit (Astralis 5, Ivoclar Vivadent, Schaan, Liechtenstein) with an output of 650 mW/cm^2 was used to polymerize specimens. Twelve teeth were restored with each adhesive system. Restored specimens were randomly divided into 4 subgroups, according to the condition of water exposure: 24 hours of indirect water exposure (24h-IE), one year of indirect water exposure (1Yr-IE), one year of direct water exposure (1Yr-DE) or one year with no water exposure (1Yr-NoE). The presence of a composite-enamel bond surrounding the restoration determined if the water exposure was indirect or direct. Mineral oil was used as the storage media for groups not subjected to water exposure. Prior to water storage, 4 teeth from each group were sectioned in two halves: one half was assigned for direct exposure of the resin dentin interface to water (1Yr-DE) and the other half was immersed in mineral oil for no water exposure (1Yr-NoE). Water was changed weekly, in order to prevent bacterial growth and accelerate the degradation process.[14]

Elapsed storage periods, specimens were serially sectioned into 1-mm thick slabs and trimmed to an hourglass shape with a cross-sectional area of approximately 1 mm^2 at the bonded interface. Four slabs were obtained from each tooth or half-tooth (1Yr-DE and 1Yr-NoE). For the specimens assigned to groups 1Yr-DE and 1Yr-NoE, the "neck" of the hourglass specimens was situated one mm from the exposure plane. Each specimen was fixed to the grips of a universal testing machine (EZ Test, Shimazu Co., Kyoto, Japan) using a cyanoacrylate adhesive (Zapit, DVA, Corona, CA, USA) and tested in tension at a crosshead speed of 1mm/min until fracture. Maximal tensile load was divided by specimen cross-sectional area to express results in units of stress (MPa). Differences in microtensile bond strength were statistically evaluated using a two-way ANOVA and the Duncan post-hoc test at a pre-set significance level of 0.05. All statistical analysis was done using a personal computer program (SAS for windows V8, SAS Institute, Cary, NC, USA).

For determination of failure modes, fractured specimens were mounted on aluminum stubs, gold sputter coated (MED 010, BAL-TEC, Furstentum, Liechtenstein) and examined using a scanning electron microscope (SEM) (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge CB1 3QH, United Kingdom) at 200 X or higher magnification. Failure mode was classified into one of three types: cohesive failure in dentin, interfacial failure, or cohesive failure in resin.

RESULTS

Mean μTBS values are presented in Table 2. Two-way ANOVA revealed that there were statistically significant differences for the factor "adhesive system" ($p<0.0001$) and for the factor "water-exposure" ($p<0.0001$). In addition, it identified a significant interaction between the two factors ($p<0.0001$). Duncan post-hoc test showed significant differences among adhesives and water-exposure conditions ($p<0.05$).

At 24 hours, OB presented the lowest µTBS values. All other adhesives presented considerably high bond strengths at this period. After one year of indirect water exposure (1Yr-IE), the µTBS significantly decreased for the two-step self-etching primers CF and CP, and for the etch-and-rinse adhesive PB. However, similar values were observed for OB and SB between 24h-IE and 1Yr-IE. Even though a significant decrease in µTBS was observed for CP after 1Yr-IE, it presented the highest µTBS for this storage condition, not significantly different from SB.

Direct water exposure for one year (1Yr-DE) significantly reduced the µTBS for the single-step self-etching adhesive OB, and for the two-step etch-and-rinse adhesives SB and PB. Even though µTBS values observed for the self-etching primers CF and CP after 1Yr-DE were significantly lower than 24h-IE, no significant differences were observed for CF and CP between 1Yr-IE and 1Yr-DE. After one year of direct water exposure the highest µTBS were also observed for CP and SB. One year of no water exposure (1Yr-NoE) resulted in similar or increased µTBS values.

Distribution of failure modes is depicted in Figure 1. The percentage of interfacial failures increased after one year of storage in water. Figure 2 shows representative SEMs of a failure mode observed for the self-etching adhesive OB after 24h-IE. A globular structure was observed at the fractured surface, and might be related to the low µTBS values recorded for this system over the experiment. Representative SEMs of the fractured surface of CF after 1Yr-DE is presented in Figure 3. The fractured adhesive resin appeared to be more porous than at 24h-IE. Figure 4 compares the failure patterns of PB after 24h-IE and 1Yr-DE. A more porous hybrid layer was observed after 1 year of storage in water.

DISCUSSION

Ideally, an adhesive system should provide adequate bond strength and sealing of dentin surfaces and be long-lasting. Much information has already been generated, but the mechanisms of resin-dentin interfaces degradation are not yet fully understood. Degradation of the bonds has been attributed to hydrolytic breakdown of the resin adhesive and/or dentin collagen.[2,3,11] This study evaluated the effects of different conditions of water exposure on the long-term behavior of resin-dentin bonds. The present findings confirm other reports that a significant reduction can occur in resin-dentin bond strengths following long-term water immersion.[15-17] However, the different adhesive systems tested presented distinct behaviors with respect to direct or indirect exposure to water for 1 year.

Water sorption within resin-tooth interfaces has been quoted as one of the dominant factors involved in the adhesion degradation.[13] Adhesive systems are largely exposed to dentinal fluids and to a lower extent, to salivary fluids in the oral environment. The water sorption and solubility characteristics of adhesive materials are important in determining the longevity and marginal quality of a restoration. Water plays an important role in the chemical degradation process of polymer materials.[18,19] Thus, more hydrophobic materials tend to take up lower quantities of water and consequently, present a lower degradation velocity.

The present study demonstrated that the presence of a composite-enamel bond adjacent to a composite-dentin bond might help protect the resin-dentin interface against degradation for some adhesives. The etch-and-rinse adhesive SB, as well as the single-step self-etching adhesive OB presented stable μTBS after one year of indirect water exposure (1Yr-IE). Also, even though the acetone-based etch-and-rinse adhesive PB presented decreased μTBS after 1Yr-IE, when it was directly exposed to water (1Yr-DE) the reduction in μTBS was even more pronounced. Figure 4 depicts a more porous hybrid layer for PB after 1Yr-DE, demonstrating that elution of resin probably occurred at this interface. One year direct water exposure was also detrimental for the resin-dentin interfaces produced by SB and OB. These results are in accordance with a previous investigation that demonstrated the benefits of a surrounding resin-enamel bond on the protection against degradation.[6]

Direct or indirect water exposure did not produce different effects in the μTBS of the two-step self-etching primers CF and CP. Bond strength decreased similarly for both adhesives in both water-storage conditions. Even though a significant reduction in bond strength was detected for CP after one year of storage in water, it still presented high μTBS values at 1Yr-IE and 1Yr-DE. The incorporation of an antibacterial monomer into the primer and surface-treated NaF into the bonding solution of CP has shown promising results with respect to long-term bond stability both *in vitro* and *in vivo*.[20,21] It has been speculated that fluoride release might inhibit some enzymes activities within the interface.[21] It might also be important in the prevention of secondary caries around restorations.[22-24] The μTBS values observed for CF after 1Yr-DE were quite reduced (45.57% lower), if compared to the 24h-IE values. A recent study demonstrated increased nanoleakage for CF after a 6-month water-storage period,[12] which might be an indication of degradation of the resin-dentin interface. The morphologic aspect observed in Figure 3 might be a sign that elution of degradation products probably occurred at these interfaces.

The one-step self-etching adhesive OB presented the lowest initial μTBS values. Observation of failure modes revealed a globular structure at the fracture plane (Fig. 2). These globules probably represent areas within the polymerized resin matrix in which water was incompletely removed, resulting in regions of incomplete polymerization and/or hydrogel formation.[8,25] Also, phase separation between adhesive ingredients, might contribute to the lower bonding effectiveness of some one-step adhesives.[26] The ability of single-step adhesives to seal dentin surfaces has been questioned, as they function as permeable membranes allowing water movement between the interface and underlying dentin.[27,28] The presence of the bonded enamel adjacent to the restoration might have decreased the diffusion rate of water and elution of degradation products, which might explain why no reduction in bond strength was observed for OB after 1Yr-IE. On the other hand, a significant reduction in μTBS was observed after 1Yr-DE. Hashimoto et al.[17] also reported a reduction in bond strength values for OB after aging resin-dentin beams in water for one year. The self-etching ability of contemporary adhesives is commonly achieved by the incorporation of polymerizable, methacrylate-based resin monomers that contain carboxylic/phosphoric acid moieties or their esters.[5,29] The presence of water is also an essential component, in order to enable ionization of acidic monomers and demineralization of underlying enamel and/or dentin.[5] Besides the presence of water in their composition, the ionizable moieties of acidic monomers are hydrophilic. Thus, increased water sorption might be expected for single-step self-etch adhesives such as OB.[30,31] An important feature of two-step self-etching systems that might contribute for the better results when compared to one-step systems, is the hydrophobic resin layer that is placed over the primer, which might reduce water sorption.[12]

Environmental water is known to be detrimental for the durability of the resin-dentin interface. Even if no water is present during polymerization, environmental moisture might enter the polymer bulk,[25,32] triggering the chemical polymer degradation.[18] Our study demonstrated that if there is no environmental water to challenge the interfaces, similar or increased µTBS is observed. The stability of mechanical properties of resin specimens aged in oil has been attributed to the absence of plasticizing effect of oil on the polymers.[33,34] The higher µTBS values observed after 1 year of immersion in oil (1Yr-NoE) may not be a result of improved bond strength in this medium, rather, the control values (24h-IE) are lower because the specimens may have suffered the plasticizing effects of water during the first 24 hours of immersion.[34,35] Even though the investigations of Carrilho et al.[34] and Paul et al.[35] were performed on resin specimens, these observations could be extrapolated to our bond strength data because dentin bond strengths exhibited by some adhesive systems have been correlated with the mechanical properties of the adhesive resins.[36]

Regions of incomplete resin infiltration or incomplete resin polymerization within hybrid layers promoted by etch-and-rinse systems might represent pathways for fluid penetration and have been described as nanoleakage.[37] Degradation of bonds has been attributed to fluid penetration through these pathways.[9,10] De Munck et al.[6] reported decreased bond strengths for SB after four years of direct exposure to water. Interestingly, the reduction in µTBS after direct water exposure was higher than that of its three-step precursor Scotchbond Multipurpose. The application of the adhesive in three steps (etchant, primer and adhesive) favors copolimerization of the primer, and the lower hydrophilicity of the cured resin might result in optimized hybridization and lower sensitivity to water degradation.

In summary, μTBS decreased for all adhesives tested after direct exposure to water for one year. The presence of a surrounding resin-enamel bond helped protect the resin-dentin interface against degradation for the etch-and-rinse adhesives and for the single-step self-etching adhesive. These observations lead us to reject our null hypotheses. Although the most reliable conclusions about the performance of dental bonding systems in the environment must be derived from long-term clinical trials, storage of bonded specimens in water may give valuable information about the degradation of these materials.

Acknowledgements

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REFERENCES

1. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982;16:265-273.

2. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000;79:1385-1391.

3. Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999;78:906-911.

4. Kanca J 3rd. Resin bonding to wet substrate. 1. Bonding to dentin. Quintessence Int 1992;23:39-41.

5. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater 2001;17:296-308.

6. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003;82:136-140.

7. 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;84:118-132.

8. Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:47247-6.

9. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. Resin-tooth adhesive interfaces after long-term function. Am J Dent 2001;14:211-215.

10. Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999;78:906-911.

11. Giannini M, Seixas CAM, Reis AF, Pimenta LAF. Six-month storage-time evaluation of one-bottle adhesive systems to dentin. J Esthet Rest Dent 2003;15:43-48.

12. Reis AF, Arrais CAG, Novaes PD, Carvalho RM, De Goes MF, Giannini M. Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives: nanoleakage expression. J Biomed Mater Res Part B: Appl Biomater 2004;71:90-98.

13. Tanaka J, Ishikawa K, Yatani H, Yamashita A, Suzuki K. Correlation of dentin bond durability with water absorption of bonding layer. Dent Mater J 1999;18:11-18.

14. Kitasako Y, Burrow MF, Nikaido T, Tagami J. The influence of storage solution on dentin bond durability of resin cement. Dent Mater 2000;16:1-6.

15. Burrow MF, Satoh M, Tagami J. Dentin bond durability after three years using a dentin bonding agent with and without priming. Dent Mater 1996;12:302-307.
16. Kitasako Y, Burrow MF, Katahira N, et al. Shear bond strengths of three resin cements to dentine over 3 years in vitro. J Dent 2001;29:139-144.
17. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. Degradation patterns of different adhesives and bonding procedures. J Biomed Mater Res Part: Appl Biomater 2003;66B:324-330.
18. Göpferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996;17:103-114.
19. Reis AF, Pereira PNR & Giannini M. Water-induced nanoleakage prevention by bonding strategies. (submitted)
20. Nakajima M, Okuda M, Ogata M, Pereira PN, Tagami J, Pashley DH. The durability of a fluoride-releasing resin adhesive system to dentin. Oper Dent 2003;28:186-192.
21. Donmez N, Belli S, Pashley DH, Tay FR. Ultrastructural correlates of in vivo/in vitro bond degradation in self-etch adhesives. J Dent Res 2005;84:355-359.
22. Tsuchiya S, Nikaido T, Sonoda H, Foxton RM, Tagami J. Ultrastructure of the dentin-adhesive interface after acid-base challenge. J Adhes Dent 2004;6:183-190.
23. Itota T, Torii Y, Nakabo S, Tashiro Y, Konishi N, Nagamine M, Yoshiyama M. Effect of fluoride-releasing adhesive system on decalcified dentin. J Oral Rehab 2003;30:178-183.
24. Pereira PN, Inokoshi S, Tagami J. In vitro secondary caries inhibition around fluoride releasing materials. J Dent 1998;26:505-510.
25. Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent 2002;4:255-269.
26. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, et al. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res 2005;84:183-188.

27. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002;30:371-382.

28. 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;83:459-464.

29. Hasegawa T, Manabe A, Itoh K, Wakumoto S. Investigation of self-etching dentin primers. Dent Mater 1989;5:408-410.

30. Burrow MF, Inokoshi S, Tagami J. Water sorption of several bonding resins. Am J Dent 1999;12:295-298.

31. Mortier E, Gerdolle DA, Jacquot B, Panighi MM. Importance of water sorption and solubility studies for couple bonding agent/resin-based filling material. Oper Dent 2004;29:669-676.

32. Mohsen NM, Craig RG, Filisko FE. The effects of moisture on the dieletric relaxation of urethane dimethacrylate polymer and composites. J Oral Rehab 2001;28:376-392.

33. Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. Biomaterials 2004;25:5789-5796.

34. Carrilho MRO, Tay FR, Pashley DH, Tjäderhane L, Carvalho RM. Mechanical stability of resin–dentin bond components. Dent Mater 2005;21:232-241.

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

36. Takahashi A, Sato Y, Uno S, Pereira PN, Sano H. Effects of mechanical properties of adhesives on bond strength to dentin. Dent Mater 2002;18:263-268.

37. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20:18-25.

Legends to Figures

Figure 1. Distribution of failure modes for all experimental groups.

Figure 2. SEM micrographs of the failure mode of a specimen restored with the single-step self-etching adhesive OB and stored in water for 24 hours. A globular structure was observed at the fractured surface, and might be related to the low μTBS values recorded for this system over the experiment. (Magnification: A - 210X, B – 5,000X).

Figure 3. SEM micrographs of the interfacial failure of a specimen restored with the two-step self-etching primer CF and stored in water for 1 year with direct water exposure (1Yr-DE). A porous resin layer was observed at the fractured surface, and might be due to resin elution after water immersion for 1 year. (Magnification: A - 202X, B – 2,000X).

Figure 4. SEM micrographs of the interfacial failure of a specimen restored with the etch-and-rinse adhesive PB after (A) 24 hours of indirect exposure to water (dentin side) and (B) 1 year of indirect exposure to water (resin side). It seems that resin elution occurred during the water storage period, because unprotected collagen fibrils can be observed after water exposure for 1 year. (Magnification A,B- 10,000X).

Table 1. Materials, Brand (Lot #), composition, application technique and manufacturers of adhesive systems used in this study: (MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; MDPB, 12-methacryloyloxydodecylpyridinium bromide; MMA, methyl methacrylate; MAC-10, methacryloyloxydecamethlene malonic acid; PENTA, dipentaerythritol pentacrylate phosphoric acid ester; UDMA, urethane dimethacrylate; Bis-GMA, bisphenol-A diglycidil ether dimethacrylate.)

Material	Brand (Lot #)	Composition	Application Technique	Manufacturer
Two-step Self-etching primers	Clearfil Protect Bond (primer: 00002A bond: 00004A)	*Primer*: MDP, HEMA, MDPB, dimethacrilates, photoinitiator, water *Adhesive*: MDP, HEMA, dimethacrylates, photoinitiator, NaF, silanated colloidal silica	f (20 s); e; g; i	Kuraray Medical, Kurashiki, Japan
	Clearfil SE Bond (primer:00400A bond:00541A)	*Primer*: Water, ethanol, MDP, HEMA, dimethacrylate hydrophilic, canphorquinone, N,N-diethanol p-toluidine *Adhesive*: MDP, Bis-GMA, HEMA, dimethacrylate hydrophobic, canphorquinone, N,N-diethanol p-toluidine, silanated colloidal silica	f (20 s); e; g; i	Kuraray Medical, Kurashiki, Japan
One-step Self-etching adhesive	One-up Bond F (Bonding A: 084 Bonding B: 578)	Water, MMA, HEMA, coumarin dye, metacryloyloxyalkyl acid phosphate, MAC-10, multifuntional methacrilic monomer, fluoraluminosilicate glass, photoinitiator (aryl borate catalyst)	h (leave undisturbed 20 s); i	Tokuyama Corp., Tokyo, Japan
Two-step total-etch adhesives	Prime&Bond NT (030822)	*Etchant*: 35% H3PO4 *Adhesive*: PENTA, UDMA, resin R5-62-1, resin T, resin D, silica nanoparticles, photoinitiators, cetilamine hidrofluoride and acetone	a (15s); b (15s); c; d; e; i	Dentsply Caulk, Milford, DE, USA
	Single Bond (3JL)	*Etchant*: 35% H3PO4 *Adhesive*: water, ethanol, Bis-GMA, HEMA, UDMA, Bisphenol A glycerolate, polyalkenoic acid copolymer, dimethacrylate, canphorquinone	a (15s); b (15s); c; d; e; i	3M ESPE, St Paul, MN, USA

Application technique – a: acid etch; b: rinse surface; c: dry with absorbent paper; d: apply one-bottle adhesive; e: gently air dry; f: apply primer; g: apply adhesive; h: apply mixture; i: light cure.

Table 2. Mean values (MPa) and standard deviation of the microtensile bond strength to dentin of the different adhesive resins tested after different storage conditions. 24h-IE - 24 h of indirect water exposure, 1Yr-IE - 1 year of indirect water exposure, 1Yr-DE - 1 year of direct water exposure, 1Yr-NoE - 1 year of no water exposure.

	24h-IE		1Yr-IE		1Yr-DE		1Yr-NoE	
One-up Bond F	28.1 (7.8)	Db	28.6 (6.3)	Bb	19.9 (4.5)	Dc	34.3 (8.4)	Ca
Clearfil SE Bond	57.6 (7.4)	Aab	33.7 (10.9)	Bc	31.4 (7.8)	Bc	47.9 (11.3)	ABb
C. Protect Bond	50.8 (7.5)	ABa	41.3 (8.7)	Ab	39.8 (9.5)	Ab	44.7 (7.3)	Bab
Single Bond	45.7 (10.2)	BCb	40.4 (9.8)	Ab	33.7 (7.9)	ABc	53.0 (12.0)	Aa
Prime&Bond NT	43.0 (9.3)	Ca	33.7 (7.9)	Bb	25.3 (8.0)	Cc	41.1 (8.9)	Ba

Means followed by different letters (upper case – column, lower case – row) differ among them by Duncan test at the 0.05 confidence level.

Figure 1.

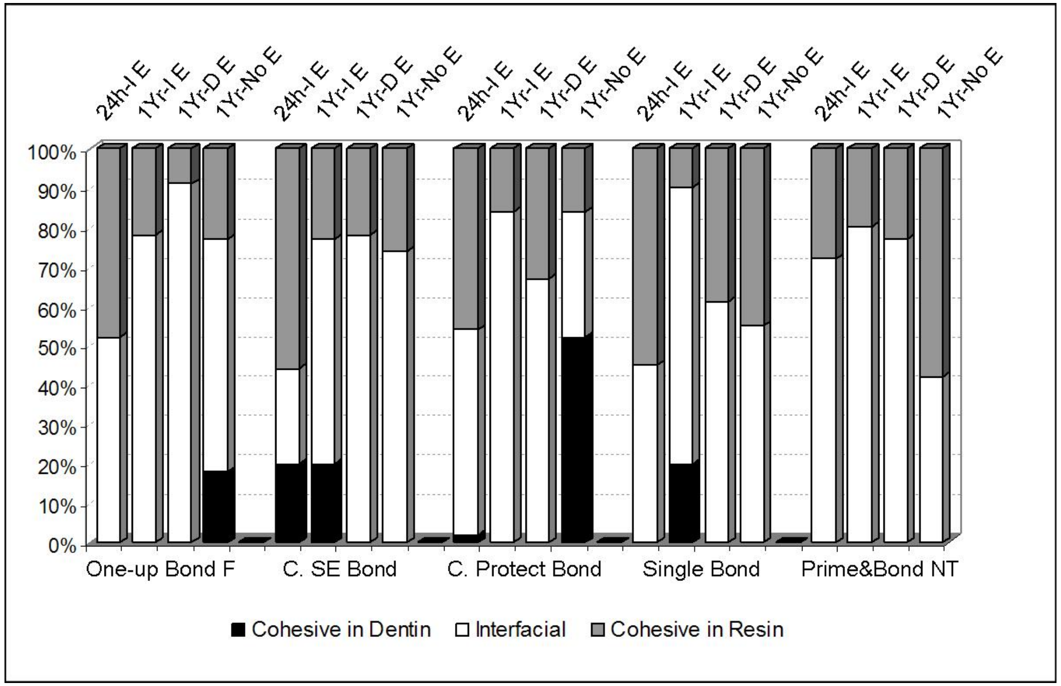


Figure 2.

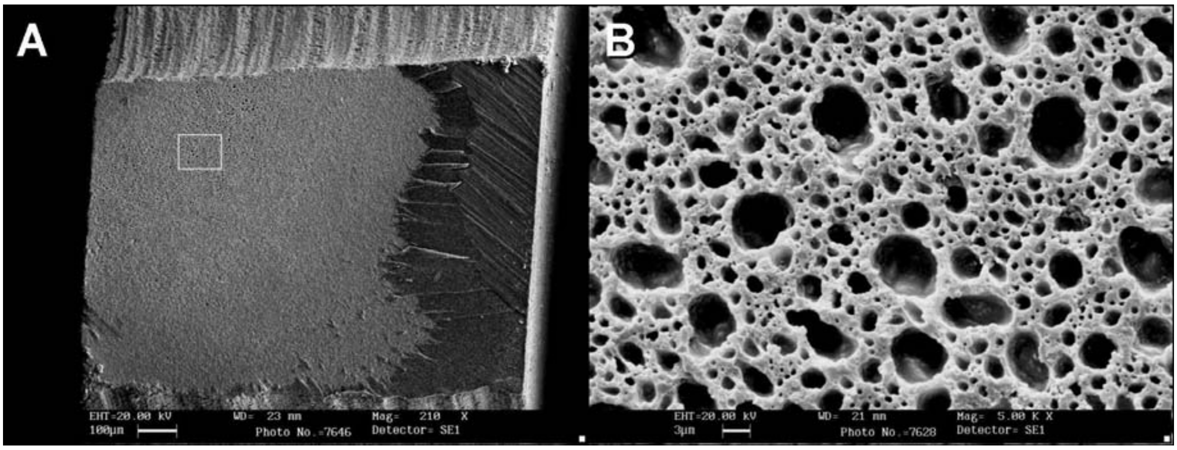


Figure 3.

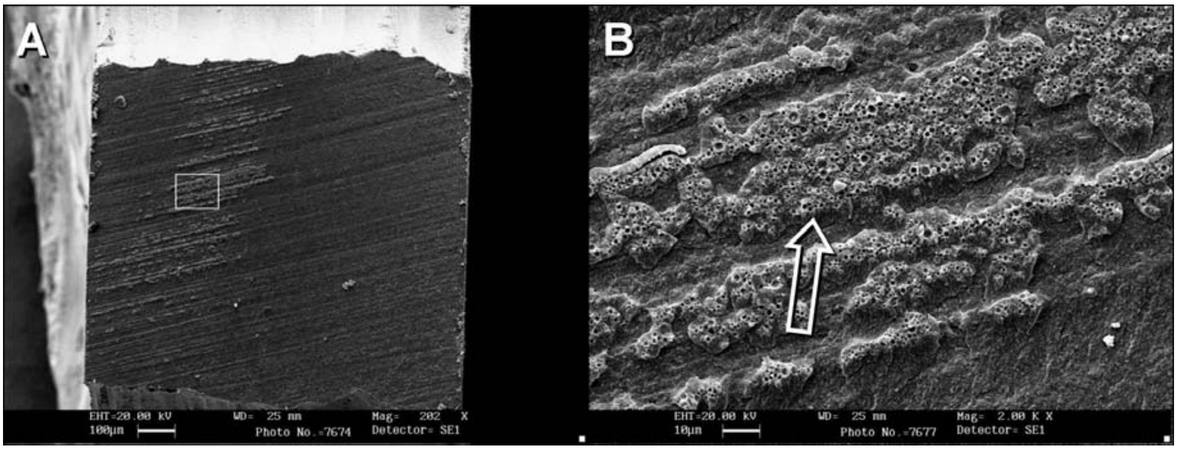
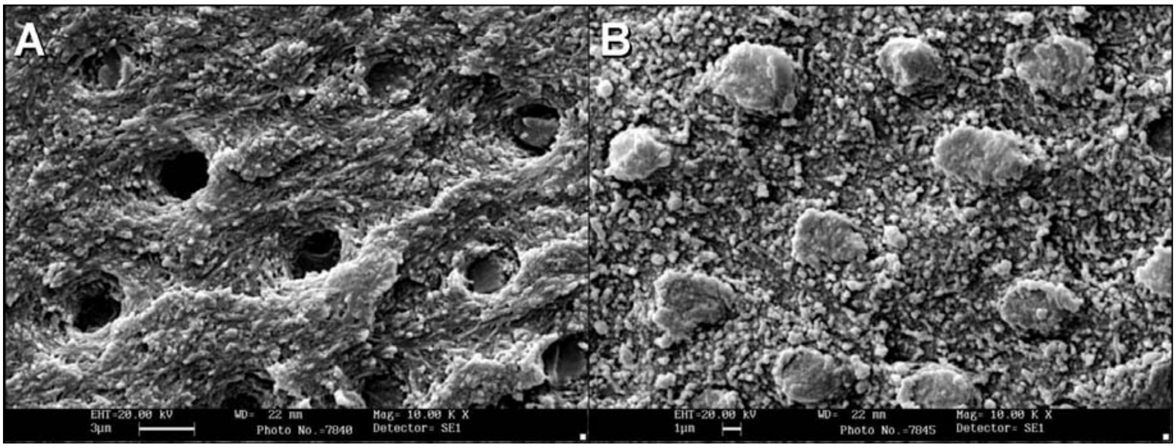


Figure 4.



4. DISCUSSÃO GERAL

O principal objetivo da odontologia restauradora adesiva é a promoção de um selamento marginal durável e efetivo dos tecidos dentais. Diversos estudos foram, têm sido e serão realizados com o intuito de se desvendar os mecanismos da união aos substratos dentais, e de desenvolver técnicas e biomateriais capazes de mimetizar e substituir de forma duradoura a estrutura dental perdida. Apesar da utilização de dentes humanos ser preferida nos estudos de adesão, este fato inviabilizaria a realização de diversas pesquisas, devido à dificuldade de obtenção de dentes hígidos de origem humana (NAKAMICHI et al., 1983; RETIEF et al., 1990). O primeiro capítulo deste trabalho demonstrou através do ensaio de microtração e da análise micromorfológica, que os tecidos dentais de origem bovina são melhores substitutos para os dentes humanos quando comparados aos dentes de origem suína.

Apesar de ser grande o número de investigações sobre o tema, os mecanismos de degradação da interface resina-dentina ainda não foram totalmente elucidados. A degradação da interface de união resina-dentina tem sido atribuída à degradação hidrolítica da resina adesiva e/ou das fibrilas colágenas (HASHIMOTO et al., 2000; SANO et al., 1999; GIANNINI et al., 2003). A adesão a um substrato vital e úmido como a dentina tem se mostrado deficiente (MARSHALL et al., 1997; CARVALHO et al., 2004). Para que se consiga uma união ao substrato dentinário com resinas adesivas, é preciso que a fase mineral seja totalmente ou parcialmente removida e substituída pela solução do adesivo. O agente de união precisa infiltrar esta camada rica em fibrilas colágenas e polimerizar *in situ*, formando o que se denomina camada híbrida (NAKABAYASHI et al., 1982). A técnica que emprega o condicionamento com ácido fosfórico para remoção da *smear layer* e desmineralização da dentina subjacente tem sido considerada crítica (PEREIRA et al., 1999; SPENCER et al., 1999; SPENCER et al., 2000; HASHIMOTO et al., 2002), pois uma infiltração incompleta pode ocorrer se a dentina for demasiadamente seca, ou o "*overwet phenomenon*" pode ocorrer na presença de umidade excessiva (KANCA, 1992; TAY et al., 1996). Como observado no Capítulo 2, a presença dos solventes orgânicos como o etanol e a acetona mostrou-se de fundamental importância para que ocorra a remoção da água existente na região de dentina desmineralizada e subsituição pela resina adesiva. Por outro lado, a união ao esmalte mostrou-se menos sensível a presença destes solventes. Apesar deste papel crucial na infiltração dos monômeros na rede de colágeno exposta pelo condicionamento ácido (Fig. 1, Capítulo 1), a evaporação incompleta destes solventes e da água pode ocorrer. A presença de água e solventes residuais pode interferir na polimerização dos adesivos, diminuindo a qualidade da união e permitindo a ocorrência de porosidades nanométricas na camada híbrida (PASHLEY et al., 1998).

Neste trabalho de tese, pode-se observar a ocorrência da nanoinfiltração nas interfaces resina/dentina produzidas por todos os sistemas adesivos testados. A manifestação dos padrões de nanoinfiltração variou consideravelmente, dependendo principalmente da composição, do modo de aplicação dos materiais utilizados e do tempo de armazenamento dos espécimes. A utilização do nitrato de prata amoniacal permite a visualização não somente de regiões onde existem espaços não infiltrados pelo adesivo, mas também de grupos polares hidrófilos que permitem a formação de pontes de hidrogênio com moléculas de água (SANO et al., 1995a; TAY et al., 2002a). Inicialmente, o conceito de nanoinfiltração foi atribuído às regiões da camada híbrida que não haviam sido completamente infiltradas pela resina adesiva. Desta forma, não era de se esperar que a nanoinfiltração fosse observada em interfaces de união produzidas por sistemas adesivos auto-condicionantes que desmineralizam e infiltram a dentina simultaneamente. No entanto, foi provado que este conceito não se aplica à realidade (TAY et al., 2002c; PEREIRA et al., 2001; OKUDA et al., 2002; CARVALHO et al., 2005). Assim, nossos resultados também demostraram a deposição de prata nas interfaces produzidas tanto pela técnica do condicionamento ácido prévio quanto na técnica auto-condicionante.

As diferenças na hidrofilia e no conteúdo de água dos adesivos influenciam diretamente os padrões de nanoinfiltração observados. A presença de água tem um papel importante tanto na técnica que utiliza o condicionamento ácido prévio quanto na técnica auto-condicionante (PASHLEY et al., 2002). A água é um componente essencial nos sistemas auto-condicionantes, pois ela viabiliza a ionização dos monômeros ácidos, que desmineralizam o esmalte e/ou dentina subjacente (TAY & PASHLEY, 2001). Além da presença de água na sua composição, os grupos ionizáveis dos monômeros ácidos são hidrófilos. Portanto, diferentes taxas de sorção de água podem ser esperadas para as interfaces de união produzidas pelos adesivos testados neste trabalho (BURROW et al., 1999; MORTIER et al., 2004). Os resultados do Capítulo 6 vêm confirmar estas observações. Foi observada uma tendência para o aumento tanto da sorção de água, quanto da solubilidade dos materiais com o aumento no tempo de armazenagem.

A difusão de moléculas de água para o interior de matrizes poliméricas é controlada pela Lei de Fick (YIU et al., 2004; TAY et al., 2003a). Os padrões de absorção de água são governados por duas teorias que ocorrem simultaneamente: a “teoria do volume livre”, na qual moléculas de água se difundem através de porosidades nanométricas ou defeitos morfológicos do material sem relação mútua com os grupos polares do material; e a “teoria da interação”, na qual moléculas se difundem através do material se ligando sucessivamente aos grupamentos hidrófilos (BELLENGER et al., 1989). Existem diversos fatores envolvidos na sorção de água e degradação dos polímeros, como: o pH do meio de armazenagem (ÖRTENGREN et al., 2001; PRAKKI et al., 2005); grau de conversão (PEARSON et al., 1989); polaridade da estrutura molecular e presença de grupos hidroxila capazes de formar pontes de hidrogênio com a água; quantidade de ligações cruzadas (BEATTY et al., 1993); presença de água residual; presença e tipo de partículas de carga (MORTIER et al., 2004; TAY et al., 2004; MARCOVICH et al., 1999). Após penetrar na matriz polimérica, a água desencadeia o processo de degradação química, resultando na formação de oligômeros e monômeros. A composição dos materiais estudados é inerentemente susceptível à hidrólise (GÖPFERICH, 1996).

As taxas de sorção de água e solubilidade apresentadas pelos sistemas adesivos após a sua polimerização são importantes na determinação indireta da longevidade e qualidade marginal da restauração (BURROW et al., 1999). Sabe-se que a umidade presente no meio oral ou de armazenagem tem um papel importante no processo de degradação química dos polímeros, apresentando um efeito deletério para a interface resina-dentina (GÖPFERICH, 1996). Mesmo se não houver água presente no interior da interface durante a polimerização, a água pode se difundir para o interior do polímero (MOHSEN et al., 2001; TAY et al., 2002c), desencadeando a degradação hidrolítica da cadeia polimérica (GÖPFERICH, 1996). Em nossos estudos (Capítulo 4, 7 e 8), verificamos que se não há água para desafiar as interfaces ou os sistemas de união, a ocorrência da degradação é praticamente inexistente. Estes resultados corroboram com outros estudos que demonstram uma degradação da interface de união na presença de água. Em contrapartida, na ausência de água, as propriedades das interfaces ou espécimes de resina permanecem inalteradas ou aumentam após armazenagem (TAY et al., 2003a; YIU et al., 2004; CARRILHO et al., 2005).

Verificou-se também que a armazenagem dos espécimes restaurados em água afetou a estabilidade da união resina-dentina em diferentes níveis para todos os grupos testados. Os adesivos auto-condicionantes de dois passos Clearfil Protect Bond (Capítulo 4) e Unifil Bond (Capítulo 3) apresentaram uma certa estabilidade da interface quanto à nanoinfiltração após armazenagem em água. A incorporação de um monômero antibacteriano no *primer* e de partículas de Fluoreto de Sódio na solução adesiva do adesivo Clearfil Protect Bond mostrou resultados promissores em relação à estabildade da união ao longo do tempo tanto *in vitro* quanto *in vivo* (NAKAJIMA *et al.*, 2003; DONMEZ *et al.*, 2005). A liberação de Flúor pelo Clearfil Protect Bond foi confirmada por imagens de Microscopia Eletrônica de Transmissão (Fig. 4, Capítulo 7), pois espaços vazios foram observados próximo à superfície do material, e representam espaços que se formaram devido à ionização e solubilização dos cristais de Fluoreto de Sódio (TAY et al., 2002c). Esta liberação de fluoretos pode ser importante na prevenção e controle de cáries secundárias ao redor de restaurações (TSUCHIYA et al., 2004; ITOTA et al., 2003; PEREIRA et al., 1998).

Ficou claro que os adesivos auto-condicionantes de passo único apresentam susceptibilidade maior à nanoinfiltração, sendo também menos resistentes à degradação hidrolítica. Dentre os adesivos auto-condicionantes de passo único, o adesivo Clearfil Tri-S Bond (Capítulo 5) foi o que apresentou menor nanoinfiltração. Todos os outros adesivos auto-condicionantes de passo único apresentaram altos valores de nanoinfiltração, alguns inicialmente, outros após armazenagem em água. A nanoinfiltração observada nas interfaces produzidas pelos adesivos auto-condicionantes não ocorre necessariamente pela infiltração incompleta na região de dentina desmineralizada. Este fenômeno ocorre devido à presença de regiões dentro da matriz resinosa polimerizada onde a água não é totalmente removida, resultando em regiões de polimerização incompleta e/ou formação de hidrogéis. A deposição de prata na camada adesiva também pode ocorrer devido à presença de monômeros ácidos que são mais susceptíveis à absorção de água (TAY et al., 2002a; TAY et al., 2002c). Somando-se a estes fatos, a separação de fases dos componentes da solução adesiva pode contribuir para a menor qualidade da adesão verificada para alguns adesivos auto-condicionantes de passo único (VAN LANDUYT et al., 2005).

A capacidade dos sistemas adesivos de passo único de selar as superfícies dentinárias efetivamente tem sido questionada, pois eles funcionam como membranas permeáveis, permitindo a movimentação de água entre a interface e a dentina subjacente (TAY et al., 2002b; CHERSONI et al., 2004). O adesivo auto-condicionante de passo único One-up Bond F apresentou os valores mais altos de sorção de água e solubilidade (Capítulo 6). Observou-se também uma intensa deposição de prata não só na interface de união (Capítulos 3 e 4), mas também nos espécimes de adesivo (Capítulo 7). Apesar de não ter sido verificado um decréscimo nas propriedades mecânicas deste adesivo após 6 meses de armazenagem em água, os valores de resistência de união à dentina foram baixos, e diminuiram após um ano de armazenagem com exposição direta à agua

(Capítulo 8). A Figura 4 (Capítulo 8) demonstra o plano de fratura de um espécime restaurado com o adesivo One-up Bond F, após 24 horas de armazenagem em água. Uma estrutura globular foi observada, e pode ser correlacionada aos aspectos de nanoinfiltração na interface resina-dentina observados nos Capítulos 3 e 4, mostrando uma deficiência na produção de uma selamento efetivo da estrutura dental. Observando os resultados obtidos com os adesivos auto-condicionantes de passo único, pode-se notar que ao se aumentar a acidez da solução do adesivo, o conteúdo dos monômeros ácidos e de água são aumentados, o que resulta em maior hidrofilia, conduzindo à maior taxa de sorção de água, e consequentemente, menor estabilidade hidrolítica (TAY & PASHLEY 2001; TAY et al., 2002a; TAY et al., 2003c; YIU et al., 2004). Os resultados apresentados nos Capítulos 3 e 4 salientam que uma característica importante dos adesivos auto-condicionantes de dois passos que pode contribuir para a melhor performance destes sistemas quando comparados aos sistemas de passo único, é a camada de resina hidrófoba que é aplicada sobre o *primer*, e pode reduzir a sorção de água.

A composição da solução adesiva bem como a presença de partículas de carga podem ter participação importante na eficiência da união (TAY et al., 2004). Cada adesivo auto-condicionante (tanto os de dois passos quanto os de passo único) apresentam em sua composição um monômero funcional específico, que exerce um papel fundamental na performance do adesivo (YOSHIDA et al., 2004). Acredita-se que a fórmula molecular específica do monômero funcional e a taxa de dissolução do sal de cálcio formado influenciam a eficiência e a estabilidade da união. O potencial de interagir químicamente com a hidroxiapatita presente na interface pode ser um auxílio importante na obtenção de uma união duradoura. Esta interação ocorre somente com adesivos auto-condicionantes mais brandos, que desmineralizam a superfície dentinária parcialmente. Foi relatado que o 10-MDP, monômero funcional dos adesivos auto-condicionantes de dois passos Clearfil SE Bond (Capítulos 3, 4, 6, 7 e 8), Clearfil Protect Bond (Capítulo 4, 6, 7 e 8) e do adesivo auto-condicionante de passo único Clearfil Tri-S Bond (Capítulo 5), pode interagir quimicamente com a hidroxiapatita formando um sal bastante estável. Por outro lado, o potencial de união química do 4-MET, monômero funcional do adesivo auto-condicionante de dois passos Unifil Bond (Capítulo 3) e do adesivo de passo único i-Bond (capítulo 5), foi descrito como substancialmente menor (YOSHIDA et al., 2004).

Uma redução no número de passos também ocorreu para os adesivos que utilizam o condicionamento ácido prévio. No entanto, foi demonstrado que os adesivos de dois passos estão mais susceptíveis à degradação que os sistemas de três passos (DE MUNCK et al., 2003). A aplicação do adesivo em três passos (ácido, *primer* e adesivo) favorece a copolimerização do *primer*, e a menor hidrofilia da resina polimerizada pode resultar em uma melhor hibridização e menor susceptibilidade à degradação hidrolítica. A deposição de prata nas camadas híbridas produzidas pelos adesivos que utilizam o condicionamento ácido prévio podem ser atribuídas às regiões onde a infiltração e/ou polimerização foram incompletas, representando possíveis caminhos para a penetração de fluidos. A degradação da união tem sido atribuída a penetração de fluidos por estes caminhos (HASHIMOTO et al., 2001). O adesivo Prime&Bond NT apresentou menor susceptibilidade à nanoinfiltração que o Single Bond. No entanto, quando se avaliou a resistência de união à dentina após um ano de armazenagem em água, observou-se uma redução significativa nos valores de união, que se acentuaram ainda mais na ausência da margem de esmalte ao redor da restauração (Capítulo 8). Esta margem de esmalte ao redor da restauração pode ajudar a proteger a interface resina-dentina contra a degradação para alguns adesivos (DE MUNCK et al., 2003). Segundo TAY et al. (2003a), em algumas situações, as regiões não infiltradas da camada híbrida podem não corresponder às regiões de degradação hidrolítica, o que justificaria a não correlação entre nanoinfiltração e resistência de união ao longo do tempo (OKUDA et al., 2001; OKUDA et al., 2002). Apesar dos dois adesivos terem se mostrado susceptíveis à degradação após armazenagem em água por 6 meses, as propriedades mecânicas do Prime&Bond NT também se mostraram inferiores ao

Single Bond (Capítulo 7). Um fato que pode vir a apoiar estas observações foi a maior solubilidade em água observada para o Prime&Bond NT quando comparado ao Single Bond (Capítulo 6). A Figura 4 (Capítulo 8) compara os padrões de fratura observados inicialmente e após 1 ano de exposição indireta à água para o adesivo Prime&Bond NT. Verifica-se uma camada híbrida mais porosa, mostrando que provavelmente ocorreu uma lixiviação dos produtos de degradação nesta interface.

DE MUNCK et al. (2005), após realizarem uma extensa revisão da literatura a respeito da durabilidade da união produzida por diferentes técnicas de desmineralização e infiltração no substrato dental, concluíram que a técnica mais eficiente para se produzir uma união duradoura aos tecido dentais ainda é a técnica de três passos que utiliza o condicionamento ácido prévio. Assim, qualquer simplificação nos procedimentos adesivos resultaria em uma redução na eficiência da união. Segundo os autores, a única técnica que se aproxima deste padrão é a técnica auto-condicionante de dois passos.

As interfaces de união resina-dentina se degradam em três estágios. Primeiro, a água é absorvida pelo polímero, desencadeando a degradação química (GÖFPERICH, 1996). Segundo, produtos de degradação, monômeros não reagidos e oligômeros são removidos da camada híbrida e da camada de adesivo (HASHIMOTO et al., 2003a; HASHIMOTO et al., 2003b). Terceiro, as fibrilas de colágeno expostas podem ser degradadas por metaloproteinases (MMPs) presentes na dentina ou saliva (PASHLEY et al., 2004). A degradação dos materiais poliméricos sintéticos está relacionada especificamente à arquitetura da corrente polimérica (STRIEGEL 2003), à estrutura química, ao peso molecular, à presença de microorganismos e condições ambientais (GU, 2003; YOURTEE et al., 2001).

Um entendimento físico-químico dos processos de degradação e erosão dos polímeros, assim como da degradação do colágeno do tipo I é a chave para a melhor compreensão dos problemas relacionados à longevidade das restaurações adesivas, e provavelmente, para a sua solução. Um outro aspecto importante que não pode ser negligenciado são os potenciais efeitos que os produtos de degradação liberados no meio oral podem ter sobre as funções fisiológicas humanas (YOURTEE et al., 2001; SANTERRE et al., 2001). O esforço combinado da biologia molecular e química de polímeros poderia certamente contribuir bastante na promoção de um selamento durável dos tecidos dentais (TAY & PASHLEY, 2002).

5. CONCLUSÕES GERAIS

Com base nos resultados obtidos e dentro dos limites dos presentes experimentos, conclui-se que:

- Os dentes bovinos são melhores substitutos para os dentes humanos nos testes de união ao esmalte e dentina do que os dentes de origem suína;
- A presença de solventes orgânicos não influencia na resistência de união ao esmalte; no entanto, é de fundamental importância na união ao substrato dentinário;
- Nenhum dos adesivos testados foi capaz de prevenir totalmente a nanoinfiltração;
- Sistemas de união que apresentam diferentes modos de aplicação e composições apresentam padrões de nanoinfiltração distintos;
- A armazenagem em água por um período prolongado de tempo influencia negativamente os padrões de nanoinfiltração, aumentando a deposição de prata;
- O sistema adesivo auto-condicionante One-up Bond F e as misturas de primer/adesivo apresentaram altas taxas de sorção de água e solubilidade;
- Verificou-se um aumento na sorção de água e solubilidade para todos os materiais testados ao longo do tempo;
- A armazenagem em água influenciou as propriedades mecânicas dos adesivos testados;
- A presença de uma margem de esmalte ao redor da restauração pode ajudar a proteger a restauração contra a degradação para alguns sistemas adesivos.

6. REFERÊNCIAS BIBLIOGRÁFICAS

Beatty MW, Swartz ML, Moore BK, Phillips RW, Roberts TA. Effect of crosslinking agent content, monomer functionality, and repeat unit chemistry on properties of unfilled resins. J Biomed Mater Res 1993; 27:403-13.

Burrow MF, Inokoshi S, Tagami J. Water sorption of several bonding resins. Am J Dent 1999;12(6):295-8.

Carrilho MRO, Tay FR, Pashley DH, Tjäderhane L, Carvalho RM. Mechanical stability of resin–dentin bond components. Dent Mater 2005;21:232-41.

Carvalho RM, Tay FR, Giannini M, Pashley DH. Effects of pre- and post-bonding hydration on bond strength to dentin. J Adhes Dent 2004; 6:13-7.

Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, Tay FR. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomaterials 2005;26:1035-1042.

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;83:459-64.

De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four-year water degradation of total-etch adhesives bonded to dentin.J Dent Res 2003;82:136-40.

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;84:118-32.

Donmez N, Belli S, Pashley DH, Tay FR. Ultrastructural correlates of in vivo/in vitro bond degradation in self-etch adhesives. J Dent Res 2005;84:355-9.

Giannini M, Seixas CAM, Reis AF, Pimenta LAF. Six-month storage-time evaluation of one-bottle adhesive systems to dentin. J Esthtet Restor Dent 2003;15:38-43.

Göpferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996; 17:103-14.

Gu J-D. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. International Biodeterioration & Biodegradation 2003; 52:69-91.

Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000;79:1385-91.

Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. Resin-tooth adhesive interfaces after long-term function. Am J Dent 2001;14:211-5.

Hashimoto M, Ohno H, Kaga M, Sano H, Tay FR, Oguchi H, Araki Y, Kubota M. Over-etching effects on micro-tensile bond strength and failure patterns for two dentin bonding systems. J Dent 2002;30:99-105.

Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. Degradation patterns of different adhesives and bonding procedures. J Biomed Mater Res Part B: Appl Biomater 2003a; 66B:324-30.

Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. Biomaterials 2003b;24:3795-803.

Imazato S, Kinomoto Y, Tarumi H, Torii M, Russell RRB, McCabe JF. Incorporation of antibacterial monomer MDPB into dentin primer. J Dent Res 1997;76:768-72.

Itota T, Torii Y, Nakabo S, Tashiro Y, Konishi N, Nagamine M, Yoshiyama M. Effect of fluoride-releasing adhesive system on decalcified dentin. J Oral Rehab 2003; 30:178-83.

Kanca J. Resin Bonding to wet substrate. I. Bonding to dentin. Quintessence Int 1992;23:39-41.

Li H, Burrow MF, Tyas MJ. Nanoleakage patterns of four dentin bonding systems. Dent Mater 2000; 16:48-56.

Marcovich NE, Reboredo MM, Aranguren MI. Moisture diffusion in polyester-woodflour composites. Polymer 1999; 40:7313-20.

Marshall GW Jr, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent 1997; 25(6):441-58.

Mohsen NM, Craig RG, Filisko FE. The effects of moisture on the dieletric relaxation of urethane dimethacrylate polymer and composites. J Oral Rehab 2001; 28:376-92.

Mortier E, Gerdolle DA, Jacquot B, Panighi MM. Importance of water sorption and solubility studies for couple bonding agent/resin-based filling material. Oper Dent 2004; 29:669-76.

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

Nakajima M, Okuda M, Ogata M, Pereira PN, Tagami J, Pashley DH. The durability of a fluoride-releasing resin adhesive system to dentin. Oper Dent 2003; 28:186-92.

Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible human teeth substitutes. J Dent Res 1983; 62:1076-1081.

Okuda M, Pereira PN, Nakajima M, Tagami J. Relationship between nanoleakage and long-term durability of dentin bonds. Oper Dent 2001; 26:482-90.

Okuda M, Pereira PNR, Nakajima M, Tagami J, Pashley DH. Long-term durability of resin dentin interface: nanoleakage vs microtensile bond strength. Oper Dent 2002; 27:289-96.

Örtengren U. On composite resin materials: Degradation, erosion and possible adverse effects in dentists. Swed Dent J Suppl 2000 ;(141):1-61.

Örtengren U, Andersson F, Elgh U, Terselius B, Karlsson S. Influence of pH and storage time on the sorption and solubility behaviour of three composite resin materials. J Dent 2001; 29:35-41.

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

Pashley DH, Zhang Y, Agee KA, Rouse CJ, Carvalho RM, Russell CM. Permeability of demineralized dentin to HEMA. Dent Mater 2000;16:7-14.

Pashley DH, Carvalho RM, Tay FR, Agee KA, Lee K-W. Solvation of dried matrix by water and other polar solvents. Am J Dent 2002; 15:97-102.

Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004; 83:216-21.

Pearson GJ, Longman CM. Water sorption and solubility of resin-based materials following inadequate polymerization by a visible-light curing system. J Oral Rehab 1989; 16:57-61.

Perdigão J, Lambrechts P, Van Meerbeek B, Tome AR, Vanherle G, Lopes AB. Morphological field emission-SEM study of the effect of six phosphoric acid etching agents on human dentin. Dent Mater 1996; 12:262-71.

Pereira PNR, Inokoshi S, Tagami J. In vitro secondary caries inhibition around fluoride releasing materials. J Dent 1998; 26:505-10.

Pereira PNR, Okuda M, Sano H, Yoshikawa T, Burrow MF, Tagami J. Effect of intrinsic wetness and regional difference on dentin bond strength. Dent Mater 1999; 15:46-53.

Pereira PNR, Okuda M, Nakajima M, Sano H, Tagami J, Pashley DH. Relationship between bond strengths and nanoleakage: evaluation of a new assessment method. Am J Dent 2001; 14:100-4.

Prakki A, Cilli R, Mondelli RF, Kalachandra S, Pereira JC. Influence of pH environment on polymer based dental material properties. J Dent 2005; 33:91-8.

Reis AF, Oliveira MT, Giannini M, De Goes MF, Rueggeberg FA. The effect of organic solvents on one-bottle adhesives' bond strength to enamel and dentin. Oper Dent. 2003; 28(6):700-6.

Retief DH, Mandras RS, Russell CM, Denys FR. Extracted human versus bovine teeth in laboratory studies. Am J Dent 1990; 3:253-258.

Saito A. Effect of fluoride in adhesion to dentin. Japan Soc Dent Mater Dev 1996; 15:78-88.

Sano H, Shono T, Takatsu T, Hosoda H.. Microporous dentin zone beneath resin-impregnated layer. Oper Dent. 1994 Mar-Apr; 19(2):59-64.

Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995a; 20:18-25.

Sano H, Yoshiyama M, Ebisu S, Burrow MF, Takatsu T, Ciucchi B, et al. Comparative SEM and TEM observations of nanoleakage within the hybrid layer. Oper Dent 1995b; 20:160-67.

Sano H, Yoshikawa T, Pereira PN, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999; 78:906-11.

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; 12:136-51.

Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int 1999; 30:501-7.

Spencer P, Wang Y, Walker MP, Wieliczka DM, Swafford JR. Interfacial chemistry of the dentin/adhesive bond. J Dent Res 2000; 79:1458-63.

Striegel AM. Influence of chain architecture on the mechanochemical degradation of macromolecules. J Biochem Biophys Methods 2003; 56:117-39.

Tanaka J, Ishikawa K, Yatani H, Yamashita A, Suzuki K.Correlation of dentin bond durability with water absorption of bonding layer. Dent Mater J 1999; 18:11-8.

Tay FR, Gwinnett AJ, Wei SH. The overwet phenomenon: an optical, micromorphological study of surface moisture in the acid-conditioned, resin-dentin interface. Am J Dent 1996; 9:43-8.

Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I. Depth of penetration beyond dentin smear layers. Dent Mater 2001; 17:296-308.

Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002a; 81:471-6.

Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002b; 30:371-382.

Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent 2002c; 4(4):255-69.

Tay FR, Pashley DH. Dental adhesives of the future. J Adhes Dent 2002;4(2):91-103.

Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SC, Yiu CK, Cheong C. Aging affects two modes of nanoleakage expression in bonded dentin. J Dent Res 2003a; 82:537-41.

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

Tay FR, Pashley DH. Dentine adhesives: have they become too hydrophilic. J Can Dent Assoc 2003c; 69:724-31.

Tay FR, Pashley DH, Yiu C, Cheong C, Hashimoto M, Itou K, et al. Nanoleakage types and potential implications: evidence from unfilled and filled adhesives with the same resin composition. Am J Dent 2004; 17:182-90.

Tsuchiya S, Nikaido T, Sonoda H, Foxton RM, Tagami J. Ultrastructure of the dentin-adhesive interface after acid-base challenge. J Adhes Dent 2004; 6:183-90.

Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, et al. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res 2005; 84:183-88.

Watanabe I, Nakabayashi N, Pashley DH. Bonding to ground dentin by a Phenyl-P self-etching primer. J Dent Res 1994; 73:1212-20.

Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. Biomaterials 2004; 25:5789-96.

Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res 2004; 83:454-8.

Yourtee DM, Smith RE, Russo, KA, Burmaster S, Cannon JM, Eick JD, Kostoryz EL. The stability of mathacrylate biomaterials when enzyme challenged: kinetic and systematic evaluations. J Biomed Mater Res 2001; 57:552-31.

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