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UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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“EFFECT OF PRE-ETCHING TO ENAMEL AND DENTIN ON BOND STRENGTH AND INTERFACIAL MORPHOLOGY OF DIFFERENT ADHESIVES SYSTEMS”

Orientadora: Profa. Dra. Mirela Sanae Shinohara
Co-Orientador: Prof. Dr. Mario Fernando de Goes

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Materiais Dentários da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas para obtenção do titulo de Mestra em Materiais Dentários.

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Mirela Sanae Shinohara

Profa. Dra. MIRELA SANAE SHINOHARA

Profa. Dra. PATRÍCIA NÓBREGA RODRIGUES PEREIRA

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“Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível.”

Charles Chaplin

RESUMO

Estudos mostram que a resistência de união (RU) alcançada pelos sistemas adesivos autocondicionantes em superfície de esmalte pré-condicionado é significativamente maior se comparada aos valores obtidos em esmalte não condicionado. A partir destas evidências, este estudo teve como objetivo avaliar a RU e a morfologia da interface adesiva (MI) em esmalte e dentina de um adesivo autocondicionante de passo único *multi-mode* e um adesivo autocondicionante de dois passos com e sem pré-condicionamento ácido da superfície comparados a dois sistemas adesivos de condicionamento total, de dois e três passos. Terceiros molares humanos recém-extraídos foram seccionados para obtenção de superfícies planas em esmalte e dentina, as quais foram desgastadas com lixa SiC #600 para padronização da *smear layer*. Estas amostras foram divididas aleatoriamente em 6 grupos: (SBU) Scotchbond Universal; (SBUcond) SBU pré-condicionado; (CSE) Clearfil SE Bond; (CSEcond) CSE pré-condicionado; (SBMP) Scotchbond Multi-Purpose; e (EX) Excite F. Os grupos de condicionamento total e os autocondicionantes a serem avaliados com pré-condicionamento receberam a aplicação do ácido fosfórico por 15s em dentina e 30s em esmalte. Todos os sistemas adesivos foram aplicados de acordo com as recomendações dos fabricantes. Em seguida, um bloco de resina composta (Filtek Supreme Plus) foi construído incrementalmente sobre cada superfície preparada para o teste de RU, e para a MI, as superfícies foram restauradas com resina *flow* (Filtek Z-350 Flowable Restorative) formando um "sanduíche", de esmalte ou dentina . Após 24h, para avaliação da RU, as amostras foram seccionadas em palitos com área transversal de 0,8mm^2 e submetidos ao teste de microtração com velocidade de 1,0mm/min. Os resultados foram analisados estatisticamente pelos testes *one-way* ANOVA e Fisher's PLSD (α=0.05) e os padrões de fratura analisados em Microscopia Eletrônica de Varredura (MEV). Para análise da MI, duas amostras de cada grupo foram incluídas em resina epóxica, polidas e observadas em MEV. Para esmalte, os resultados de RU mostraram que o SBUcond e CSEcond obtiveram os maiores valores, seguidos dos grupos SBMP, SBU e CSE que não apresentaram diferenças entre si. O grupo EX obteve os menores valores comparados aos outros grupos. Na análise da MI, os grupos SBUcond e CSEcond apresentaram nítidas extensões do adesivo penetrado no esmalte desmineralizado, semelhante aos sistemas de condicionamento total. Já para dentina, os resultados de RU mostraram que o condicionamento ácido prévio diminuiu significativamente os valores de RU dos sistemas autocondicionantes. Os maiores valores foram obtidos pelo SBU, CSE e SBMP, com valores de RU estatisticamente equivalentes entre si. Como em esmalte, o sistema adesivo EX obteve os menores valores. A análise em MEV da MI mostraram que o condicionamento prévio da dentina com adesivos autocondicionantes forma uma camada híbrida mais espessa com formação de *tags* resinosos, semelhantes aos sistemas adesivos de condicionamento total. Pode-se concluir que em esmalte, o condicionamento prévio aumentou significativamente os valores de RU para os sistemas autocondicionantes. Contudo, estes sistemas adesivos não apresentaram diferença estatística do adesivo convencional SBMP. E para dentina, o pré-condicionamento dos adesivos autocondicionantes diminuiu significativamente os valores de RU, entretanto, os valores de SBUcond não foram diferentes significativamente dos obtidos pelo sistema adesivo convencional SBMP.

Palavras-chave: Microscopia Eletrônica de Varredura; Resistência à Tração, Ataque Ácido Dentário

ABSTRACT

Studies show that the bond strength (BS) achieved by self-etching adhesive systems on pre-etched enamel surface is significantly higher compared with the values obtained on unetched enamel. From this evidence, this study aimed to assess the BS and morphology of the adhesive interface (AI) on enamel and dentin of a one-step self-etching adhesive multi-mode and a two-step self-etching adhesive with and without pre-etching of surface compared with two total-etching adhesive systems, two and three steps. Recently extracted human third molars were sectioned to obtain flat surfaces in enamel and dentin, which were ground with #600 SiC sandpaper to standardize the smear layer. These samples were randomly assigned into 6 groups: (SBU) Scotchbond Universal; (SBU-et) SBU pre-etched; (CSE), Clearfil SE Bond, (CSE-et) CSE pre-etched; (SBMP) Scotchbond Multi-Purpose, and (EX) Excite F. The groups total-etching and self-etching to be evaluated with pre-etching received the application of phosphoric acid for 15s in dentin and 30s in enamel. All adhesive systems were applied according to manufacturers' recommendations. Then, a block of composite resin (Filtek Supreme Plus) was constructed incrementally on each surface prepared for BS testing. For AI, the areas were filled with flowable resin (Filtek Flowable Restorative Z-350) forming a "sandwich" of enamel or dentin. After 24 hours, to review the BS, the specimens were sectioned into sticks with cross-sectional area of 0.8mm² and submitted to microtensile test at a speed of 1.0mm/min. The results were statistically analyzed by one-way ANOVA test and Fisher's PLSD (α=0.05) and fracture patterns were analyzed through Scanning Electron Microscopy (SEM). For analysis of AI, two samples from each group were embedded in epoxy resin, polished and observed through SEM. For enamel, the results of BS showed that SBU-et and CSE-et obtained the highest values, followed by SBMP groups, SBU and CSE with no differences among them. The EX group obtained the lowest values of all other groups. In the analysis of AI, and CSE-et and SBU-et groups showed sharp tag extensions of the adhesive penetrated in the demineralized enamel, similar to total-etching systems. As for dentin, BS results showed that acid etching significantly reduced the BS values of self-etching systems. The highest values were obtained by the SBU, CSE and SBMP with BS values statistically equivalent to each other. As in enamel, the adhesive system EX obtained the lowest values. SEM analysis showed that BS for pre-etched dentin with self-etching adhesive forms a thicker hybrid layer with resin tags formation, similar to total-etching adhesive systems. It can be concluded that on enamel, the pre-etching significantly increased BS for self-etching systems. However, these adhesive systems showed no statistical difference from conventional adhesive SBMP. And to dentin, pre-etching of sel-etching adhesives significantly reduced BS, however, SBU-et values were not significantly different from those obtained by the conventional adhesive system SBMP.

Keywords: Scanning Electron Microscopy; Tensile Strength; Acid Etching, Dental

SUMÁRIO

INTRODUÇÃO

Até os dias atuais um dos grandes desafios da Odontologia Restauradora é alcançar uma perfeita adesão dos materiais restauradores à estrutura dental. Com o desenvolvimento tecnológico e avanço das pesquisas científicas, a evolução dos materiais restauradores adesivos tem permitido alcançar sucesso com as restaurações estéticas. Porém, uma união efetiva e durável simultânea em esmalte e dentina continua sendo a meta dos pesquisadores.

O procedimento adesivo feito a partir da aplicação do ácido fosfórico tem sido considerado essencial para se alcançar sucesso na adesão em esmalte, desde que este conceito foi descrito por Buonocore (Buonocore, 1955). A partir do desenvolvimento da técnica do condicionamento ácido, alcançou-se maior perspectiva para o sucesso dos procedimentos restauradores. Entretanto, nem sempre as margens de uma restauração estão somente em esmalte, podendo haver envolvimento do substrato dentinário ou grande parte do preparo ser composto por dentina (Fusayama *et al*., 1979, Goracci *et al*., 1994). Porém, o condicionamento ácido realizado neste tecido não obteve o mesmo sucesso que no esmalte, devido à pouca afinidade do material restaurador com a dentina, pela necessidade de manter este substrato úmido, por sua diferente e complexa morfologia. Entretanto, o conceito de condicionamento ácido total foi proposto por Fusayama *et al*. em 1979 e aliado posteriormente à hibridização da dentina, descrita em 1982, por Nakabayashi *et al*. Nesta, mostrou-se a presença da interpenetração do adesivo em dentina desmineralizada por meio do condicionamento por ácido fosfórico, sendo denominada de “camada híbrida”, uma estrutura resultante da combinação da infiltração dos monômeros resinosos e das fibrilas colágenas expostas (Nabayashi *et al*., 1982, Pashley *et al*., 2011).

Em decorrência de sua evolução, os sistemas adesivos são disponíveis no mercado de acordo com o tratamento do substrato e o número de passos clínicos. Os adesivos de condicionamento total ou convencionais são classificados em 3 ou 2 passos. Desta forma, ácido fosfórico, *primer* e adesivo são aplicados separadamente ou a partir da união de *primer* e adesivo em um mesmo frasco. Entretanto, para eliminar a sensibilidade da técnica devido ao condicionamento ácido e reduzir o tempo clínico de aplicação, foram introduzidos os sistemas adesivos autocondicionantes (Watanabe *et al*., 1994). Diferente dos sistemas adesivos convencionais, estes sistemas não requerem um passo separado para o condicionamento ácido, pois contém em sua composição um monômero funcional, que em contato íntimo com o cálcio da hidroxiapatita do esmalte e/ou dentina, interage e condiciona o substrato simultaneamente (Van Meerbeek *et al*., 2011). Estes sistemas podem ser comercializados em dois passos (*primer* autocondicionante e adesivo) ou em apenas um passo clínico (*primer* autocondicionante e adesivo contidos em um frasco), chamados de sistemas *all-in-one* ou de passo único (Toledano *et al*., 2001, Cho *et al*., 2004, Perdigão *et al*., 2006, Perdigão *et al*., 2009),

Os adesivos autocondicionantes, embora apresentem pH ácido e não serem removidos do substrato dental após sua aplicação, provocam desmineralização limitada dos tecidos dentários. Diante disso, a efetividade desses materiais sobre o esmalte é menor, devido ao alto conteúdo de cálcio existente neste tecido. (Van Meerbeek *et al*., 2003, Kenshima *et al*., 2005, Perdigão *et al*., 2006, Salz *et al*., 2006),

Recentemente, foi introduzido no mercado, um sistema adesivo de passo único contendo em sua composição a combinação do copolímero *Vitrebond*™ e o monômero bifuncional 10-MDP (10-metacriloxidecil di-hidrogênio fosfato). Segundo o fabricante, este sistema adesivo pode ser utilizado na técnica de condicionamento total e na autocondicionante, considerado um sistema adesivo *multi-mode*. Desta forma, é necessária a avaliação da eficiência deste sistema de nova abordagem de aplicação, quanto às suas propriedades mecânicas com o substrato dental.

Este estudo foi separado em 2 capítulos, no formato alternativo, tendo como objetivo avaliar a resistência de união e a morfologia da interface adesiva em esmalte e dentina de um adesivo autocondicionante de passo único *multi-*

mode e um adesivo autocondicionante de dois passos com e sem pré-condicionamento ácido da superfície, comparados a dois sistemas adesivos de condicionamento total, de dois e três passos.

CAPÍTULO 1

Performance of a One-step Multi-mode Adhesive on Pre-etched Enamel on Bond Strength and Interfacial Morphology

Artigo a ser submetido à revista *Journal of Adhesive Dentistry*

ABSTRACT

Purpose: To compare bond strength (μTBS) and interfacial morphology of a one-step multi-mode adhesive and 2-step self-etching adhesive on pre-etched and non-etched enamel surface with two total-etching adhesives systems.

Materials and Methods: Thirty human third molars were sectioned to obtain two enamel fragments. For μTBS, forty eight enamel surfaces were ground using 600-grit SiC paper and randomly assigned into 6 groups (n=8): [SBU] Scotchbond Universal, 3M ESPE; [SBU-et] pre-etched SBU; [CSE] Clearfil SE Bond, Kuraray Dental; [CSE-et] pre-etched CSE; [SBMP] Scotchbond Multi-Purpose, 3M ESPE; and [EX] Excite F, Ivoclar Vivadent. The pre-etched specimens were conditioned with 37% phosphoric acid for 30s, each adhesive system was applied according to manufacturers' instructions, and composite resin blocks (Filtek Supreme Plus, 3M ESPE) were incrementally built up. Specimens were sectioned in $0.8mm^2$ beams and subjected to tension test. The data were analyzed with one-way ANOVA and Fisher's PLSD (α=0.05). For interface analysis, two samples of each group were embedded in epoxy resin, polished, and then observed through scanning electron microscopy (SEM).

Results: The μTBS values and the standard deviations were: CSE-et=34.2(9.0); SBU-et=33.6(9.3); SBMP=30.4(11.0); CSE=28.5(8.3); SBU=27.4(8.5); and EX=23.3(8.2). CSE-et and SBU-et presented highest bond strength values, followed by SBMP, CSE, and SBU, whose statistical difference was not significant. EX showed the lowest statistical bond strength values. SEM images of interface from pre-etched samples showed clear extensions of penetrated adhesive tags into demineralized enamel.

Conclusions: Pre-etched grounded enamel significantly increased bond strength for one multi-mode adhesive SBU and 2-step self-etching adhesive CSE with clear tags.

Keywords: self-etching adhesive, total-etching adhesive, phosphoric acid, interface, functional monomer, scanning electron microscopy.

INTRODUCTION

The technological advancement has brought improvements in performance of materials in bonding strength and sealing of composite resin restorations to dental substrate. Buonocore has established the concept of adhesion to enamel in 1955[2]. But, it has become a challenge in restorative dentistry to achieve a durable and stable adhesion of resin-based materials simultaneously to enamel and to dentin.

Currently, clinicians are increasingly using simplified adhesive systems with fewer application steps and less technique sensitivity. Self-etching adhesives consist mainly in a chemical interaction of acidic functional monomer to the calcium of hydroxyapatite. Depending on the acidic functional monomer[9,26,34,36] present in the adhesive system, the interface formed between adhesive and tooth substrate has been considered more resistant to biodegradation[8,9,34,36]. Previous studies have confirmed that 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) is the best acidic functional monomer showing stable and durable interaction with hydroxyapatite for both enamel and dentin[6,9,26,29]. However, in general, the demineralization capability of self-etching system is limited[14] and may compromise the adhesion to enamel[12].

As a known fact, self-etching adhesive systems have less acidity and aggressiveness compared with phosphoric acid[12,14]. Several investigations have showed controversial results for self-etching adhesive systems to enamel.

According to Tsujimoto *et al.*[22], the surface free energy of the solid and the surface tension of the liquid should affect the wetting of a solid, consequently interfering in bonding strength. Thus, not only the degree of enamel etching, but also the chemical activity and the mechanical properties of the adhesives should play important roles in determining bond strength. In their study, they found that the total surface free energy increased as the surface roughness decreased for Clearfil Tri-S Bond, a mild-self-etching adhesive that contains the acidic functional monomer 10-MDP in its composition. In other words, 10-MDP reacts with calcium ions from tooth substrate. If the smear layer becomes thinner, 10-MDP can react better with calcium from enamel. In contrary, for etched enamel with phosphoric acid, there were no differences in the surface free energy regardless of the surface roughness (180, 600, 2000-grit silicon carbide-paper). Therefore, for mild-self-etching adhesives the adhesion mechanism seems to be different. Since it depends more on the chemical interaction, less surface roughness (grit-2000) permits better ions binding of acidic functional monomer to calcium from grounded enamel[22]. These findings are in corroboration to the results of Mine *et al.*[12]. They found that the surface-preparation method significantly affected the nature of the smear layer and the interaction with ultra-mild-self etching adhesive.

Despite the important role of that chemical, several studies showed that the enamel bond strengths of self-etching adhesives were lower than total-etching adhesives[7,24,30]. Theoretically, the phosphoric acid creates more porosity on enamel[15] and increases the bonding area and the wettability of the substrate so resin would infiltrate better in etched enamel. Therefore, some authors have recommended the use of phosphoric acid associated with self-etching adhesive systems to ease enamel dissolution, to increase the bonding procedure, and consequently to improve the bond strength[3-5,7,10,11,16,21,23,24,30]. Peumans *et al.*[19] in a five-year clinical follow-up of Class V composite resin restorations using a mild-self-etching adhesive system showed that selective enamel etching with phosphoric acid resulted in an improved marginal adaptation. However, pre-etching enamel did not influence on the overall clinical performance of the restorations.

Recently, a one-step multi-mode adhesive system -Scotchbond Universal- has been introduced in the market combining the acidic functional monomer 10-MDP with ‘Vitrebond copolymer’. Both compounds have interaction with calcium from hydroxyapatite[32] and the latter is less affected by moisture contamination[13]. Considering the composition, in accordance with manufacturer, that one-step multi-mode adhesive can be used in self-etching mode, in total-etching technique or selective enamel etching mode.

Based on these considerations, the purpose of the present study was to compare bond strength and interfacial morphology of a one-step multi-mode adhesive and a 2-step self-etching adhesive on pre-etched and non-etched enamel surface with two total-etching adhesives systems. The null hypothesis tested was that the pre-etching of enamel with phosphoric acid does not influence the laboratory performance of self-etching adhesive systems.

MATERIALS AND METHODS

This study was reviewed and approved by the research Ethics Committee (Protocol #113/2011). Thirty caries-free extracted human third molars were collected, cleaned, and stored in 0.1% thymol solution under refrigeration before using in the experiment. Twenty-four teeth (n=8) were used for bond strength test and six teeth (n=4) for interface morphology by SEM.

Specimen Preparation for Microtensile Bond Strength

For each tooth, the root was removed (Fig1.A) and the crown was sectioned on occlusal-cervical direction to obtain two enamel surface fragments (Fig1.B) using a low-speed diamond saw (Extec Corp.; Enfield, CT, USA) mounted on a precision cut-off machine (Isomet 1000, Buehler; Lake Buff, IL, USA). The enamel surface from the fragments was polished using 600-grit SiC (silicon carbide) abrasive paper under water cooling for 60 seconds to produce a standardized smear layer (Fig1.C-a). After that, the samples were randomly assigned into 6 groups: [SBU] Scotchbond Universal Adhesive; [SBU-et] pre-etched SBU; [CSE] Clearfil SE Bond; [CSE-et] pre-etched CSE; [SBMP] Scotchbond Multi-Purpose; and [EX] Excite F. The compositions, manufacturers, and batch numbers of the adhesive systems are shown on Table 1.

The adhesive application was according to the bonding procedures shown on Table 2. Each adhesive system was applied following the manufacturers instructions, except for the groups SBU-et and CSE-et, that were pre-etched (Fig1.C-b) with 37% phosphoric acid gel (Condac 37% - FGM Produtos Odontológicos Ltda; Joinville, SC, Brazil – Batch n^{o}. 060911) for 30 seconds, rinsed with running water (Fig1.C-c), and gently air-dried (Fig1.C-g) prior application of the adhesive system (Fig1.C-e).

After the bonding procedure, a composite resin block (Filtek Supreme Plus 3M ESPE; St Paul, MN, USA) was incrementally built up until approximately 6mm thick (Fig1.C-g). Each increment was light activated for 20 seconds using the Elipar S10 LED Curing Light (3M ESPE; St Paul, MN, USA) with a power density of 800 mW/cm². The light output intensity periodically analyzed using a radiometer (Sybron Kerr, Orange, CA, USA) during the experiment. Then, the restored samples were stored in distilled water at 37^{o}C for 24 hours.

Microtensile Bond Strength Test

After 24 hours storage, the restored samples were longitudinally sectioned in both 'x' and 'y' directions (Fig1.D-b) across to the adhesive interface using a low-speed diamond saw in a precision cut-off machine to obtain beams of approximately 0.8mm^2 of bonding area (Fig1.D-c). The specimens were attached to a testing jig with cyanoacrylate adhesive (Super Bonder Loctite Henkel; São Paulo, SP, Brazil) and subjected to a tension force in a universal testing machine (EZ Test, Shimadzu; Kyoto, Japan) at a crosshead speed of 1mm/min (Fig1.D-d). The cross-sectional areas were calculated in order to obtain μTBS values in

Megapascal units (MPa). The data were statistically analyzed (StatView software) by one-way ANOVA and Fisher's PLSD (α=0.05).

The fracture modes from enamel sides were gold sputter coated and analyzed under SEM (JSM 5600LV, JEOL; Tokyo, Japan). Failure modes were categorized into: cohesive failure in enamel; mixed failure; adhesive failure in enamel or adhesive; and cohesive failure in resin composite.

Interfacial Morphology Analysis under Scanning Electron Microscopy

Six human third molars were prepared (Fig2.A) to obtain enamel-adhesive interfaces. The roots were removed and the crowns were sectioned on occlusal-cervical direction to obtain two enamel surface samples (Fig2.B) using a low-speed diamond saw mounted on a precision cut-off machine under water cooling. Each enamel surface was polished with 600-grit SiC abrasive paper for 60 seconds to create a standardized smear layer (Fig2.C). After that, two enamel fragments were used to obtain one enamel disk 'sandwich'. Therefore, four fragments were prepared to obtain two enamel disks 'sandwich' per group. The bonding procedure was performed (Fig2.D) in the same way as previously described in specimen preparation for microtensile bond strength test. Then, a thin layer of a low-viscosity composite resin (Filtek Z-350 Flowable Restorative; 3M ESPE; St Paul, MN, USA) was placed between two enamel surface fragments and light activated to produce an enamel disk 'sandwich' (Fig2.E). The resin-tooth bonded specimens were stored in distilled water at 37^{o}C for 24h. Subsequently, the enamel disk 'sandwich' was vertically sectioned at the enamel-adhesive interface (Fig2E) and the two slices were embedded in epoxy resin (Epoxy Resin – UK Buehler LTD, Lake Bluff, USA) (Fig2.F).

The enamel-adhesive interfaces were polished with 600, 800, 1200, and 2000-grit SiC abrasive papers (Carborundum Abrasives, Recife, PE, Brazil) under running water (Fig2.F). Next, 1.0, 0.3, and 0.05μm diamond pastes (UK Buehler LTD, Lake Bluff, IL, USA) on polishing felts were used to complete the polishing procedure. To remove any diamond paste debris, the last polishing procedure took 20 minutes under water cooling. The interface samples were polished for 10 minutes on each diamond paste. Between each diamond paste polishing procedure, the samples were ultrasonically cleaned (Unique Ind. Co. and Electronic Products Ltda, Sao Paulo, SP, Brazil) for 10 minutes. Then, the enamel-adhesive interface samples were mounted on stubs, gold sputtered coated and analyzed under SEM.

TABLE 1. Composition, manufacturers, and batch numbers of the adhesive systems used in the study

Adhesive Systems / Code	Composition	Technique
SBU: Scotchbond Universal Adhesive (Batch #148785) 3M-ESPE; St Paul, MN, USA	10-MDP, HEMA, Vitrebond™ Copolymer, filler, ethanol, water, initiators, silane (pH=2.7)	Self-etching Selective etching enamel Total-etching
CSE: Clearfil SE Bond (Primer Batch #01089A Bond Batch #01628A) Kuraray Noritake Dental Inc.; Tokyo, Japan	Primer: water, 10 MDP, HEMA, hydrophilic aliphatic dimethacrylate, accelerators, dl-camphorquinone (pH=2.0) Adhesive: 10 MDP, bis-GMA, HEMA, initiators, colloidal silica, dl-camphorquinone, accelerator	2-step self-etching Selective etching enamel
SBMP: Scotchbond Multi-Purpose (Primer Batch #N322814 Bond Batch #N322814) 3M-ESPE; St Paul, MN, USA	Primer: HEMA, water, polyalkenoic acid polymer (pH=3.3) Adhesive: Bis-GMA, HEMA, tertiary amines, photo-initiator	3-step total-etching adhesive
EX: Excite F (Batch #N198012) Ivoclar Vivadent; Schaan, Liechtenstein	Phosphonic acid acrylate, HEMA, DMA, ethanol, silicone dioxide, initiators, stabilizers, potassium fluoride (pH=2.5)	2-step total-etching

10-MDP (methacryloyloxy decyl di-hydrogenphosphate), HEMA (hydroxyethyl methacrylate), Vitrebond™ Copolymer (copolymer of acrylic and itaconic acid), bis-GMA (bisphenol A glycidylmethacrylate), DMA (dimethacrylate).

TABLE 2. Bonding Procedures

Group	Application Procedure
SBU	Apply adhesive (rubbing) for 20s Gently air dry for 5s Light cure for 10s
SBU-et	Acid etching for 30s Rinse with water for 20s Gently air dry for 5s Apply adhesive (rubbing) for 20s Gently air dry for 5s Light cure for 10s
CSE	Apply primer (rubbing) for 20s Gently air dry for 5s Apply adhesive for 20s Gently air dry for 5s Light cure for 10s
CSE-et	Acid etching for 30s Rinse with water for 20s Gently air dry for 5s Apply primer (rubbing) for 20s Gently air dry for 5s Apply adhesive for 20s Gently air dry for 5s Light cure for 10s
SBMP	Acid etching for 30s Rinse with water for 20s Gently air dry for 5s Apply primer for 20s Gently air dry for 5s Apply adhesive for 20s Gently air dry for 5s Light cure for 10s
EX	Acid etching for 30s Rinse with water for 20s Apply adhesive for 20s Gently dry Light cure for 10s

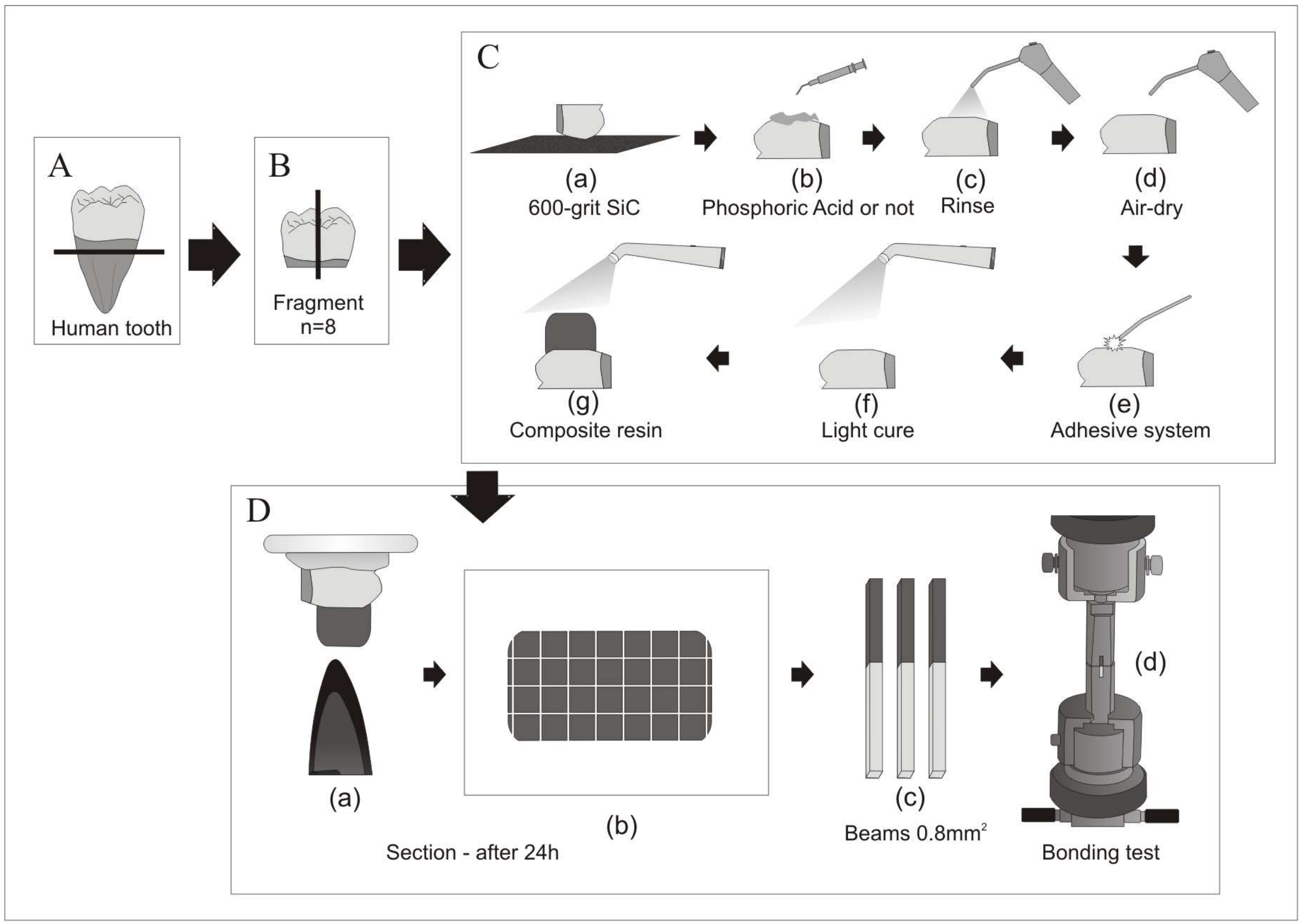

Figure 1. Specimen preparation for microtensile bond strength.

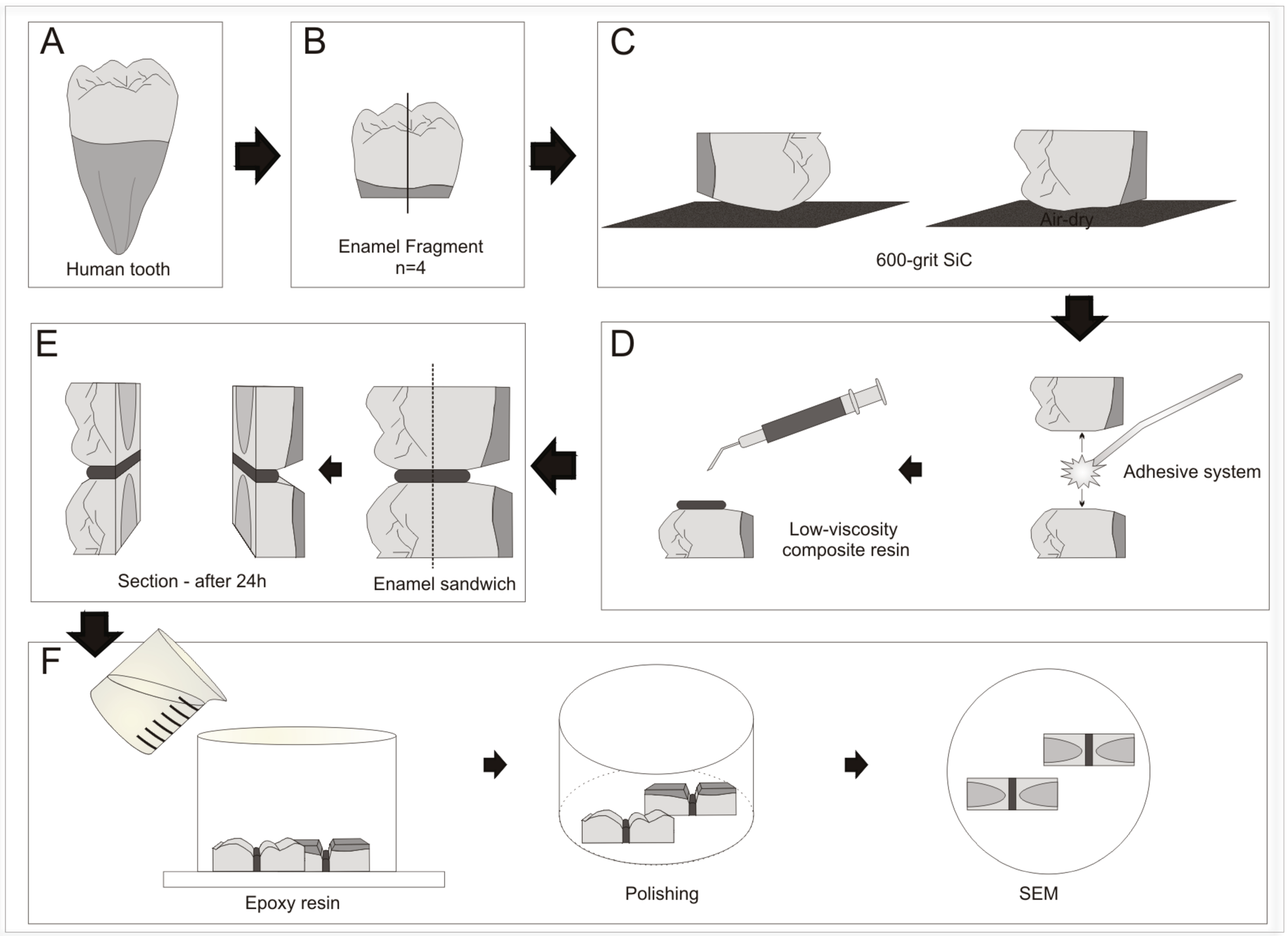

Figure 2. Specimen preparation for interfacial morphology.

RESULTS

Microtensile Bond Strength Results

Mean and standard deviations of microtensile bond strength values of all groups are shown on Table 3. The one-way ANOVA and Fisher's PLSD ($p<0.05$) statistical analysis revealed a significant difference between pre-etched (SBU-et and CSE-et) and non-etched groups (SBU and CSE). Pre-etched groups presented the higher bond strength values. The results of one-step multi mode SBU and the 2-step self-etching adhesive system CSE did not differ from bond strength values of the gold standard 3-step total-etching adhesive system SBMP. The 2-step total-etching adhesive EX showed the lowest bond strength value compared with all other groups. Most of the observed modes of fracture on enamel-etched groups were mixed failure in adhesive/resin, regardless of type of adhesive system; except for both self-etching adhesive systems the failure modes were predominantly adhesive in enamel.

Interfacial Morphology Analysis under Scanning Electron Microscopy

Representative SEM images of the enamel-adhesive interfaces from each group are shown on Figure 3. The 3-step and 2-step total-etching adhesive systems (Fig 3.E and 3.F) showed similar enamel-adhesive interfaces. For both we could be observe clear extensions of penetrated adhesive tags into demineralized enamel. For SBU and CSE in self-etching mode (Fig 3.A and 3.C), it could detect an interaction between enamel and adhesive without gaps. However, there was no evidence of adhesive tags formed in the interface. It could detect slightly non-continuous demineralization and penetration of the adhesive showing that the SBU and CSE attached to grounded enamel surface. When the pre-etching procedure was performed, both SBU-et and CSE-et (Fig 3.B and 3.D) (Fig 3.E and 3.F) interfaces showed slight infiltration of the adhesive in grounded enamel surface.

TABLE 3. Means and standard deviations (SD) of microtensile bond strength values of all groups.

Groups		MPa (SD)
One-step multi mode	SBU	27.4 (8.5) b
	SBU-et	33.6 (9.3) a
2-step self-etching	CSE	28.5 (8.3) b
	CSE-et	34.2 (9.0) a
3-step total-etching	SBMP	30.4 (11.0) b
2-step total-etching	EX	23.3 (8.2) c

Same letters indicate no statistical difference among the groups.

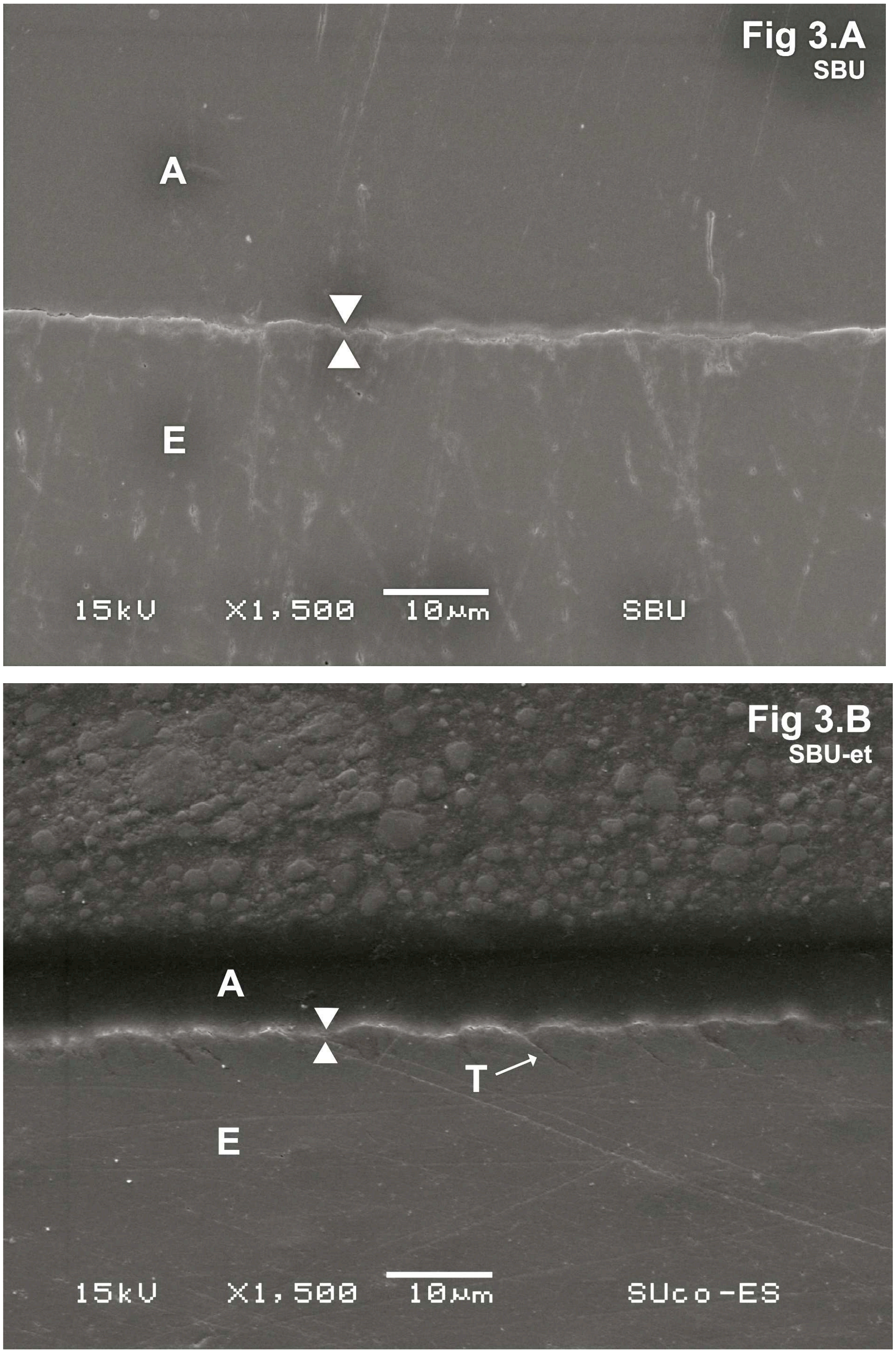

Figure 3. SEM imagens of the ultrastructure of the interfaces. (A)SBU and (B)SBU-et. The adhesive-enamel interface is indicated between the arrows.
A= adhesive layer; E= enamel; T=tags.

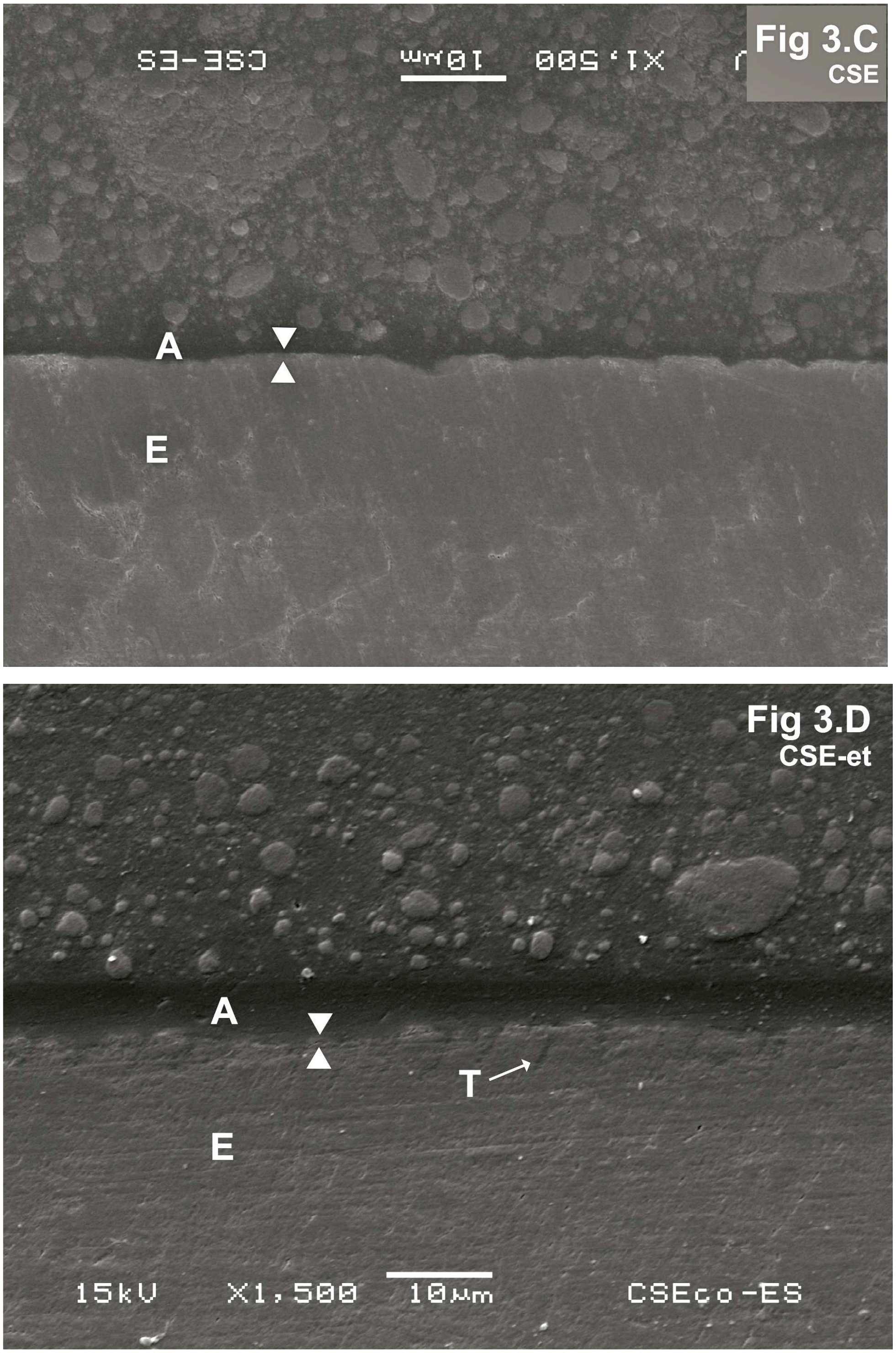

Figure 3. SEM imagens of the ultrastructure of the interfaces. (C)CSE and (D)CSE-et. The adhesive-enamel interface is indicated between the arrows.
A= adhesive layer; E= enamel; T=tags.

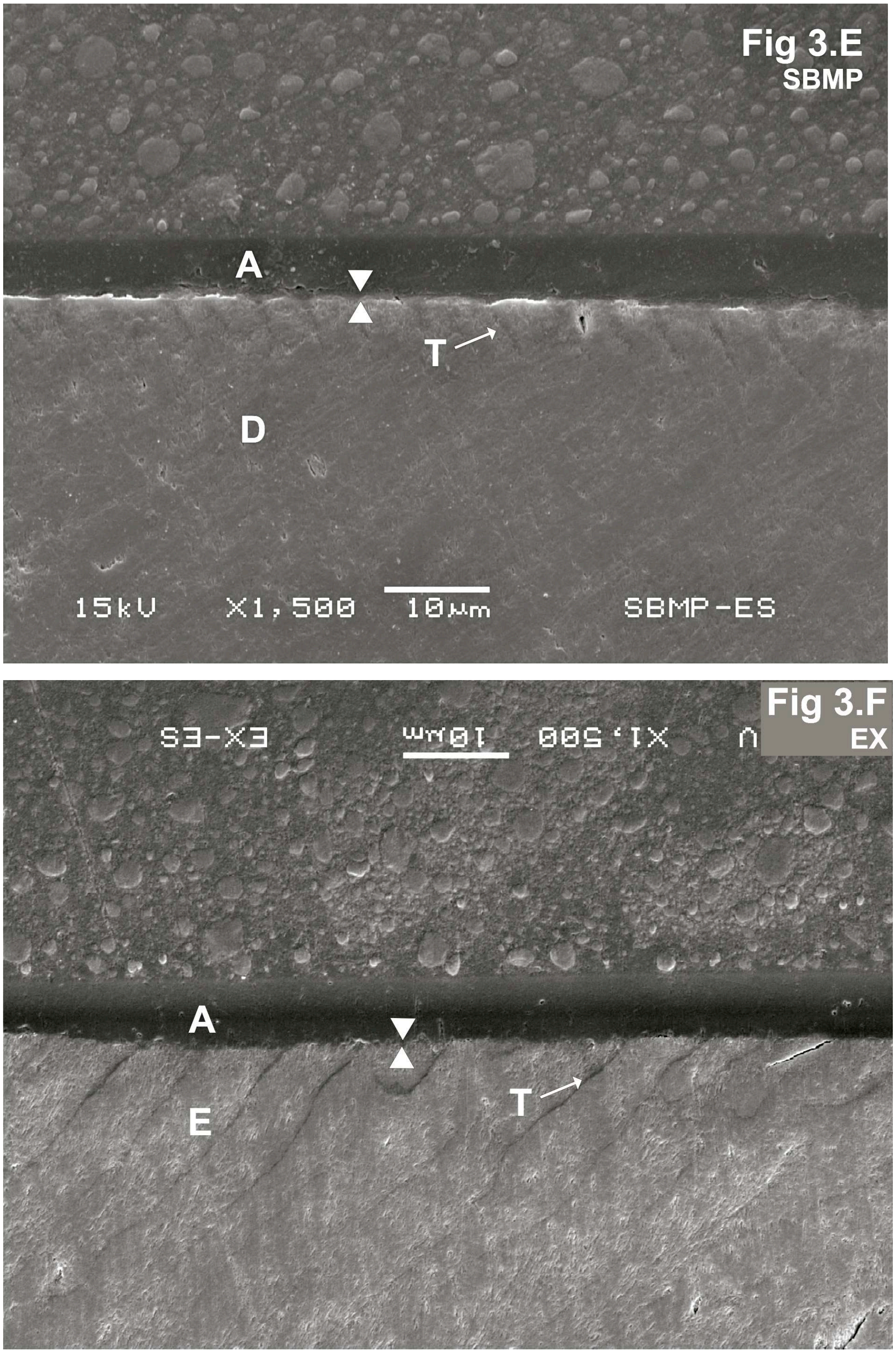

Figure 3. SEM imagens of the ultrastructure of the interfaces. (E)SBMP and (F)EX. The adhesive-enamel interface is indicated between the arrows.
A= adhesive layer; E= enamel; T=tags.

DISCUSSION

This study compared a one-step multi-mode adhesive system that contains 10-MDP and 'Vitrebond copolymer' on grounded enamel with a 2-step self-etch primer/adhesive system and other two total-etching adhesive systems. Different from other mild-self-etching systems, SBU manufacturer's recommendation is to be used in either self-etching or total-etching mode on both enamel and dentin. In accordance with previous investigations[3-5,7,10,11,16,21,23,24,30], the effectiveness of etching prior to applying self-etching adhesive systems to enamel was confirmed in the present study. The bond strength results from enamel pre-etching with 37% phosphoric acid to SBU-et (33.6MPa) and CSE-et (34.2MPa) were statistically the highest of all other adhesive systems including the 3-step total etching system SBMP (30.4MPa), considered the gold standard adhesive. Therefore, the null hypothesis was rejected. Pre-etching the enamel significantly increased bond strength values for one-step multi-mode SBU and 2-step self-etching CSE. Possibly the presence of polyalkenoic acid copolymer called 'Vitrebond copolymer' in SBMP increased the interaction of the adhesive with the enamel, since that copolymer has interaction with calcium (Ca) from hydroxyapatite (HAp)[11,32]. The carboxyl groups of the polyalkenoic acid dissociate to release protons in aqueous solutions and be able to interact in acid-base reactions[25]. Carboxyl groups can replace phosphate ions of the substrate and form ionic bonds with Ca[11]. The SEM image from SBMP showed a uniform tag penetration into grounded enamel, which was expected to total etching system[7]. Further, a hydrophobic layer is applied last in order to preserve the bond interface strong and stable.

In regard to EX total-etching adhesive system, although SEM image showed micromechanical penetration of the adhesive in demineralized enamel, the bond strength values were lower among the groups. Probably the monomer and solvent composition of that adhesive system compromised the quality of bonding to enamel substrate[20].

The acid etching to enamel increases the bonding area and the wettability of the adherent surface[22] to obtain a good micromechanical retention[3-5,7,10,11,16,21,23,24,30]. That may partially explain the best performance of self-etching systems on etched enamel. Moreover, both self-etching adhesives contain 10-MDP; this functional acidic monomer has a strong chemical affinity to Ca in HAp[35]. Therefore, the chemical and additional micromechanical interactions of SBU and CSE to etched enamel increased the microtensile bond strength values. The interface SEM micrographs (Fig 3.A and 3.C) show precise bonding of the adhesive system interacts to demineralized enamel for both SBU and CSE. Furthermore, most of the observed modes of fracture on enamel-etched groups were mixed failure in adhesive/resin, regardless of type of adhesive system. On the other hand, for both self-etching adhesive systems the failure modes were predominantly adhesive. It may be considered that micromechanical retention to enamel has an important role on bonding strength in combination to chemical interaction with HAp.

Remarkably, SBU and CSE used in self-etching mode without phosphoric acid showed no statistical bond strength values different from SBMP. We hypothesized that chemical interaction performance to enamel could be enough for a satisfactory adhesion. Van Landyut *et al*[26]*.* showed that Ca-10-MDP is the most stable salt compared with other salts formed from experimental acidic monomers. Moreover, they have demonstrated that high bond strength could be correlated to low dissolution rate of the Ca-salt due to the high chemical bonding capability. This chemical interaction concept has been called 'Adhesion-Decalcification concept'[29,33] proposed by Yoshida *et al.*[33]. Basically, the acidic monomer either adheres to or decalcifies HAp into two phases chemical interactions. A recent study by Yoshida *et al.*[34] confirmed that MDP-containing adhesives does form nanolayering at the adhesive interface, on which Ca ions released upon partial dissolution of HAp connect to 10-MDP for a high hydrolytically stable Ca-10-MDP formation[31]. In addition Yoshihara *et al.*[37] reported the strong hydrophobicity of the nanolayered structure that can protect the hybrid layer against hydrolytic bond degradation processes. However, the interaction of 10-MDP to enamel seems to be less than to HAp in dentin, according to Yoshihara *et al.*[35] characterization by X-ray diffraction confirmed nanolayering on enamel and dentin, but which was significantly greater on dentin. They supposed that enamel HAp crystals structure and size interfere on chemical bonding to 10-MDP, since the crystals within dentin is considerably smaller than that within enamel.

Nonetheless, adequate bonding could be achieved when substantial numbers of microretentive site are produced[36]. Considering that the enamel surface preparation must be carried out, in our study the enamel was grounded using 600-grit SiC-sandpaper to standardization the smear layer on surface. According to Mine *et al.*[12], the surface preparation method significantly affected the nature of smear layer and the interaction with mild self-etching adhesive. The 600-grit SiC preparation removed the aprismatic layer and fragments of crystal particles were compacted in the surface voids, in order to the adhesive be able to incorporate adequately every single crystal. For bur-preparation, a rougher surface with numerous subsurface cracks is obtained so that resin impregnation was not uniform. Therefore, the ‘resin-smear complex’ might contain many areas that were not infiltrated by resin. From these results, micromechanical interlocking of the adhesive resin into enamel surface is more dependent on the surface receptiveness. To simulate clinical situation which uses extra-fine diamond bur (15μm grit-size)[12] we used SiC sandpaper for best adhesive performance.

Another issue is the application procedure, mainly to self-etching systems. Yoshihara *et al.*[35] proved that actively rubbing the adhesive for 20s on surface promotes more intimate contact of 10-MDP molecules with HAp[1,35,38,39], since the solvent more effectively evaporates from the surface. In addition, on rubbing the 10-MDP adhesive, Ca is released from enamel/dentin and assists the nucleation and growth of Ca salts.

Though our bonding strength results showed positive values for SBU and CSE in self-etching mode, etching enamel or selective enamel margins on cavity preparation might be indicated for many reasons. Several laboratorial and clinical studies[3-5,7,10,11,16,18,19,21,23,24,27,30] provided that etching enamel prior mild-self-etching adhesive application has improved the integrity of enamel margins. In an *in vitro* enamel sealing study of self-etching adhesives[16], when restorations are thermally challenged, etching with phosphoric acid improved the enamel marginal sealing of self-etch adhesives. An 8-year clinical trial[18] using two-step self-etching adhesive with and without selective enamel etching revealed that the clinical effectiveness of CSE remained excellent; with selective acid etching of the enamel cavity margins only have some minor positive effect in regard to small marginal defects/discolorations at the enamel side. However, they did not require any restorative intervention.

Nevertheless, in clinical situation to apply the phosphoric acid only on enamel margin is very critical, so selective enamel etching is a challenge. Thus, several studies have evaluated etching dentin prior self-etching adhesives[21,23,26] application and the results have not been positive. Torii *et al.*[21] showed that phosphoric acid prior the application of self-etching primers decreases the adhesion of composite resin to dentin, although it increases enamel adhesion. Another study by Van Landuyt *et al.*[23] indicated that the bonding effectiveness of CSE can be improved by selectively etching the enamel margins. However, etching should be limited to enamel, etching the dentin formed a low-quality hybrid layer prone to nanoleakage.

On the other hand, the new one-step multi-mode SBU has been suggested to be used on multiple techniques: total-etching, selective enamel etching, and self-etching. In accordance with our bond strength results on enamel, either etching or self-etching mode may be chosen. The representative SEM images from SBU-etc (Fig 3.B) showed micromechanical interlocking when phosphoric acid was used prior the adhesive. However, without etching a homogeneous interaction could be observed on enamel surface. Besides the presence of 10-MDP in composition, SBU contains 'Vitrebond copolymer' that allows an ionic bonding to the mineral of dental substrate[13,28] and induces to tolerance to moisture contamination[32]. Therefore, both 10-MDP and 'Vitrebond copolymer' interact chemically to dental substrate. A recent laboratory study[17] indicated that SBU is not affected by the adhesion strategy or by the degree of dentin moisture. That result is favorable to indicate selective enamel etching to SBU, excluding undesirable effects on dentin substrate in case of phosphoric acid gel running over.

The adhesion strategies have been improved in the last years and the 'Adhesion-Decalcification concept' has progressively been consolidated in resin composite-tooth substrate interaction. Many studies have demonstrated that acidic functional monomer with a strong chemical affinity for the Ca in HAp is essential for the longevity of restorations[6]. Therefore, the micromechanical retention concept for long required to achieve a good adhesion has been gradually changed for chemical interaction on tooth substrate. Despite micromechanical retention can achieve adequate results, several problems regarding durability of restorations have been scientifically questioned: degradation of exposed collagen non-infiltrated by resin monomers, non-polymerized infiltrated resin, and postoperative sensitivity. Technique sensitivity and number of steps of application must be considered critical. It is expected that chemical interaction concept could minimize these difficulties in conventional micromechanical adhesion concept and increase the durability of restorations.

CONCLUSION

Within the limitations of our study, the etching procedure with phosphoric acid on grounded enamel prior application of a one multi-mode adhesive SBU and a 2-step self-etching adhesive CSE significantly increased bond strength results and clear resin tags could be observed under SEM interface images. However, when SBU and CSE were applied in self-etching mode, the bond strength values were not different from those of gold standard 3-step total-etching adhesive system SBMP.

CLINICAL RELEVANCE

The pre-etching procedure with phosphoric acid on grounded enamel using self-etching adhesive systems could be beneficial to enhance bond strength values.

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CAPÍTULO 2

Effect of Pre-etching to Dentin on Bond Strength and Interfacial Morphology of a One-Step Multi-mode Adhesive

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ABSTRACT

Objectives: To compare bond strength (μTBS) and interfacial morphology (IM) of a one-step multi-mode adhesive and a 2-step self-etching adhesive on pre-etched and non-etched dentin with two total-etching adhesives.

Methods: For μTBS test: forty-eight middle dentin flat surface were obtained and randomly assigned into 6 groups (n=8): [SBU] Scotchbond Universal; [SBU-et] pre-etched SBU; [CSE] Clearfil SE Bond; [CSE-et] pre-etched CSE; [SBMP] Scotchbond Multi-Purpose; and [EX] Excite F. For pre-etched samples, phosphoric acid was applied for 15s, each adhesive system was used according to manufacturers' instructions, and composite resin blocks were incrementally built up. After 24h, specimens were sectioned in 0.8mm^2 beams and subjected to tension test. The data were analyzed with one-way ANOVA and Fisher's PLSD. For IM analysis, two dentin disks from six teeth (n=4) were obtained, the adhesive system was applied on dentin surface according to the groups, and a low-viscosity resin was placed between two disks. The dentin 'sandwiches' were embedded in epoxy resin, polished, and observed through SEM.

Results: The μTBS values were: SBU=55.5; CSE=54.1; SBMP=53.3; SBU-et=50.7; CSE-et=46.5; and EX=41.4. SBU, CSE, and SBMP presented the highest μTBS values. Followed by SBU-et, CSE-et, and EX. SEM images from pre-etched and total-etching samples showed clear prolonged resin tags into demineralized dentin. Both SBU and CSE showed a thin hybrid layer.

Conclusions: Despite the thin hybrid layer, SBU and CSE showed a high μTBS values. Etching dentin prior application of one-step multi-mode SBU decreased the μTBS, but did not differ from the gold standard total-etching adhesive SBMP.

Clinical Significance: The application of phosphoric acid on dentin prior self-etching adhesives is not recommended.

Keywords: dentin, microtensile bond strength, self-etching adhesive, total-etching adhesive, phosphoric acid, acidic functional monomer, 10-MDP

INTRODUCTION

Since Nakabayashi *et al.*[1] described the concept of adhesion to dentin in 1982 and named 'hybrid layer' the interface formed from monomer infiltration into demineralized dentin, the adhesive techniques have been improved and new categories of adhesive systems have been available. Currently, self-etching adhesive systems have substantially gained the confidence of clinicians. These adhesive systems have been developed to simplify usage and to reduce postoperative sensitivity[2].

Nevertheless, the success of resin composite restorations still has been a challenge for several reasons. Marginal adaptation, marginal infiltration, marginal staining, postoperative sensitivity, secondary caries, and failures on composite resin might compromise the longevity of esthetics restorations. The effort to achieve the ideal adhesion to both dentin and enamel has been the target of innumerous researches[3-6]. The most recent concept in adhesion is called 'Adhesion-Decalcification concept', first described by Yoshida *et al.*[7] in 2001. Accordingly, the adhesion not only depends on micromechanical retention on demineralized dentin and enamel, but mainly on chemical interaction of an acidic functional monomer to calcium of hydroxyapatite. As stated by Yoshida *et al.*[8] in 2004, 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) ionically bond most effectively to hydroxyapatite. In this way, simplified adhesive systems containing 10-MDP have been developed and improved in the market.

Recently, it came into the market a one-step multi-mode adhesive system that contains 10-MDP and 'Vitrebond copolymer', both may chemically interact with dental substrate. According to Yoshihara *et al.*[9], the chemical stability of the 10-MDP-Ca salts could contribute to the bond durability. Moreover, the 'Vitrebond copolymer' may provide to the adhesive to be more tolerant to moisture contamination and permit also a good sealing to dentin[10]. However, these self-etching adhesives containing 10-MDP have been questioned in regard to enamel surface. Some studies showed that self-etching systems do not bond adequately to enamel[11-14], because of their low aggressiveness and acidity compared with phosphoric acid, used in total-etching adhesive systems. Considering these studies, some authors have recommended to etch the enamel prior application of self-etching adhesive systems[11,13-23]. In clinical situation, this procedure is called 'selective enamel etching'.

During the selective enamel etching procedure, the phosphoric acid can run over to the dentin. Therefore, it is critical not to etch part of the dentin adjacent to the enamel edge. The purpose of the present study was to compare bond strength and interfacial morphology of a one-step multi-mode adhesive and a 2-step self-etching adhesive on pre-etched and non-etched dentin surface with two total-etching adhesives systems. The null hypothesis tested in the present study was that the pre-etching of dentin does not affect the laboratory performance of self-etching adhesive systems.

MATERIALS AND METHODS

This study was reviewed and approved by the research Ethics Committee (Protocol #113/2011). Fifty-four caries-free extracted human third molars were collected, cleaned, and stored in 0.1% thymol solution under refrigeration before using in the experimental. Forty-eight teeth (n=8) were prepared for bond strength test and six teeth (n=4) for interface morphology by scanning electron microscopy.

Specimen Preparation for Microtensile Bond Strength

For μTBS, the root of each tooth was removed (Fig1.A) and the crown was cut to obtain a middle-dentin flat surface (Fig1.B) using a low-speed diamond saw (Extec Corp.; Enfield, CT, USA) mounted on a precision cut-off machine (Isomet 1000, Buehler; Lake Buff, IL, USA). The dentin surfaces were polished using 600-grit SiC (silicon carbide) abrasive paper under water cooling for 60 seconds to produce a standardized smear layer (Fig1.C-a). After that, the samples were randomly assigned into 6 groups: [SBU] Scotchbond Universal Adhesive; [SBU-et] pre-etched SBU; [CSE] Clearfil SE Bond; [CSE-et] pre-etched CSE; [SBMP] Scotchbond Multi-Purpose; and [EX] Excite F. The compositions, manufacturers, and batch numbers of the adhesive systems were shown on Table 1.

The adhesive application was according to the bonding procedures shown on Table 2. Each adhesive system was applied following the manufacturers instructions (Fig1.C-e), except for the groups SBU-et and CSE-et, that was pre-etched (Fig1.C-b) with 37% phosphoric acid gel (Condac 37% - FGM Produtos Odontológicos Ltda; Joinville, SC, Brazil – Batch n^{o}. 060911) for 15 seconds, rinsed with running water (Fig1.C-c), and gently dried prior application of the adhesive system.

After the bonding procedure, a composite resin block (Filtek Supreme Plus 3M ESPE; St Paul, MN, USA) was incrementally built up until approximately 6 mm thick. Each increment was light activated for 20 seconds using the Elipar S10 LED Curing Light (3M ESPE; St Paul, MN, USA) with a power density of 800 mW/cm^2. The light output intensity periodically analyzed using a radiometer (SybronKerr, Orange, CA, USA) during the experiment. Then, the restored samples were stored in distilled water at 37^{o}C for 24 hours.

Microtensile Bond Strength Test

After 24 hours storage, the restored samples were longitudinally sectioned in both ‘x’ and ‘y’ directions across to the adhesive interface using a low-speed diamond saw on a precision cut-off machine to obtain beams of approximately $0.8mm^2$ of bonding area. The specimens were attached to a testing jig with cyanoacrylate adhesive (Super Bonder Loctite Henkel; São Paulo, SP, Brazil) and subjected to a tension force in a universal testing machine (EZ Test, Shimadzu; Kyoto, Japan) at a crosshead speed of 1 mm/min. The cross-sectional areas were calculated in order to obtain µTBS values in MegaPascal units (MPa). The data were statistically analyzed by one-way ANOVA and Fisher’s PLSD (α=0.05) using Statview statistical software.

The fracture modes from dentin sides were gold sputter coated and analyzed under SEM (JSM 5600LV, JEOL; Tokyo, Japan). Failure modes were categorized into: cohesive failure in dentin; mixed failure; adhesive failure in dentin or adhesive; and cohesive failure in resin composite.

Interfacial Morphology Analysis under Scanning Electron Microscopy

Six human third molars were prepared in order to analyze the morphology of dentin-adhesive interface (n=4). The roots were removed and two dentin disks (approximately 1.5-mm thick) were obtained from each crown using a low-speed diamond saw mounted on a precision cut-off machine under water cooling. Each dentin surface disk was polished with 600-grit SiC abrasive paper for 60 seconds to create a standardized smear layer. After that, two dentin disks were used to obtain one ‘sandwich’ disk sample. Therefore, four dentin disks were used to obtain two dentin ‘sandwich’ disks per group. In other words, four dentin-adhesive interfaces were produced to be analyzed under SEM. The bonding procedure was performed in the same way as previously described in specimen preparation for microtensile bond strength test. Then, a thin layer of a low-viscosity composite resin (Filtek Z-350 Flowable Restorative; 3M ESPE; St Paul, MN, USA) was placed between two dentin disks and light activated to produce a dentin ‘sandwich’ disk. The resin-tooth bonded specimens were stored in distilled water at 37^oC for 24h.

Subsequently, the dentin 'sandwich' disk was vertically sectioned at the dentin-adhesive interface and the two slices were embedded in epoxy resin (Epoxy Resin – UK Buehler LTD, Lake Bluff, USA).

The dentin-adhesive interfaces were polished with 600, 800, 1200, and 2000-grit SiC abrasive papers (Carborundum Abrasives, Recife, PE, Brazil) under running water. Next, 1.0, 0.3, and 0.05μm diamond pastes (UK Buehler LTD, Lake Bluff, IL 60044, USA) on polishing felts were used to complete the polishing procedure. To remove any diamond paste debris, the last polishing felt was used for 20 minutes under water cooling without diamond paste. The interface samples were polished for 10 min on each diamond paste. Between each diamond paste, the samples were ultrasonically cleaned (Unique Ind. Co. and Electronic Products Ltda, Sao Paulo, SP, Brazil) for 10 minutes. Then, the dentin-adhesive interface samples were mounted on stubs, gold sputtered coated and analyzed under SEM.

TABLE 1. Adhesive systems used in the study with composition and manufacturer's information

Adhesive Systems / Code	Composition	Technique
SBU: Scotchbond Universal Adhesive (Batch #148785) 3M-ESPE; St Paul, MN, USA	10-MDP, HEMA, Vitrebond™ Copolymer, filler, ethanol, water, initiators, silane (pH=2.7)	Self-etching Selective etching enamel Total-etching
CSE: Clearfil SE Bond (Primer Batch #01089A Bond Batch #01628A) Kuraray Noritake Dental Inc.; Tokyo, Japan	Primer: water, 10 MDP, HEMA, hydrophilic aliphatic dimethacrylate, accelerators, dl-camphorquinone, (pH=2.0) Adhesive: 10 MDP, bis-GMA, HEMA, initiators, colloidal silica, dl-camphorquinone, accelerator	2-step self-etching Selective etching enamel
SBMP: Scotchbond Multi-Purpose (Primer Batch #N322814 Bond Batch #N322814) 3M-ESPE; St Paul, MN, USA	Primer: HEMA, water, polyalkenoic acid polymer (pH=3.3) Adhesive: Bis-GMA, HEMA, tertiary amines, photo-initiator	3-step total-etching adhesive
EX: Excite F (Batch #N198012) Ivoclar Vivadent; Schaan, Liechtenstein	Phosphonic acid acrylate, HEMA, DMA, ethanol, silicone dioxide, initiators, stabilizers, potassium fluoride (pH=2.5)	2-step total-etching

10-MDP (methacryloyloxy decyl di-hydrogenphosphate), HEMA (hydroxyethyl methacrylate), Vitrebond™ Copolymer (copolymer of acrylic and itaconic acid), bis-GMA (bisphenol A glycidylmethacrylate), DMA (dimethacrylate).

TABLE 2. Bonding Procedures

Group	Application Procedure
SBU	Apply adhesive (rubbing) for 20s Gently air dry for 5s Light cure for 10s
SBU-et	Acid etching for 15s Rinse with water for 20s Apply adhesive (rubbing) for 20s Gently air dry for 5s Light cure for 10s
CSE	Apply primer (rubbing) for 20s Gently air dry for 5s Apply adhesive for 20s Gently air dry for 5s Light cure for 10s
CSE-et	Acid etching for 15s Rinse with water for 20s Apply primer (rubbing) for 20s Gently air dry for 5s Apply adhesive for 20s Gently air dry for 5s Light cure for 10s
SBMP	Acid etching for 15s Rinse with water for 20s Apply primer for 20s Gently air dry for 5s Apply adhesive for 20s Gently dry Light cure for 10s
EX	Acid etching for 15s Rinse with water for 20s Apply adhesive for 20s Gently dry Light cure for 10s

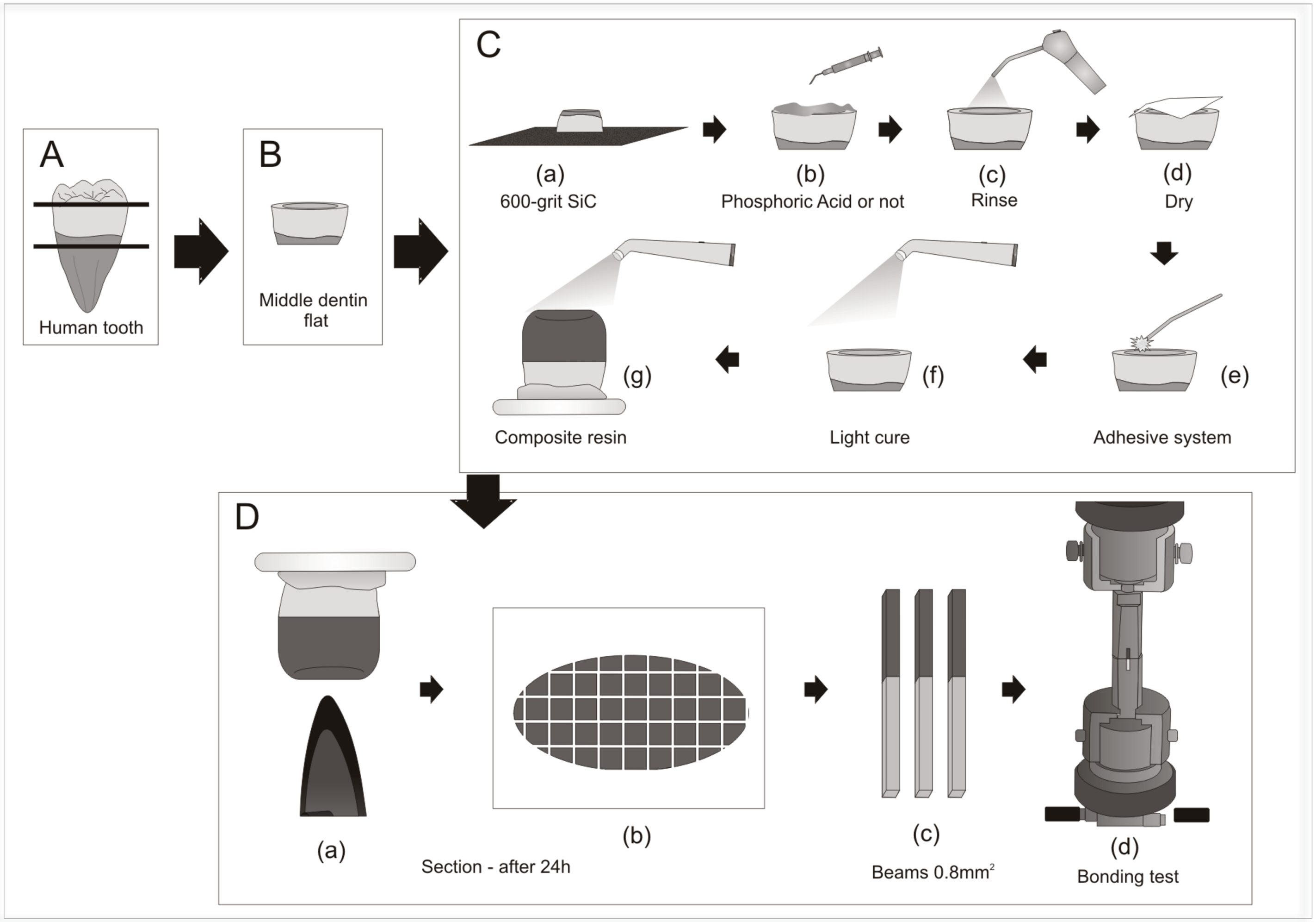


Figure 1. Specimen preparation for microtensile bond strength.

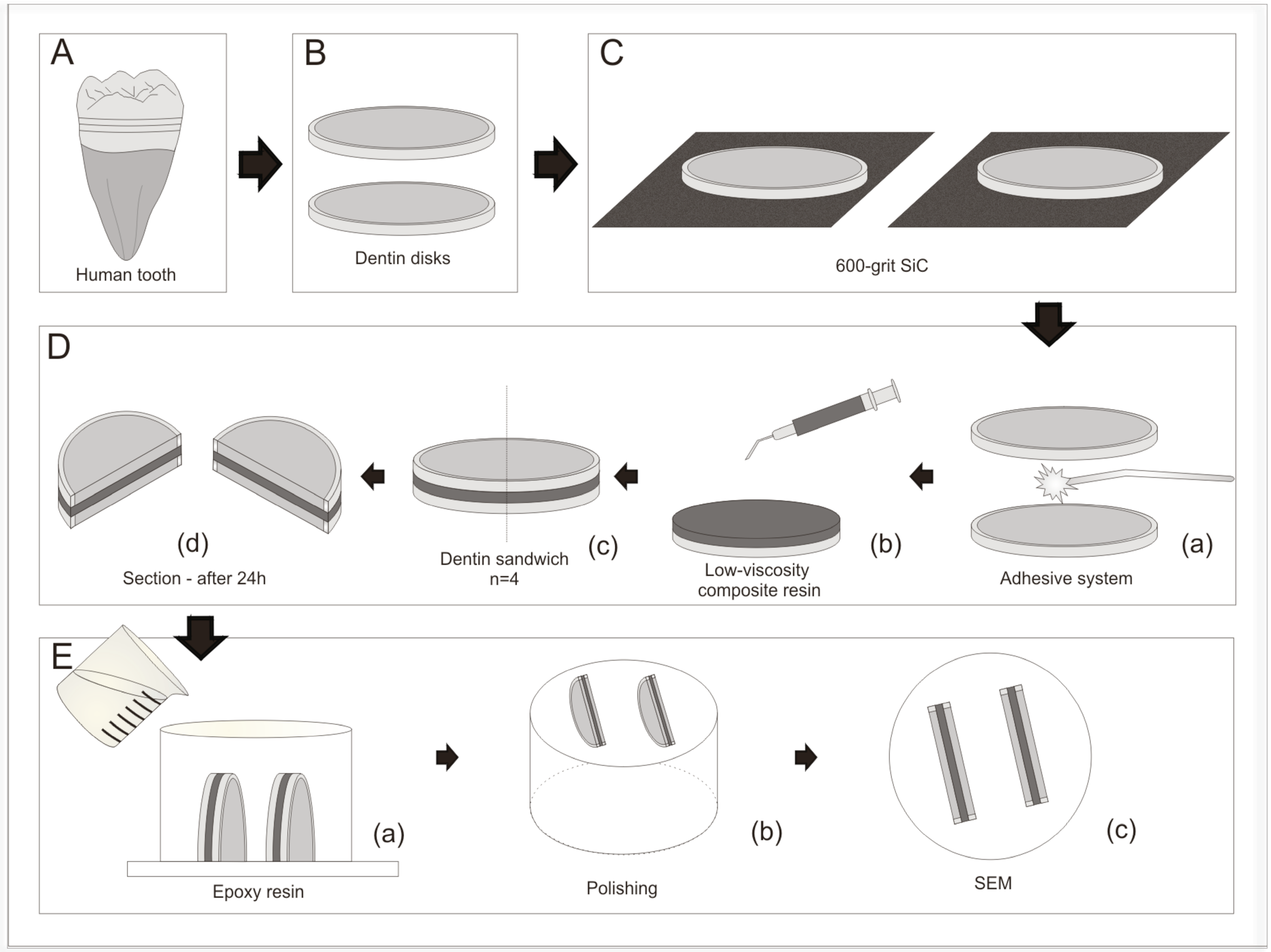


Figure 2. Specimen preparation for interfacial morphology.

RESULTS

Microtensile Bond Strength Results

Mean and standard deviations of microtensile bond strength values of all groups are shown on Table 3. The one-way ANOVA and Fisher's PLSD ($p<0.05$) statistical analyses revealed significant high bond strength values to SBU, CSE, and SBMP. Followed by SBU-et, CSE-et, and EX, whose μTBS values were different among each other. Remarkably, the one-step multi-mode adhesive SBU and 2-step self-etching adhesive CSE did not differ from SBMP results, considered the gold standard total-etching adhesive system. Moreover, the values of SBU-et were not statistically different from the values of SBMP. The 2-step total-etching adhesive EX showed the lowest bond strength value compared with all other groups. Most of the observed modes of fracture on dentin-adhesive interface were mixed failure in adhesive/resin, regardless of type of adhesive system; except for CSE-et, whose failures were predominantly adhesive in dentin.

Interfacial Morphology Analysis under Scanning Electron Microscopy

SEM images from dentin-adhesive interfaces of each group are shown on Figure 3. The 3-step and 2-step total-etching adhesive systems (Fig 3.E and 3.F) showed similar features at the interface. For both SBMP and EX we could observe a thick hybrid layer with lengthy resin tags penetrated into demineralized dentin, regardless the different bond strength values. For SBU and CSE in self-etching mode (Fig 3.A and 3.C), it could detect an interaction between dentin and adhesive without gaps, that might be considered attached to dentin surface. The resin tags were less evident in SBU and CSE than in SBMP and EX. When the pre-etching procedure was performed, both SBU-et and CSE-et interfaces (Fig 3.B and 3.D) showed similar features of interface formed with SBMP and EX. Clearly resin tags infiltrated into demineralized dentin were detected and the hybrid layer produced was thicker than in self-etching mode techniques.

TABLE 3. Means and standard deviations (SD) of microtensile bond strength values of all groups.

Groups		**MPa (SD)**
One-step multi mode	SBU	55.5 (11.4) a
	SBU-et	50.7 (11.7) b
2-step self-etching	CSE	54.1 (10.6) a
	CSE-et	46.5 (10.2) c
3-step total-etching	SBMP	53.3 (12.6) ab
2-step total-etching	EX	41.4 (9.1) d

Different letters indicate statistical significant difference among the groups.

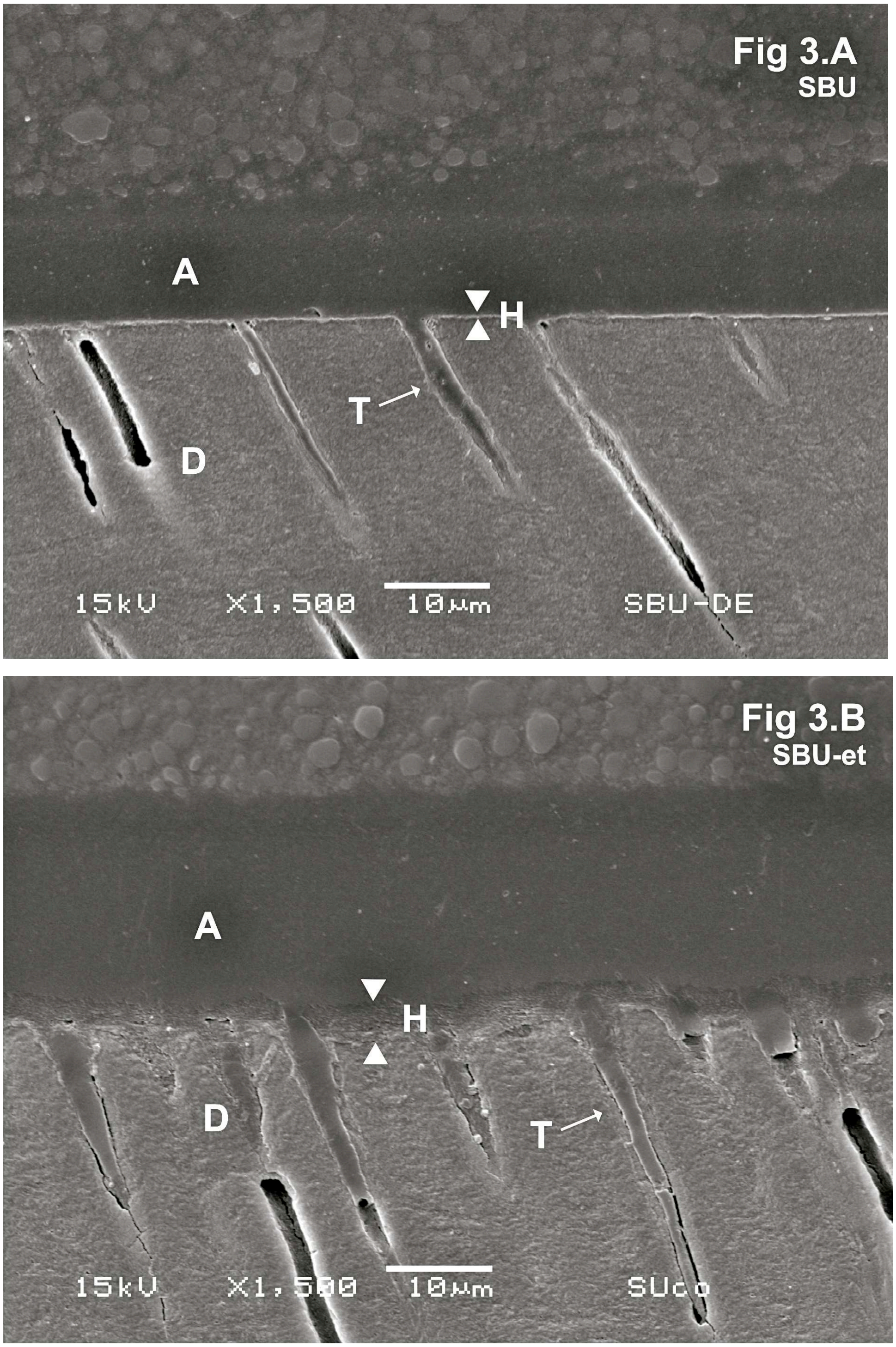


Figure 3. SEM imagens of the ultrastructure of the interfaces. (A)SBU and (B)SBU-et. The dentin-adhesive interface is indicated between the arrows.
A= adhesive layer; D= dentin; H= hybrid layer; T=tags.

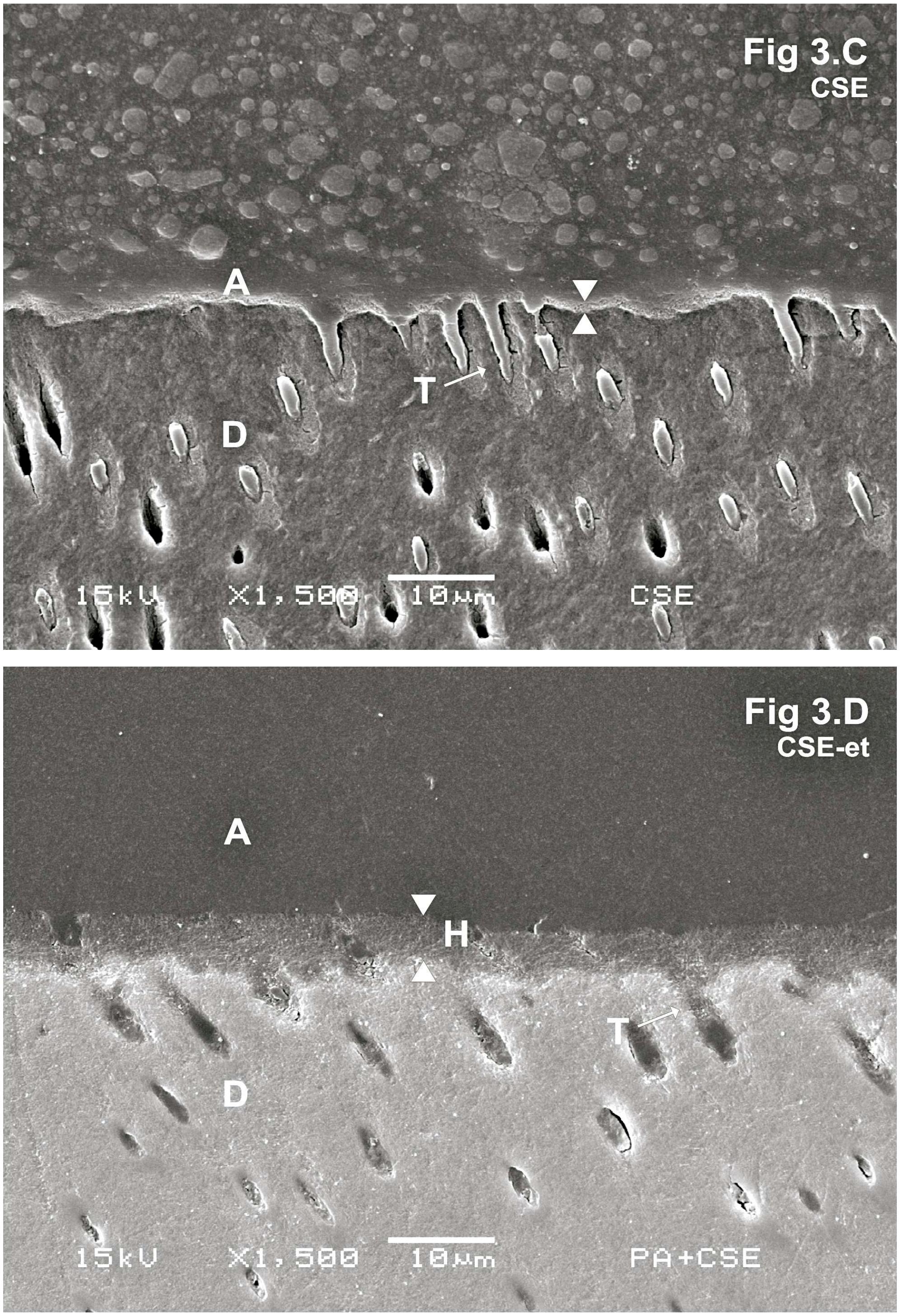
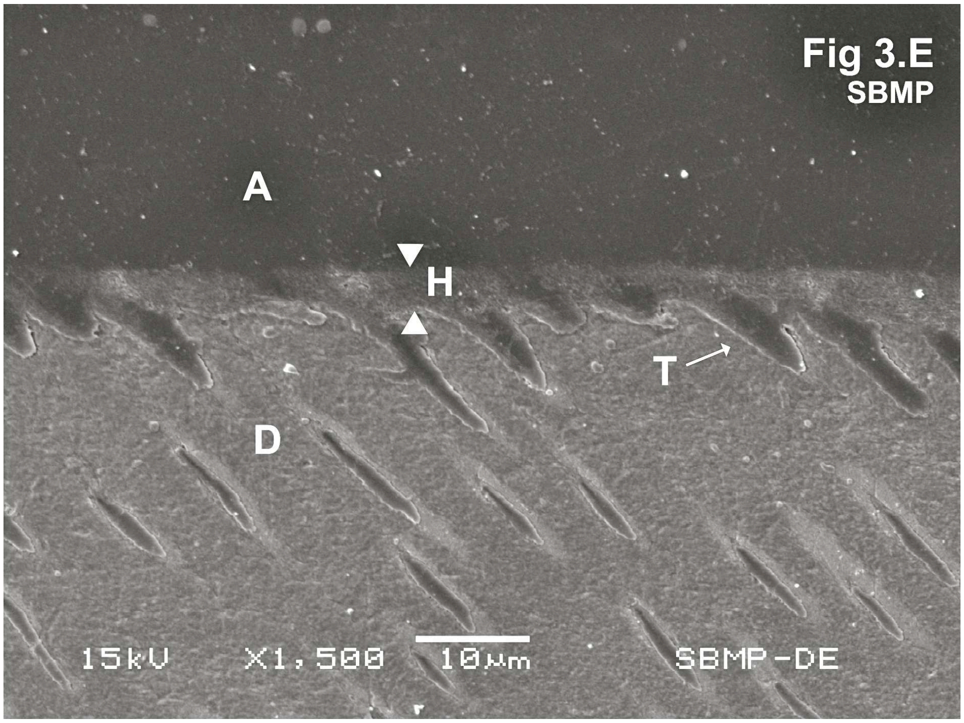


Figure 3. SEM imagens of the ultrastructure of the interfaces (C)CSE and (D)CSE-et. The dentin-adhesive interface is indicated between the arrows.
A= adhesive layer; D= dentin; H= hybrid layer; T=tags.



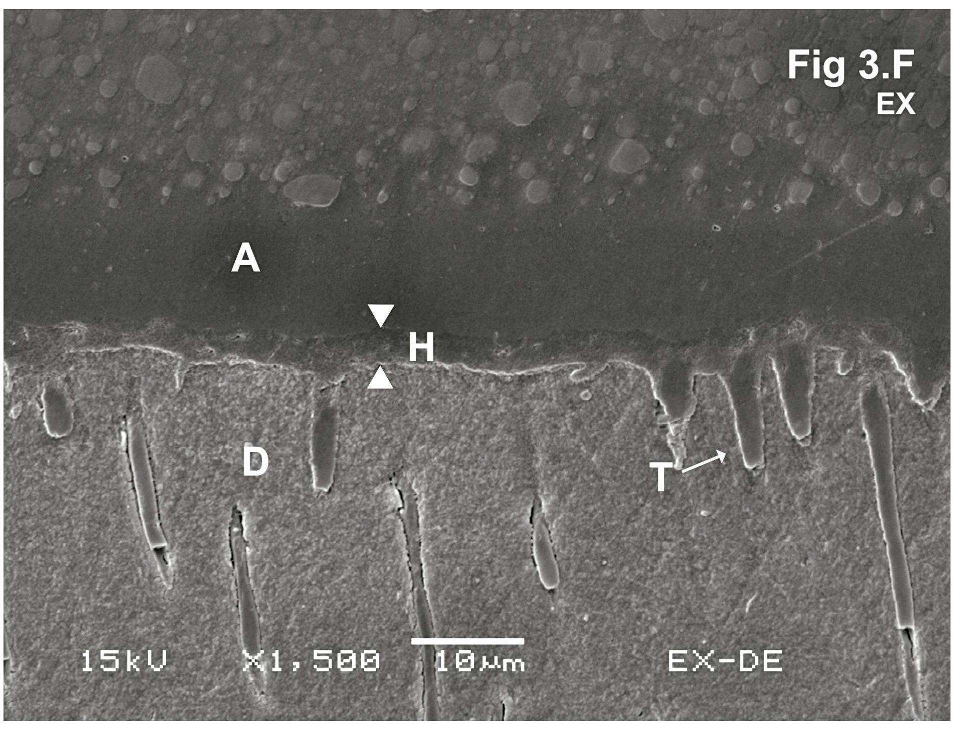


Figure 3. SEM imagens of the ultrastructure of the interfaces.(E)SBMP and (F)EX.
The dentin-adhesive interface is indicated between the arrows.
A= adhesive layer; D= dentin; H= hybrid layer; T=tags.

DISCUSSION

According to several studies, self-etching adhesive systems that contain the acidic functional monomer 10-MDP have shown exceeding bonding results[2,17,21-27]. The effectiveness of a 2-step mild-self-etching adhesive CSE that contains 10-MDP has been consolidated in many clinical and laboratorial studies[2,20,22-24,26-30]. CSE has been considered the gold standard among self-etching adhesive systems in the last years[3], based mainly on its best chemical interaction to calcium from hydroxyapatite (HAp) compared with other acidic functional monomers such as 4-MET (4-methacryloyloxyethyl-trimellitic acid) and Phenyl-P (2-methacryloxyethyl phenyl hydrogen phosphate). Hence, in order to reduce clinical steps, SBU - a one-step multi-mode adhesive - which also contains 10-MDP - has recently been introduced in the market to be used as self-etching or total-etching mode for both dentin and enamel. Therefore, the present study compared a one-step multi-mode (SBU) adhesive system and a 2-step self-etching primer/adhesive system (CSE) on pre-etched and non-etched dentin with two total-etching adhesive systems. From the results, SBU (55.5MPa), CSE (54.1MPa), and SBMP (53.3MPa) had the highest bond strength values and were statistically not different among them. Followed by SBU-et (50.7MPa), CSE-et (46.5MPa), and EX (41.4MPa), these values were statistically different among each other. Therefore, the null hypothesis was rejected. Pre-etching the dentin significantly decreased bond strength values for one-step multi-mode SBU and 2-step self-etching CSE. Conversely, a recent study from Perdigão *et al.*[28] showed no difference in bond strength results between pre-etched and non-etched dentin using SBU. That is, SBU acted properly in both situations: dried non-demineralized dentin and wet demineralized dentin. The authors attributed those results to the fact that SBU contains a polyalkenoic acid copolymer called 'Vitrebond copolymer' that allows an ionic bonding to the mineral of the dental substrate[31,32] and induces to tolerance to moisture contamination[10]. Although our bond strength values from SBU were higher than SBU-et, the values from SBU-et did not statistically differ from gold standard total-etching adhesive system SBMP and they were higher than CSE-et and EX. Moreover, in accordance with other findings, the chemical interaction of 10-MDP and Ca of the dental substrate is fundamental to achieve a good and stable adhesion between resin-based material and tooth. Several researches have shown that the presence of 10-MDP in adhesive systems can improve bonding by increasing wettability and demineralization of dental substrate and by ionic bonding to Ca. This chemical interaction is the 'Adhesion-Decalcification concept' proposed by Yoshida *et al.*[7]. Basically, the acidic monomer either adheres to or decalcifies HAp into two-phasis-chemical interactions. Thus, we agree with the proposal of Perdigão *et al.*[28] in regard to the Vitrebond copolymer compound into SBU.

The highest values for SBU and CSE on non-etched dentin may be supported by essentially the presence of that acidic functional monomer 10-MDP, following the concept described above. Yoshida *et al.*[27] confirmed that MDP-containing adhesives do form nanolayers at the adhesive interface. These authors evaluated if CSE and SBU would be able to produce nanolayers at their adhesive/dentin interface and observed that for CSE (an ultrastructurally relatively easily nanolayer) at the transition of the hybrid layer to the adhesive interface. For SBU, the nanolayer was found primarily near the dentin tubules, most likely having made use of calcium present within the remained smear plugs. Also Yoshihara *et al.*[33] reported the strong hydrophobicity of the nanolayered structure that can protect the hybrid layer against hydrolytic bond degradation processes[34,35]. Considering the features of the two self-etching adhesive systems and previous investigations, the satisfactory results obtained on non-etched dentin in our study were expected. When the dentin is pre-etched probably the superficial mineral content is removed, thus the interaction of 10-MDP with HAp is lesser when the dentin is not etched.

It is important to emphasize the application procedure of these self-etching systems. Yoshihara *et al.*[9] proved that actively rubbing the adhesive for 20s on dental surface promotes more intimate contact of 10-MDP molecules with HAp, since the solvent more effectively evaporates from the surface. In addition, on rubbing the 10-MDP adhesive, calcium is released from enamel/dentin and assists the nucleation and growth of calcium salts. Therefore, in our study the application procedure for both self-etching adhesives was rubbing on the dentin surface according to manufacturers' instructions[30,36-38]. That rubbing procedure additionally should produce slightly more demineralization to enable diffusion of the monomers, which subsequently leads to formation of more resin tags[38].

Whereas the result for CSE-et on pre-etched dentin was lower than SBU, CSE, SBMP, and SBU-et, in corroboration with numerous other previous studies. Van Landuyt *et al.*[20] indicated that the bonding effectiveness of CSE can be improved by selectively etching the enamel edges. However, etching should be limited to enamel, etching the dentin formed a low-quality hybrid layer prone to nanoleakage. We hypothesized that since CSE is composed of the acidic primer and bond separately, probably the acidic primer may not infiltrate adequately into the pre-etched dentin and the bond consequently, leaving non-polymerized areas in the interface. Another assumption concerns CSE is a 2-step system and the bond, considered more hydrophobic, is applied later, may turn difficult to hydrophobic bond to infiltrate adequately into demineralized dentin with phosphoric acid and acidic monomer by hydrophilic features. This may be correlated in this study to the failure modes, which were predominantly adhesive in dentin only to that group. For these reasons, it is not recommended to etch dentin prior the application of CSE.

In SBMP, for long considered the gold standard among all categories of adhesive systems, the high bond strength values may be related basically to the micromechanical interaction. The SEM image from SBMP confirmed the micromechanical interaction with uniform and lengthy tag penetrations into demineralized dentin compared with other adhesive systems. Moreover, SBMP contain polyalkenoic acid copolymer, which has chemical interaction to dentin substrate, through calcium from HAp. Whereas, the EX total-etching adhesive system showed the lowest bond strength values, despite SEM image of interface indicated clearly tag penetrations into demineralized dentin. Possibly, the monomer and solvent composition of EX compromised the bonding strength and quality. According to an *in vivo* study by Perdigão *et al.*[17], the application of EX resulted in statistically lower bond strength than Single Bond. They explain the unfavorable performance of EX to the lack of water in the adhesive. This condition may play a more important role than the type of solvent itself. Another investigation from Bouillaguet *et al.*[39] justified the lower bond strengths of EX for the incomplete polymerization of the adhesive layer, since such adhesive layers are more sensitive to water and oxygen contamination.

In the present study SBU is the adhesive system that probably contains the least HEMA. This might be a positive concept in terms of durability of adhesive-dentin interface. HEMA is a water-soluble monomer with its hydroxyl group, which does not interact ionically with HAp[32,33,40,41]. According to Yoshida *et al.*[41] HEMA inhibits the formation of interfacial nanolayers, this occurs because of the suppression of nucleation and growth of MDP-Ca salts by intermolecular interactions between HEMA and MDP. Despite the little amount of HEMA in the SBU, the presence of 'Vitrebond copolymer' allows to have more chemical interaction with HAp, consequently more stable chemical structures are formed with an important role in the longevity of bonding interface. In the SEM images from SBU, we could observe a thin hybrid layer compared with other adhesive systems. However, that interface seems to be an adequate interaction to the dentin and no evidence of gaps were found.

From our results, it is advisable to use both self-etching adhesives without etching the dentin surface. Nevertheless, the best clinical situation of self-etching systems still recommends the application of phosphoric acid on enamel edge despite the risk of this acid running over to dentin. Although SBU-et has demonstrated lower bond strength values than SBU, our supposition is that the combination of functions of 'Vitrebond copolymer' and 10-MDP allows to avoid the side effect of the phosphoric acid running over to dentin.

Clearly, the adhesion strategies have been improved by 'Adhesion-Decalcification concept'. This concept has progressively been consolidated in resin composite-tooth substrate interaction using 10-MDP contain self-etching adhesive systems. Several investigations with 10-MDP have demonstrated that acidic functional monomer has a strong chemical affinity for the calcium in HAp, which is essential for the longevity of restorations. Despite total-etching systems with micromechanical retention can achieve adequate results, several problems regarding durability of restorations have been scientifically questioned for a long time, such as dentin characteristics[42], degradation of exposed collagen non-infiltrated by resin monomers, non-polymerized infiltrated resin, and postoperative sensitivity. Technique sensitivity and a number of steps of application must also be considered critical for clinicians. Therefore, it is expected that chemical interaction concept could minimize those difficulties from conventional micromechanical adhesion concept and increase the durability of esthetics restorations.

Based on these considerations, we can conclude that 37% phosphoric acid etching for 15s prior application of SBU and CSE self-etching adhesive systems decrease the bonding strength on dentin. However, the SBU-et did not differ from the gold standard total-etching adhesive system SBMP. Despite the thin hybrid layer, the one-step multi mode SBU and 2-step self-etching adhesive CSE showed high μTBS values on dentin. Further studies must be conducted regarding aging and interaction to demineralized dentin substrate.

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CONSIDERAÇÕES GERAIS

O surgimento dos sistemas adesivos autocondicionantes teve como objetivo superar as dificuldades e otimizar a qualidade de união obtida com os sistemas adesivos convencionais, tais como reduzir os passos clínicos por meio da simplificação do procedimento restaurador e eliminar a sensibilidade da técnica (Perdigão *et al.,* 2003, Van Meerbeek *et al*., 2011). A incorporação de componentes que contribuam para o aumento na resistência e durabilidade de união (Fukuda *et al.,* 2003) revelam resultados promissores, de forma que o desempenho dos adesivos tem mostrado boas propriedades mecânicas imediatas, independentemente da estratégia de união abordada (Perdigão *et al.,* 2003, De Munck *et al.,* 2005). Podendo-se considerar a técnica e a composição do sistema adesivo utilizado, os fatores mais importantes quanto à efetividade de união (Fu *et al.,* 2005).

Desta forma, o conceito de que a adesão se baseia principalmente na retenção micromecânica vem dando espaço ao novo conceito de interação química chamado de "*Adhesion-Decalcification Concept*", proposto por Yoshida *et al.* (2001), que indica que a descalcificação da hidroxiapatita por meio dos ácidos carboxílicos são dependentes da solubilidade dos sais carboxílicos, independentemente da concentração e do pH (Yoshida *et al.,* 2001).

Neste estudo, compararam-se sistemas adesivos convencionais e autocondicionantes, compostos por diferentes números de passos, a partir da análise da influência do condicionamento ácido prévio em dentina e em esmalte, associados aos sistemas autocondicionantes. O fabricante do sistema adesivo de passo único SBU afirma que este produto pode ser utilizado em dentina/esmalte e com/sem condicionamento ácido prévio, diferentemente do que é preconizado pelos outros sistemas autocondicionantes (Perdigão *et al.,* 2003). Este sistema possui o monômero 10-MDP e o copolímero *Vitrebond*™, ambos apresentando capacidade de interagir quimicamente com os substratos dentais (Yoshida *et al.,* 2012).

Estudos prévios demonstraram que o monômero funcional acídico 10-MDP e o copolímero *Vitrebond*™ possuem afinidade química ao cálcio da hidroxiapatita (Yoshida *et al.,* 2012). Acredita-se que essas interações contribuam para melhores resultados de resistência de união (Yoshida *et al.,* 2012*)*, gerando inclusive maior longevidade (Moszner *et al*., 2005). O bom desempenho imediato obtido com os adesivos SBU e CSE no presente estudo pode ser relacionado à presença do monômero 10-MDP na composição desses adesivos autocondicionantes. (Yoshida *et al.,* 2012). Porém, quando associados ao condicionamento ácido prévio em dentina, houve diminuição dos valores médios de resistência de união. A partir dos achados da literatura, esse resultado já era esperado, uma vez que o conteúdo mineral superficial é removido, diminuindo as ligações do 10-MDP ao cálcio da hidroxiapatita (Yoshida *et al*., 2012*)*.

Em esmalte, o condicionamento ácido prévio aumentou os valores de resistência de união para os sistemas autocondicionantes, uma vez que uma maior área de superfície com cálcio ficou disponível para ligar-se aos monômeros 10-MDP e maior embricamento mecânico foi obtido (Yoshida *et al.,* 2012*)*. Devido à composição predominantemente inorgânica do esmalte (Gwinnett *et al.,* 1967), o uso de condicionamento ácido não consiste em uma etapa técnica crítica quanto à durabilidade de união nesse substrato. Além disso, esta etapa expõe maior área de superfície dos cristais de hidroxiapatita e melhora a molhabilidade do adesivo, permitindo a formação de uma área de união considerável e resistente. O embricamento mecânico em esmalte apresenta ainda melhores resultados quando associado à uma adesão química (Yoshida *et al*., 2004). As imagens em MEV dos grupos SBU e CSE com pré-condicionamento evidenciaram esta melhoria na união, pois mostraram nítidas extensões do adesivo penetrado no esmalte desmineralizado. Apesar dos maiores resultados de resistência de união associados ao uso dos sistemas adesivos SBU e CSE em esmalte condicionado, os valores obtidos na ausência do condicionamento prévio do esmalte não apresentaram diferença estatisticamente significante com o grupo SBMP, considerado um sistema adesivo “padrão-ouro”, podendo portanto ser considerados satisfatórios. O que permite concluir que é possível utilizar os sistemas autocondicionantes desse estudo associados ou não ao condicionamento prévio do esmalte.

No substrato dentinário, a degradação das fibrilas colágenas devido à incompleta penetração dos monômeros e a degradação adesiva advinda da polimerização resinosa insuficiente têm afetado a estabilidade de união da técnica com condicionamento ácido prévio (Perdigão *et al*., 2003). Com a técnica autocondicionante, essas dificuldades são minimizadas uma vez que os monômeros ácidos desmineralizam e penetram no substrato simultaneamente, gerando mínima exposição da camada orgânica desmineralizada e melhor polimerização resinosa do adesivo (Van Landuyt *et al*., 2006, Van Meerbeek *et al*., 2008).

Quanto ao tratamento deste substrato, o pré-condicionamento provocou redução dos valores de resistência de união dos grupos SBUcond e CSEcond. A hipótese para os valores obtidos em CSEcond é que o *primer* ácido provavelmente não conseguiu infiltração adequada na dentina pré-condicionada e após a aplicação do adesivo, ficaram áreas não polimerizadas na interface. Porém, o SBUcond não obteve diferença estatística do grupo do adesivo convencional SBMP. Ao analisar as imagens em MEV, foi possível observar que o adesivo SBMP produziu interações micromecânicas por meio da penetração uniforme dos *tags* resinosos na dentina desmineralizada. O SBMP, assim como os grupos SBU e CSE obtiveram os maiores valores de resistência de união, seguido do grupo CSEcond e do EX, que apresentou os menores valores de resistência de união. Provavelmente, os monômeros e solventes na composição deste último adesivo comprometeram a resistência de união, embora as imagens em MEV desse grupo tenham mostrado com clareza a penetração dos *tags* na dentina desmineralizada.

Portanto, supõe-se que a técnica de condicionamento seletivo em esmalte, apesar de ser uma técnica crítica pela dificuldade clínica da limitação do substrato, pois pode atingir dentina, é a mais adequada quando utilizam-se os sistemas adesivos autocondicionantes CSE e SBU. Considerando-se as limitações dos dois estudos, para melhores previsões de interação micromecânica e química, se faz necessário ampliá-los no âmbito clínico e laboratorial, com o envolvimento do envelhecimento da interface, na contínua busca pela obtenção de resultados de baixos índices de degradação marginal e melhor união ao substrato dental.

CONCLUSÃO

Pelos resultados encontrados nos dois estudos realizados pode-se concluir que:

- O pré-condicionamento com ácido fosfórico na superfície de esmalte com aplicação do sistema adesivo de passo único *multi-mode* SBU e do sistema adesivo autocondicionante de 2 passos CSE possibilitou o aumento significativo dos valores de RU. Nas imagens de MEV de ambos os grupos, observou-se nítida formação de camada híbrida e penetração de *tags* resinosos. Porém, quando estes adesivos são aplicados na técnica autocondicionante, os valores de RU não foram significativamente diferentes do sistema adesivo de condicionamento total de 3 passos SBMP, considerado “padrão-ouro” na literatura.
- O pré-condicionamento com ácido fosfórico na superfície de dentina com aplicação do sistema adesivo de passo único *multi-mode* SBU e do sistema adesivo autocondicionante de 2 passos CSE reduziu significativamente os valores de RU. Entretanto, os resultados obtidos para o SBUcond não foram diferentes estatisticamente dos obtidos pelo sistema adesivo convencional considerado padrão-ouro, SBMP. Apesar das imagens em MEV apresentarem fina camada híbrida formada, o sistema adesivo *multi-mode* SBU e o sistema adesivo autocondicionante CSE obtiveram os maiores valores de RU.

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

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Avaliação da morfologia e resistência de união de adesivos contemporâneos sobre a superfície de esmalte e dentina"**, protocolo nº 113/2011, dos pesquisadores Mario Fernando de Goes, Marcela Santiago Freitas e Renata Fernandes de Souza Lacerda, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 03/11/2011.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Morfology and bond strenght evaluation of comtemporary adhesives on the surface of enamel and dentin"**, register number 113/2011, of Mario Fernando de Goes, Marcela Santiago Freitas and Renata Fernandes de Souza Lacerda, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 11/03/2011.

Lívia M. A. Tenuta

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

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
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