MIRELA SANAE SHINOHARA

# EFEITO do FLÚOR de um SISTEMA ADESIVO na RESISTÊNCIA DE UNIÃO e no DESAFIO ÁCIDO em DENTINA

Faculdade de Odontologia de Piracicaba - UNICAMP

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

Orientador: Prof. Dr. Mario Fernando de Goes

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# RESUMO

Apesar do avanço na odontologia adesiva, não é possível conseguir uma união perfeita da resina composta ao substrato dental. Diversos são os adesivos dentinários atualmente presentes, mas nenhum é capaz de impedir a infiltração marginal, degradação e formação de cárie secundária na interface dente-resina ao longo do tempo. Dessa forma, como alternativa, o flúor foi incorporado a um adesivo com o objetivo de promover uma ação antibacteriana e impedir a formação de cárie secundária, consequentemente aumentar a longevidade da restauração. Portanto, este estudo teve como objetivos: (1) avaliar a influência do flúor presente no adesivo na resistência de união em dentina e analisar a estrutura da interface dente-adesivo após desafio ácido por meio de microscopia eletrônica de varredura (MEV); (2) avaliar a influência do flúor no adesivo na resistência de união em dentina após 24 horas e 3 meses de estocagem de água, observar a formação de uma zona ácido-resistente na interface dentina-resina após 24 horas e 3 meses de estocagem em água por meio de microscopia de luz polarizada (MLP), e comparar o grau de conversão do adesivo que contém flúor com dois adesivos sem flúor após 24 horas, 1 semana e 1 mês. Tanto para o teste de resistência à microtração, como para análise das interfaces: terceiros molares humanos hígidos recém-extraídos foram usados para realização das restaurações, de acordo com os grupos analisados em cada estudo específico. Para a análise do grau de conversão, pastilhas dos adesivos foram preparados em temperatura controlada e atmosfera de nitrogênio para análise de espectrofotômetria de infravermelho pela técnica transformada de Fourier em modo de transmissão (FTIR). Os resultados mostraram que o flúor presente no adesivo apresenta um efeito positivo na resistência de união, embora apresente menor grau de conversão comparado aos adesivos sem flúor. As imagens de MEV e MLP mostraram que o adesivo com flúor forma uma zona de inibição adjacente à camada híbrida após desafio ácido.

Palavras-chave: sistemas adesivos, flúor, dentina e zona ácido-resistente

# ABSTRACT

Despite of the advance in adhesive dentistry, it is not possible to achieve an ideal bonding between composite resin and dental substrate. Currently, a variety of adhesive systems have been used. However, any adhesive system is capable to hamper the marginal leakage, degradation, and secondary caries formation at dentin-resin interfaces in long-term. For that reason, as an alternative, fluoride was incorporated in adhesive in order to promote an antibacterial action and to impede the secondary caries formation, consequently increasing the longevity of restoration. Thus, the objectives of this study were: (1) to evaluate the influence of a fluoride-containing adhesive on the microtensile bond strength (μTBS) in dentin, and to analyze the ultra-structure of the dentin-adhesive interface after acid challenge; (2) to evaluate the influence of fluoride-containing adhesive on μTBS after 24 hours and 3 months water-storage; to observe an acid-resistant zone at resin-dentin interface using polarized light microscopy (PLM) after 24 hours and 3 months water storage, and to compare the degree of conversion of fluoride-containing adhesive with non-fluoride containing adhesives after 24 hours, 1 week, and 1 month. For both, to the μTBS test and to analyze the ultra-structure of interfaces: free-caries human third molars were used for restorations, in accordance with of the analyzed groups in each specific study. In order to measure the degree of conversion, chips of adhesives were prepared at a constant temperature and in a nitrogen atmosphere for the analysis at Fourier Transform-Raman spectroscopy (FTIR). The results showed that the present fluoride-containing adhesive has a positive effect in bonding strength, although the lower degree of conversion compared with non-fluoride adhesives. The photomicrographs of SEM and PLM demonstrated that fluoride-containing adhesive allow the formation of an inhibition zone adjacent to the hybrid layer after acid challenge.

Key-words: adhesive systems, fluoride, dentin, acid-resistant zone

# SUMÁRIO

# INTRODUÇÃO

O sucesso de uma restauração depende de uma união estável e durável entre o substrato dental e o material restaurador (De Munck *et al.* 2005). Embora o avanço da odontologia restauradora e adesiva tenha possibilitado o surgimento de inúmeros materiais com características estéticas e propriedades mecânicas adequadas, ainda são diversos os problemas subseqüentes ao procedimento restaurador, tais como: microinfiltração marginal, queda na resistência de união, hipersensibilidade e possível ocorrência de cárie secundária, comprometendo a longevidade das restaurações (Kidd *et al.*1992; Fontana *et al.*2000).

Dessa forma, estudos voltados à adesão da resina ao dente vêm sendo um dos grandes desafios científicos. Sabe-se que a união da resina composta ao substrato dental depende de um terceiro componente indispensável: o sistema adesivo. Nakabayashi, em 1982, denominou a região da dentina desmineralizada por um ácido e infiltrada pelos monômeros adesivos como camada híbrida. Portanto, teoricamente, uma efetiva adesão depende da formação de uma camada híbrida ácido-resistente capaz de barrar qualquer infiltração de fluidos e bactérias da cavidade bucal.

Apesar da grande variedade de sistemas adesivos existentes no mercado, sendo que os últimos lançamentos surgiram para simplificar a técnica e diminuir o tempo de trabalho, estudos têm mostrado que a durabilidade e a longevidade produzida por esses materiais são de curto-prazo, já que o sucesso máximo depende de uma restauração com características físico-químicas não degradáveis à ação bioquímica e mecânica da cavidade bucal.

Atualmente, têm-se basicamente dois tipos de técnicas adesivas da resina composta ao substrato dental: a técnica do condicionamento ácido total, também chamada de técnica úmida e a técnica autocondicionante. Na primeira técnica aplica-se o ácido fosfórico 35-37% previamente à aplicação dos agentes de união (primer + adesivo), o qual produz uma desmineralização do substrato dental promovendo a remoção da camada de esfregaço sobre o preparo cavitário; seguido da aplicação do primer, que facilita a infiltração dos monômeros resinosos provenientes do adesivo. Enquanto que a técnica autocondicionante envolve um processo simultâneo de dissolução da camada de esfregaço e desmineralização do substrato dental juntamente com a infiltração dos monômeros resinosos. Embora, essas técnicas possibilitem uma resistência de união com valores *in vitro* e *in vivo* satisfatórios (De Munck *et al.* 2005; Eick *et al.* 1997), a imcopatibilidade dos monômeros hidrófilos e hidrófobos, além da presença da água tanto no substrato dental como no adesivo têm sido características negativas para uma efetiva adesão (Spencer *et al.* 2002; Tay *et al.* 2004).

Visto que ainda não se tem um sistema adesivo capaz de formar uma união dente-resina durável sem alterações ao longo tempo, estudos direcionados à incorporação de componentes que permitam aumentar a longevidade da restauração têm sido propostos. Atualmente, sistemas adesivos que apresentam em sua composição um monômero antibacteriano e liberação de flúor têm mostrado serem positivos em manter uma integridade marginal, diminuir a degradação da união dente-restauração e conseqüentemente prevenir possíveis cáries secundárias (Han *et al.* 2002; Nakajima *et al.* 2003; Itota *et al.* 2003). Imazato *et al.,* em 1997, incorporaram o monômero antibacteriano MDPB *(methacryloxydodecylpyridinium bromide)* no primer de sistemas adesivos e verificaram que o MDPB apresentou um efeito antibacteriano, além de não interferir na polimerização do adesivo e na resistência de união.

Dessa forma, surgiu um sistema adesivo autocondicionante que apresenta em sua composição o MDPB no primer e o flúor no adesivo, Clearfil Protect Bond (Kuraray Medical Inc.). Estudos prévios têm relatado a ação positiva do flúor presente nos materiais restauradores na inibição de cáries secundárias e remineralização do substrato dentinário após desafio cariogênico artificial. Em relação à presença do flúor no adesivo, não é completamente compreendido o mecanismo de ação do mesmo na interface dente-adesivo-resina.

Ferracane *et al.*, em 1998, verificou que um adesivo dentinário contendo flúor foi capaz de liberar o flúor nos microespaços presentes na cavidade restaurada, e esse flúor é capaz de penetrar na camada híbrida. Observou também que o flúor não foi detectado nas regiões da camada híbrida e dentina que não apresentavam áreas de infiltração, sugerindo que a água é necessária para a dissolução e transporte do íon flúor.

A literatura mostra que os adesivos que aplicam a técnica do condicionamento ácido total levam a formação de uma camada de dentina desmineralizada não infiltrada pelo monômero resinoso, pois o adesivo não é capaz de penetrar totalmente na dentina condicionada (De Munck *et al.* 2005). Ou seja, formando uma área susceptível a infiltração de fluidos e bactérias e posterior degradação, agindo como uma membrana permeável. Da mesma forma, essa membrana permeável é existente mesmo quando se utiliza os adesivos autocondicionantes, devido a presença de nanoespaços na camada híbrida. Por conseguinte, os sistemas adesivos ainda não são capazes de formar uma interface impermeável e resistente contra os fluidos do substrato dentinário e da cavidade bucal. Visto que os monômeros hidrófobos e hidrófilos são susceptíveis à hidrólise, assim como as fibrilas colágenas da dentina são à degradação.

Diferentes composições de sistemas adesivos estão presentes, mas nenhuma ainda permite um selamento marginal estável da restauração, principalmente devido à presença da água no agente adesivo, assim como no substrato dental. Perante os estudos prévios, este trabalho teve como objetivo avaliar o efeito do flúor presente em um sistema adesivo na adesão à dentina.

# CAPÍTULO 1

Evaluation of Antibacterial and Fluoride-releasing Adhesive System on Dentin – Microtensile Bond Strength and Acid-base Challenge

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TITLE

# Evaluation of Antibacterial and Fluoride-releasing Adhesive System on Dentin - Microtensile Bond Strength and Acid-base Challenge

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## SYNOPSIS

This study evaluated the influence of a fluoride-containing adhesive on microtensile bond strength (µTBS) to dentin, as well as analyzed the dentin-adhesive interface after acid-base challenge. Experimental groups were: G1 - Clearfil SE Bond control (SE); G2 - Clearfil Protect Bond control (PB); G3 - Primer[SE]/Adhesive[PB]; G4 - Primer[PB]/Adhesive[SE]. For µTBS evaluation, dentin surfaces were ground, bonded, and composite resin crowns were built up to obtaining beams to be tested. For interfacial analysis, adhesive system was applied on dentin surface and a low -viscosity resin was placed between two dentin disks. Then, the specimens were subjected to acid-base challenge, sectioned, and polished to be observed by SEM. µTBS data showed no statistical differences among the groups (G1: 51.3, G2: 47.6, G3: 55.0, G4: 53.9; mean in MPa). Through SEM, it was observed that a thick acid-base resistant zone adjacent to the hybrid layer was created only when the fluoride-releasing adhesive was used. In conclusion, the presence of fluoride in an adhesive contributed significantly to preventing secondary caries, and did not interfere with dentin-adhesive bond strength.

# INTRODUCTION

The success of restorations hinges on the adhesion stability between composite resin and tooth structure. To maintain this adhesion stability, the presence of a hybrid layer is essential in that it forms a resistant structure against bacterial invasion. Currently, simplified systems such as self-etching primer/adhesive systems have demonstrated good clinical performance to dentin. This approach is less technique-sensitive and reduces the time required for the bonding procedure. Although advances in adhesive dentistry have brought improvements in bonding systems and techniques, bond failures at the tooth-restoration interface remain a challenge in the dental field.

Microgaps between restorative materials and the cavity wall permit invasion of fluids and bacteria, leading to secondary caries. This occurs probably due to inadequate marginal adaptation of composite restorations 1). According to previous reports 2,3), secondary caries is the commonly cited reason for failure and replacement of restorations. Hence, antibacterial activity is considered to be an important beneficial property of dentin bonding systems for successful restorative treatments.

From this point of view, new versions of adhesive system containing fluoride in composition have been introduced in order to inhibit the action of secondary caries arising from enamel cracks or microleakage on the tooth-restoration interface. With regard to these fluoride-releasing restorative materials, some researchers have extensively demonstrated their significant cariostatic and antibacterial effects 4,5).

An experimental two-step self-etching primer/adhesive system, ABF (former name Clearfil Protect Bond) - composed of an antibacterial primer containing MDPB (12-methacryloyloxydodecylpyridinium bromide) and a fluoride-releasing adhesive - has shown the potential in artificial secondary caries inhibition around restorations 6-8). However, there is little information about the influence of antibacterial components on bonding strength. Likewise, information is scarce concerning antibacterial primer performance and fluoride-releasing adhesive effect against artificial caries challenge.

Tsuchiya *et al.* 9) described the formation of an acid-base resistant zone adjacent to the hybrid layer after acid-base challenge. Results of the study clearly showed the influence of adhesive material composition in the formation of the acid-base resistant zone. Although the characteristics of that resistant zone are still unclear, their results suggested the potential effect of self-etching primer adhesive systems in inhibition secondary caries.

Therefore, the purpose of the present study was to evaluate the influence of primer and bond agent – by interchanging between a self-etching primer adhesive system with antibacterial properties (Clearfil Protect Bond) and a non-fluoride containing self-etching primer adhesive system (Clearfil SE Bond) - on microtensile bond strength. In addition, *in vitro* inhibitory effect on artificial secondary caries inhibition around adhesive restorations was observed using a SEM.

## MATERIALS AND METHODS

Table 1 lists the compositions, manufacturers, and batch numbers of the materials employed in this study.

Sixteen caries-free extracted human third molars were obtained under a protocol (#023/2004) approved by the Ethical Committee at the Piracicaba School of Dentistry, State University of Campinas. Before use, the teeth were cleaned of debris and stored in physiological saline containing 0.1% thymol.

TABLE 1

Materials used in this study.

| Material | Components | Batch Number | Manufacturer |
|---|---|---|---|
| Adhesive System | | | |
| *Clearfil SE Bond (SE)* | Primer: MDP, HEMA, water, PI, accelerators, CA<br>Adhesive: MDP, HEMA, dymethacrylates, PI, CA, microfiller | Primer: 00507A<br>Adhesive: 00712A | Kuraray Medical Inc., Tokyo, Japan |
| *Clearfil Protect Bond (PB)* | Primer: MDPB, MDP, HEMA, MFM, PI, water<br>Adhesive: MDP, HEMA, MFM, PI, microfiller, NaF | Primer: 000010<br>Adhesive: 000017 | Kuraray Medical Inc., Tokyo, Japan |
| Restorative Material | | | |
| *Clearfil AP-X* | Bis-GMA, TEG-DMA, barium glass filler (85wt%), PI, accelerators | 01063A | Kuraray Medical Inc., Tokyo, Japan |
| *Metafil flo* | 2,2-Bis[4-(methacryloxy-polyethoxy)phenyl-]propane, bi-functional methacrylate monomers, silica, 3-(trimethoxysilyl) propyl methacrylate | GS1 | Sun Medical, Moriyama, Japan |

**Abbreviations:** MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; PI: photoinitiator; CA: catalyst; MDPB: methacryloexydodecyl pyridinium bromide; MFM: multifunctional methacrylate; NaF: sodium fluorite; Bis-GMA: bisphenyl glycidyl methacrylate; TEG-DMA: triethylene glycol dimethacrylate

### Experiment 1 - Microtensile bond test

### 1. Tooth specimen preparation

For each tooth, the coronal portion was removed to expose a flat, midcoronal dentin surface using a low speed diamond saw under water refrigeration (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) (Fig.1A). The exposed dentin surface was ground using 600-grit silicon carbide abrasive paper under water stream for 60 seconds to produce a standardized smear layer (Fig.1B).

### 2. Bonding and restorative procedures

Prior to adhesive application, the teeth were randomly assigned into four groups, according to the bonding procedures shown in Table 2. After applying the bonding resin, a composite resin, Clearfil AP-X, was built up using increments approximately 1 mm thick. Each increment was light-activated for 40 seconds (Optilux 500/Demetron-Kerr, Danbury, CT, USA).

TABLE 2

Application procedures of adhesive systems.

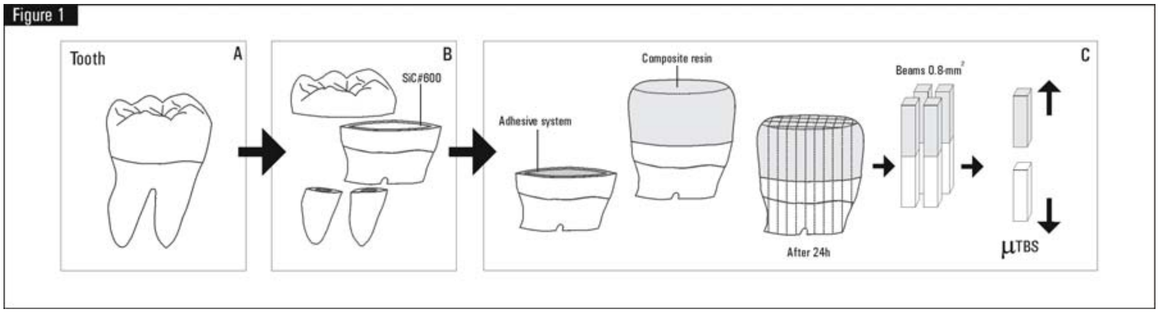
| Groups | Application Procedure |
|---|---|
| *Clearfil SE Bond (SE)* | Primer: apply 20s, air-dry<br>Adhesive: apply and 10s light-cure |
| *Clearfil Protect Bond (PB)* | Primer: apply 20s, air-dry<br>Adhesive: apply and 10s light-cure |
| Primer: *SE*<br>Adhesive: *PB* | Primer: apply 20s, air-dry<br>Adhesive: apply and 10s light-cure |
| Primer: *PB*<br>Adhesive: *SE* | Primer: apply 20s, air-dry<br>Adhesive: apply and 10s light-cure |

### 3. Microtensile test

After the specimens had been stored in distilled water at 37°C for 24 hours, the bonded samples were sectioned perpendicular to the adhesive interface into serial slabs with a diamond saw under water lubrication. Each slab was further sectioned to obtain beams with an adhesive area of approximately 0.8 mm$^2$.

Then, the specimens were fixed to a testing apparatus with a cyanocrylate adhesive (Zapit, Dental Ventures of American, Anaheim, USA) and subjected to microtensile testing at a crosshead speed of 1mm/min (EZ – test, Shimadzu Co, Kyoto, Japan) (Fig.1C). The mean bond strengths obtained were analyzed using the Kruskal-Wallis One-Way Analysis of Variance on Ranks test. The fractured beams of debonded specimens were sputter-coated with gold and observed under a SEM (JSM-5310LV-JEOL, Tokyo, Japan) to determine the mode of failure. Failure modes were classified into four groups: (1) failure in adhesive resin; (2) failure at hybrid layer; (3) failure in dentin; and (4) mixed failure between hybrid layer and partial failure in adhesive resin.

Fig. 1 Specimen preparation (µTBS).



Experiment 2 – SEM interfacial observation after acid-base challenge

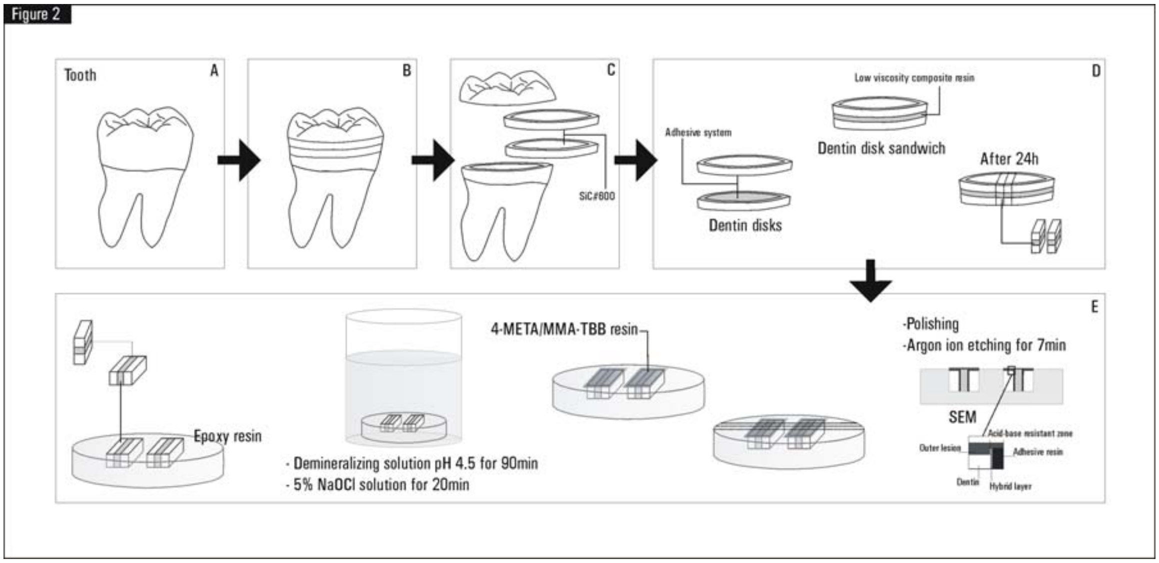
Approximately 1.5 mm-thick dentin disks were obtained from the midcoronal portion of each tooth with the use of a diamond saw under running water (Fig.2A). Two disks were obtained from each tooth (Fig.2B). Each surface of the dentin disk was ground with 600-grit silicon carbide paper under running water for 60 seconds (Fig.2C), and the adhesive system was applied. Bonding procedures were conducted in the same manner as previously described.

After applying the bonding resin, a thin layer of a low-viscosity composite resin Metafil flo (Sun Medical, Moriyama, Japan), was placed between two disks and light-activated to produce a dentin disk sandwich. The resin-tooth bonded specimens were stored in distilled water at $37^{o}$C for 24h. Subsequently, the dentin disk sandwich was vertically sectioned at the dentin-adhesive interface (Fig.2D), and the blocks of dentin disk sandwich were embedded in epoxy resin.

Specimens were subjected to an acid challenge by being immersed in a buffered demineralizing solution (2.2 mmol/L $CaCl_2$, 2.2 mmol/L $NaH_2PO_4$, and 50 mmol/L acetic acid), adjusted to pH 4.5 for 90 minutes. In a pilot study, the time for acid challenge was determined by SEM observation of the artificial caries lesion being of approximately 10 µm depth. After the acid challenge, specimens were immersed in 5% sodium hypochlorite solution for 20 minutes to remove any demineralized dentin collagen fibrils, and then rinsed with running water for 60 seconds.

After which, a 4-META/MMA-TBB resin (Super Bond C&B, Sun Medical, Moriyama, Japan) was applied to protect the demineralized surface from the polishing procedure. The samples were then vertically sectioned at the dentin-adhesive interface and polished with diamond pastes down to 0.25vμm. The polished samples were etched with an argon ion beam (EIS–IE, Elionix, Tokyo, Japan) for seven minutes for distinct ultrastructural identification of the dentin-adhesive interface (Fig.2E). Following which, the samples were sputter-coated with gold, and the morphological changes of the dentin-adhesive interface produced by acid-base challenge were observed under a SEM.

Fig. 2 Specimen preparation (SEM).



## RESULTS

### Microtensile bond strength results

Mean bond strengths and standard deviations are shown in Table 3. There were no significant differences among the groups (p=0.538). The results suggested that the incorporation of an antibacterial monomer in the primer and a fluoride-releasing material in the adhesive did not compromise the microtensile bond strength values.

Failure mode proportions of the debonded specimens are shown in Fig.3. Most of the failures were adhesive or mixed failures regardless of the group.

TABLE 3

Results of microtensile bond strength test.

| Groups | MPa (SD) |
|---|---|
| *Clearfil SE Bond (SE)* | 51.30 (19.6)* |
| *Clearfil Protect Bond (PB)* | 47.64 (10.7)* |
| Primer: *SE* / Adhesive: *PB* | 54.99 (10.0)* |
| Primer: *PB* / Adhesive: *SE* | 53.85 (12.6)* |

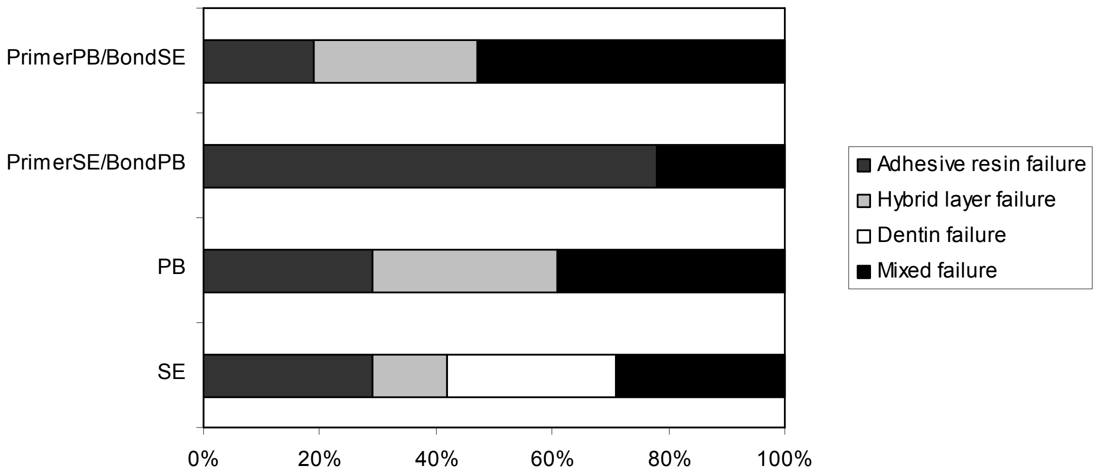
* Indicate no statistically significant difference (p=0.538)
Kruskal-Wallis One Way Analysis of Variance on Ranks

## Scanning electron microscopy analysis

Fig. 4 shows the representative SEM images from each group analyzed in this study. Outer lesions and acid-base resistant zones were observed in all specimens. Depth of the outer lesion ranged from 7 to 10 µm after acid-base challenge. In parallel, a thin hybrid layer - approximately 0.5 µm thick – was also observed in all groups. SEM analysis showed that the structures of both adhesive and hybrid layer were not damaged after acid-base challenge, regardless of the material used.

Figure 4a SE/SE shows the interface of Clearfil SE Bond adhesive system and dentin. The presence of the acid-base resistant zone (approximately 0.5µm thick) beneath the hybrid layer was clearly observed. Similarly, the acid-base resistant zone was observed when PB primer and SE adhesive were applied (Fig.4d). The observed acid-base resistant zone was homogeneous and parallel to the hybrid layer. On the other hand, the interfaces of Clearfil Protect Bond group and SE primer/PB adhesive group (Figs 4b and 4c) showed sharp formation of a thick acid-base resistant zone (over 1.0 µm thick) adjacent to the hybrid layer. Further, the acid-base resistant zone was formed from the upper slope to the end of outer lesion. Therefore, the thick acid-base resistant zone adjacent to the hybrid layer could be observed only when the fluoride-releasing adhesive was used.

Fig. 3 Incidence (%) of failure patterns according to SEM analysis.



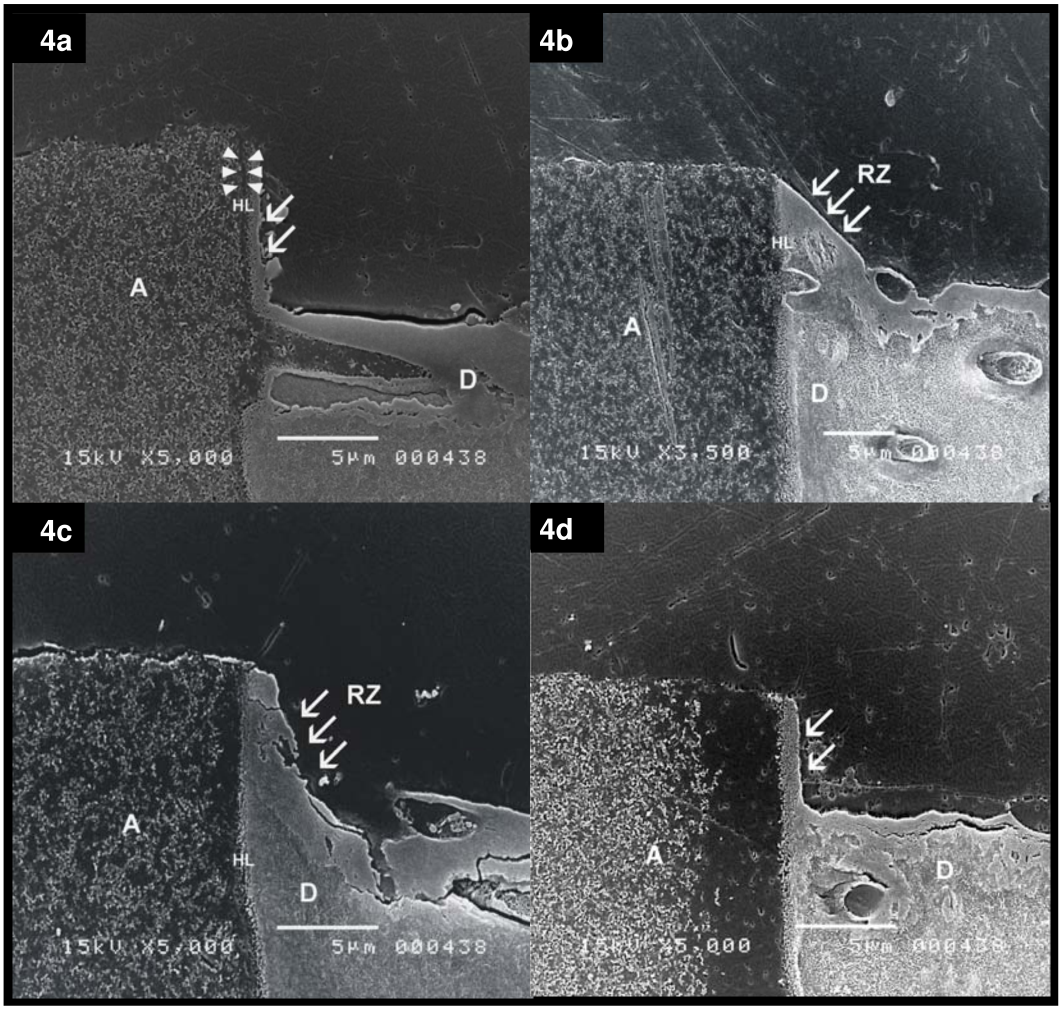


Fig. 4 SEM images of the ultrastructure of the interfaces after acid challenge.

(a) SE/SE Clearfil Se Bond: acid resistant zone of approximately 0.50 μm wide (arrows) could be clearly observed beneath the hybrid layer (center of arrowheads); it is a thin and homogeneous zone parallel to the hybrid layer;

(b) PB/PB Clearfil Protect Bond: thick acid-base resistant zone could be observed close to the hybrid layer, and which slopes from the top of the outer lesion;

(c) SE/PB Primer of CSE and Adhesive of CPB: similar to the adhesive interface of CPB, a thick acid-base resistant zone over 1.0 μm wide (arrows) was observed;

(d) PB/SE Primer of CPB and Adhesive of CSE: thin acid-base resistant zone of approximately 0.50 μm width could be observed (center of arrowheads).

A=adhesive resin; D=dentin; HL=hybrid layer; and RZ=acid-base resistant zone.

## DISCUSSION

Fluoride release from restorative materials has been extensively researched for many years [5,10,11]. This is because fluoride has been shown to exhibit anticariogenic activity by increasing enamel and dentin resistance to subsequent acid attack as well as inhibit carbohydrate metabolism in dental plaque. It has been widely accepted that fluoride could facilitate remineralization or prevent demineralization of the dental structure [10,12].

Currently, composite resins have been selected as the major direct restorative material in clinical dental practice. Against this background, manufacturers have been trying to develop various fluoride-releasing adhesive systems and composite resins [13]. Studies have reported that fluoride-containing dentin adhesive may release fluoride into marginal gaps, and thereby exert a beneficial effect on adjacent demineralized enamel and dentin [8,14].

In addition, the presence of a fluoride-releasing component in dentin bonding is advantageous in that it imparts its inherent antimicrobial properties [6]. For this reason, antibacterial monomers have been developed and incorporated in dental resins [6,15]. In particular, Imazato *et al.* [16] demonstrated that the antibacterial monomer, MDPB, synthesized from quaternary ammonium dodecylpyridinium, could be considered as the most promising candidate to be accepted as a true non-agent-releasing antibacterial monomer [13,17,18].

Several reports [19-21] have established that self-etching primer/adhesive systems could certainly be used in restorative dentistry because of their ability to provide efficient marginal sealing. However, even if an adhesive system shows high bond strength, secondary caries is still found in clinical resin restorations after long-term use.

The primer of PB has an antibacterial monomer (MDPB), and the adhesive has a fluoride-releasing component (treated sodium fluoride). On the other hand, Clearfil SE Bond is an antibacterial-free adhesive system. The objective of this study, therefore, was to verify the influence of interchanging both self-etching primer and adhesive resin (SE and PB) on microtensile bond strength, as well as in the morphology of dentin-adhesive interface after acid-base challenge. In this way, the independent effectiveness and interference from the primer and adhesive of these adhesive systems could be observed.

From the microtensile bond strength test results, there were no statistically significant differences among the groups [7,22]. These results corroborated those of Imazato *et al.* [23,24], whereby it was found that the incorporation of MDPB into dentin primer did not demonstrate any adverse effect on the bond strength or curing behavior [25] of the adhesive system. Regarding the presence of a fluoride-releasing component in the adhesive resin, several studies [6,24,26-29] have established the ability of fluoride ions to inhibit secondary caries by the remineralization of dentin around the restoration. According to Han *et al.* 8), although the mechanism of fluoride action on caries reduction is not fully understood for clinical use, fluoride-releasing adhesive resins and luting cements are useful in preventing the initiation of the caries process and the development of secondary caries in restored teeth.

Nakajima *et al.* 30) reported that the durability of dentin bonding created by a fluoride-releasing adhesive did not change after six-month storage, as compared with a fluoride-free adhesive. They hypothesized that the fluoride component somehow prevented the degradation of dentin, resulting in the improvement of long-term stability at the dentin interface.

Although this study did not specifically evaluate the influence of each component of the adhesive systems (Table 1) on microtensile bond strength, we could conclude that the presence of fluoride-releasing component in the adhesive system did not alter the bond strength values when compared with a non-fluoride adhesive. Therefore, based on the microtensile bond strength test results, PB could be considered a reliable adhesive system for clinical use – with due consideration to its antibacterial activity too.

Besides the high bond strength values, PB exhibited a significant behavior in the acid-base challenge. A demineralizing solution was used to induce the acid attack, and 5% NaOCl solution was used to remove demineralized dentin collagen fibrils 9). An acid-base resistant zone formation was clearly observed through the SEM images. This zone was formed beneath the hybrid layer with or without fluoride-releasing component. Therefore, the SEM method was useful for analyzing the ultrastructural morphology of the dentin-adhesive interface after acid-base challenge 9). Moreover, the argon ion etching technique allowed the hybrid layer to be visibly distinguished from the dentin-resin interface 31).

Toba *et al.* 32) stated that by means of confocal laser scanning microscopy, experimental ABF was found to be effective in inhibiting artificial secondary caries around restoration 29). Nevertheless, thickness of the inhibition zone was relatively thinner than those created with conventional glass-ionomer cements 4,32).

We speculated that the formation of a thick acid-base resistant zone was related to the presence of fluoride-releasing component in the adhesive resin 26,27,33). This was because the formation of a thicker acid-base resistant zone took place only when the fluoride-containing adhesive was used (Figs 4b and 4c). Therefore, the formation of acid-base resistant zone was material-dependent 34). Further, Torii *et al.* 4) suggested that fluoride-releasing adhesive systems may enhance the mineralization of decalcified dentin beneath composite resins and thereby contribute to the longevity of restorations.

With the fluoride-free adhesive system, a thin and homogeneous acid-base resistant zone (approximately 0.5 μm thick) was detected along the interface between dentin and adhesive. According to Tsuchiya *et al.* 9), the existence of the acid-base resistant zone suggested that the monomer had penetrated deeper than the hybrid layer – which was revealed by argon ion etching. Moreover, the micromechanical attachment of adhesive to dentin is acid-base resistant. This could be observed even after acid-base challenge.

Carvalho *et al.* 35) demonstrated that with some mild self-etch adhesive systems, a zone of partially demineralized but uninfiltrated dentin was formed beneath the hybrid layer. They speculated that due to the reduced etching potential of acidic monomers toward the base of hybrid layers, spaces containing products formed by dissolved calcium and phosphate ions during self-etching were created. Hence, a possible explanation for the presence of a thick acid-base resistant zone adjacent to the hybrid layer after acid-base attack, when the fluoride-releasing adhesive was used, was that fluoride was released to those spaces beneath the hybrid layer. As a result, the reaction of fluoride and other products prevented the demineralization of dental structure.

According to Itthagarun *et al.* 1), the fluoride released from bonded fluoride-releasing restorative materials and the potential benefit of artificial caries inhibition are indirect indications of the permeability of dentin adhesives and hybrid layers to water and ion movement. In addition, Ferracane *et al.* 14) reported that a fluoride-containing adhesive released fluoride into the microspaces of a restored cavity, and thus offered some degree of protection from demineralization and recurrent caries.

Although the characteristics of the acid-base resistant zone are still unclear, analysis from the current study adequately suggested that the acid-base resistant zone formation was due to monomer penetration and fluoride release. This was especially observed when the fluoride-containing adhesive of Clearfil Protect Bond was used, since its acid-base resistant zone (over 1.0 μm thick) was thicker than that formed with Clearfil SE Bond (approximately 0.5 μm thickness).

Previous experiments 36-38) have shown the evident nanoleakage within the hybrid layer from non-fluoride releasing self-etching primer/adhesive systems. Through this study, it was shown that the use of self-etching primer/adhesive systems containing an antibacterial monomer (MDPB) and a fluoride-releasing component was beneficial. Apart from rendering protection against secondary caries formation and progression 32), these materials allowed significant bond strength to be yielded. As fluoride-releasing adhesives are in direct contact with the cavity wall.

The progression rate of secondary caries is an important factor that determines the longevity of restorations. As such, further work should be carried out with regard to long-term antibacterial efficacy, as well as the quality and stability, of the acid-base resistant zone beneath the hybrid layer against caries progression.

## ACKNOWLEDGEMENTS

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# CAPÍTULO 2

Fluoride-containing adhesive: durability on dentin bonding

Artigo enviado para publicação na revista Dental Materials.

# Title: Fluoride-containing adhesive: durability on dentin bonding

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## Abstract

*Objectives:* The aim of this study was to evaluate the effect of a fluoride-containing adhesive on dentin bonding durability.

*Methods:* Flat surfaces of human were ground using 600-grit SiC paper and randomly assigned to 6 groups: (SBMP-24) Scotchbond Multi-Purpose control [SBMP], 24h; (SE-24) SBMP etch and primer + Clearfil SE Bond adhesive [SE], 24h; (PB-24) SBMP etch and primer + Clearfil Protect Bond adhesive [PB], 24h; (SBMP-3) SBMP, 3 months; (SE-3) SBMP + SE, 3 months; and (PB-3) SBMP + PB, 3 months. To evaluate the effect of the adhesive resin alone, all teeth were etched with 35% phosphoric acid and primed with SBMP primer prior to applying the adhesive resin. Bonded assemblies were prepared for microtensile bond strength (μTBS) and stored in distilled water at 37°C for 24 hours and 3 months. 0.8-mm$^2$ beams were sectioned and tested for μTBS. Sections of restored teeth of each group were exposed to an acid challenge. The specimens were sectioned, polished, and then observed with polarized light microscopy (PLM). Also, the degree of conversion (DC) of the adhesives was measured using Fourier Transform-Infrared spectroscopy (FTIR) at 24 hours, 1 week, and 1 month after polymerization.

*Results:* μTBS values obtained in MPa (24h/3m) were: (MP) 61.5/52.9, (SE) 55.5/55.6, and (PB) 50.3/61.0. The DC in percentage (24h, 1 week, 1 month) were: (MP) 60.5/65.5/61.3, (SE) 69.6/68.7/70.7, and (PB) 53.1/56.6/58.3. For interface analysis by PLM, an inhibition zone (IZ) adjacent to the hybrid layer was created only when the fluoride-containing adhesive (PB) was used.

*Significance:* The fluoride-containing adhesive demonstrated significant effects on formation of an inhibition zone in dentin after acid challenge. Despite the fact that the DC of the fluoride-containing adhesive was lower than that of SBMP and SE, the μTBS values for this adhesive increased after 3 months storage in water.

## Keywords

Adhesive system, dentin, durability, fluoride-containing material, hybrid layer, inhibition zone, microtensile bond strength, outer lesion, polarized light microscopy, and secondary caries.

## Introduction

Currently resin composites combined with an adhesive system are widely used for direct restorations because of excellent esthetics and acceptable mechanical properties. However, the efficacy of dentin bonding in preventing microleakage and the formation of marginal gaps is still a critical aspect of the clinical success of restorations and remains as a challenge for adhesive dentistry [1,2]. Microgaps at the interface of restorations allow invasion of fluids and bacteria leading to secondary caries, one of the major reasons for failure and replacement of restorations [3,4].

Several studies have demonstrated instability in the adhesion of these biomaterials to tooth tissue [1,2,5,6]. Degradation may occur in the dental substrate or in the polymer components of the resin-dentin interface or both [5,7]. For that reason, the bonding effectiveness remains time-dependent and one of the goals of dental materials science is to improve the longevity of dental restorations.

The formation of a high quality hybrid layer has been considered a key factor for obtaining durable and strong bonding protected from bacteria and the hydrolytic action of oral and dentin fluids, and bacteria [1,2]. Many attempts have been made to produce dental materials that may inhibit bacterial growth [8-11]. New versions of adhesive systems containing fluoride in composition have been suggested in order to inhibit the action of secondary caries arising from enamel cracks or microleakage in the tooth/restoration interface [10-11]. Some researchers have extensively demonstrated the significant cariostatic and antibacterial effect of fluoride-releasing restorative materials [12,13]. Fluoride ions penetrating into the dentin have been shown to enhance mineralization of the dentin [14,15]. Furthermore, Imazato et al. developed an antibacterial monomer MDPB (12-methacryloyloxydodecylpyridinium bromide) that provides antibacterial activity and could be incorporated into dental various dental resins [16-19].

Recently, a two-step self-etching primer adhesive system, composed of an antibacterial primer containing MDPB and a fluoride-releasing adhesive has shown the potential for artificial secondary caries inhibition around restoration [9,11,20]. However, there is little information on the influence of the antibacterial components on bond strength and resistance to an artificial caries challenge. Tsuchiya et al. [21] described the formation of an acid-base resistant zone adjacent to the hybrid layer after acid-base challenge, demonstrating a clear influence of the adhesive material composition on the formation of the acid-base resistant zone and inhibition zone. Although the characteristics of that resistant zone are still unclear, their results suggested the potential effect of self-etching primer adhesive systems in the inhibition secondary caries.

Our previous study [8] showed that the presence of fluoride in the self-etching primer adhesive system demonstrated significant effects on the formation of a thick acid-base resistant zone. Therefore, the objectives of the present study were: (1) to evaluate the influence of fluoride-containing adhesive on microtensile bond strength (μTBS) after 24 hours and 3 months water-storage; (2) to observe in vitro secondary caries inhibition at the resin-dentin interface after 24 hours and 3 months water storage using polarized light microscopy (PLM), and (3) to compare the degree of conversion of fluoride-containing adhesive with non-fluoride containing adhesives after 24 hours, 1 week, and 1 month.

## Materials and Methods

The compositions, manufacturers, and batch numbers of the materials used in this study are listed in Table 1.

Thirty caries-free extracted human third molars were obtained under #023/2004 and approved by the Ethical Committee at the Piracicaba School of Dentistry, State University of Campinas. Before use, the teeth were cleaned of debris and stored in physiological saline solution containing 0.1% thymol.

Table 1

Materials used in this study.

| Material | Components | Batch Number | Manufacturer |
|---|---|---|---|
| Adhesive | | | |
| *Scotchbond Multi-Purpose Plus (SBMP)* | 35% Phosphoric acid etchant<br>Primer: HEMA, polyalkenoic acid copolymer, water<br>Adhesive: HEMA, BisGMA, CQ, amines | Etchant: 4CH<br>Primer: 3AE<br>Adhesive: 3NE | 3M-ESPE, St Paul, MN, USA |
| *Clearfil SE Bond (SE)* | Adhesive: MDP, HEMA, dymethacrylates, PI, CA, microfiller | Adhesive: 00789A | Kuraray Medical Inc., Tokyo, Japan |
| *Clearfil Protect Bond (PB)* | Adhesive: MDP, HEMA, MFM, PI, microfiller, NaF | Adhesive: 00023C | Kuraray Medical Inc., Tokyo, Japan |
| Restorative Material | | | |
| *Z-250 Filtek (A3 shade)* | Microhybrid, BisGMA, Bis-EMA, UDMA, filler loading 77.6 wt% | 4AN | 3M-ESPE, St Paul, MN, USA |
| *Protect Liner F* | TEG-DMA, Bis-GMA, methacryloyl fluoride-methyl, methacrylate copolymer | 0025C | Kuraray Medical Inc., Tokyo, Japan |

Abbreviations: MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; PI: photoinitiator; CA: catalyst; MFM: multifunctional methacrylate; NaF: sodium fluorite; Bis-GMA: bisphenyl glycidyl methacrylate; UDMA: urethane dimethacrylate, CQ: camphorquinone; Bis-EMA: ethoxylated bisphenol A dimethacrylate; TEGDMA: triethylene glycol dimethacrylate.

## 1. Tooth preparation

The coronal portion of each tooth was removed using a low speed diamond saw under water cooling (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) to expose a flat midcoronal dentin surface. The dentin surface was ground by hand using 600-grit silicon carbide abrasive paper under water stream for 60s to produce a standardized smear layer.

## 2. Bonding and restorative procedures

Prior to adhesive application, the teeth were randomly assigned to six groups, according to the bonding procedure and storage time, as shown in Table 2. To evaluate the effect of the adhesive alone, all teeth were etched with 35% phosphoric acid and primed with SBMP primer prior to applying the specific adhesives. The SBMP-24 was the control group in which that the SBMP was used according to manufacturer's instructions.

After applying the adhesive resin, a resin composite, Filtek Z-250 (3M ESPE, St. Paul, MN, USA) was built up in increments approximately 1-mm-thick. Each increment was light activated for 40s (Astralis 5, Ivoclar Vivadent AG, Schaan Liechtenstein). Then, the restored teeth were stored in water at 37$^{o}$C for 24 hours or 3 months.

Table 2

Application procedures of adhesive systems.

| Groups | | Application Procedure |
|---|---|---|
| *SBMP 24 h or SBMP 3 m* | *Scotchbond Multi-Purpose Plus (SBMP)* | 35% PA: apply 15s, rinse 15s, lightly air-dry<br>Primer of SBMP: apply 20s, air-dry<br>SBMP Adhesive: apply and 10s light-cure |
| *SE 24 h or SE 3 m* | *Clearfil SE Bond (SE)* | 35% PA: apply 15s, rinse 15s, lightly air-dry<br>Primer of SBMP: apply 20s, air-dry<br>SE Adhesive: apply and 10s light-cure |
| *PB 24 h or PB 3 m* | *Clearfil Protect Bond (PB)* | 35% PA: apply 15s, rinse 15s, lightly air-dry<br>Primer of SBMP: apply 20s, air-dry<br>PB Adhesive: apply and 10s light-cure |

* 24 h: twenty four hours; 3 m: 3 months; 35% PA: 35 % phosphoric acid.

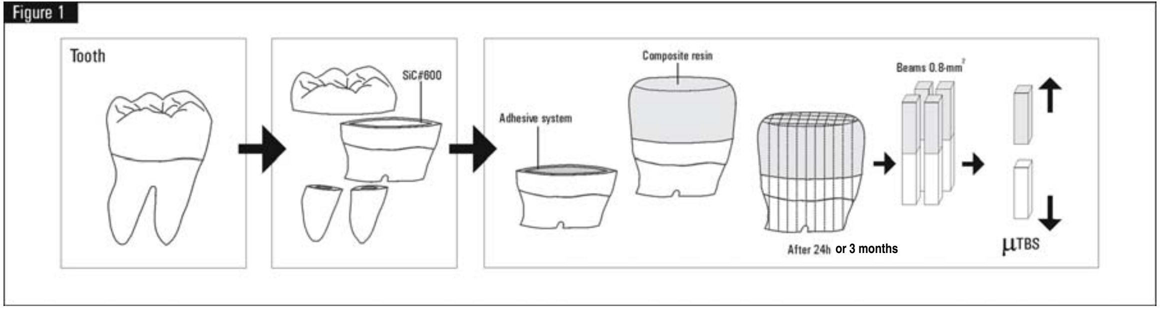
## 3. Microtensile test

After the specimens had been stored in distilled water at 37$^{o}$C for 24h or 3 months, the bonded samples were sectioned perpendicularly to the adhesive interface using a diamond saw under water lubrication to produce a series of slabs. Each slab was further sectioned to produce beams with an adhesive area of approximately 0.8 $mm^2$ (Figure 1).

The specimens were then fixed to a testing apparatus with a cyanocrylate adhesive (Super Bonder gel, Loctite, Henkel Corp., Rocky Hill, CT, USA) and subjected to microtensile testing at a crosshead speed of 1mm/min (EZ – test, Shimadzu Co, Kyoto, Japan). The mean bond strengths obtained were analyzed using the two-way ANOVA and Tukey's test ($\alpha$=0.05).

The fractured beams of debonded specimens were sputter coated with gold and observed under scanning electron microscopy (SEM – JEOL, JSM – 5600LV, Tokyo, Japan) to determine the mode of failure. Failure modes were classified into four groups: (1) failure in adhesive resin; (2) failure at the hybrid layer; (3) failure in dentin; (4) mixed failure between the hybrid layer and partial failure in adhesive resin; (5) failure between the adhesive resin and partial failure in resin composite; and (6) failure in resin composite.

Figure 1 Specimen preparation - μTBS.



## 4. Polarized light microscopy examination after artificial caries challenge

Sections from the restored teeth of each group were embedded in epoxy resin. Then, the specimens were subjected to an acid challenge by immersing it in a buffered demineralizing solution (2.2mmol/L $CaCl_2$, 2.2mmol/L $NaH_2PO_4$, and 50mmol/L acetic acid), adjusted to pH 4.5. Each group was stored in a separate container and stirred for 5 days at room temperature. The solution was changed every day to prevent saturation of the solution.

Afterward, an epoxy resin layer was applied to protect the demineralized surface from the subsequent polishing procedure. The samples were then vertically sectioned to the dentin-adhesive interface, ground by hand on 600, 1200, and 2000 grit silicon carbide paper under running water to a thickness of approximately 150μm, and polished with diamond pastes down to 0.25 μm. Sections were imbibed in water and examined using a polarized light microscopy (Olympus BH-2 Microscope, Olympus America, Inc., Two Corporate Center Drive., Melville, NY, USA).

## 5. Degree of conversion measurement

Ten (±0.5) milligrams of each adhesive was weighed and polymerized (VIP, Bisco Inc., 1100W. Irving Park Rd. Schaumburg, IL 60193, EUA) inside of aluminum pans in a Differential Scanning Calorimeter chamber (DSC 7, Perkin Elmer, 45 William Street, Wellesley, MA 02481-4078, EUA) at a constant temperature of 25$^{o}$C and in a nitrogen atmosphere (20 psi) to avoid formation of an oxygen-inhibition layer. For each material, three samples were prepared (n=3).

Twenty-four hours after the polymerization, small chips of polymerized adhesive were removed with a scalpel and were placed on a KCl crystal for analyze at Fourier Transform-Raman spectroscopy analysis (FTIR, DS20/XAD microscope, Analect Instruments, Irvine, CA, USA). Thirty scans were taken at 8 cm$^{-1}$ resolution. The uncured adhesive resin was similarly evaluated. DC was calculated from the ratio of the C=C peak (1638 cm-1) from the methacrylate group to that of the unchanging C...C peak from the aromatic ring (1610 cm-1) for the uncured and cured specimens using standard baseline techniques. Five readings were taken for each sample.

The samples were stored at constant temperature (25$^{o}$C) and protected from light and the FTIR analysis was repeated after one week and one month.

## 6. Transmission Electron Microscopy examination

Representative photographs of specimens of each adhesive (SBMP-24, SE-24, and PB-24) were processed for transmission electron microscopy (TEM – Philips CM12, Philips, Eindhoven, The Netherlands). TEM was only performed to provide additional information on the ultra-morphological structures of the dentin-adhesive interfaces. Instead of using a hybrid composite, a thin layer of a low-viscosity composite (Protect Liner F, Kuraray Medical Inc., Tokyo, Japan) was used in order to enable ultramicrotomy. After 24 hours of storage in water, the bonded specimens were coated with two layers of nail varnish applied to within 1-mm of the bonded surfaces. Ammoniacal silver nitrate was prepared and the specimens were placed in the tracer solution in total darkness for 24 hours. Then, the samples were 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 bonded interface. The specimens were sectioned into serial slabs using a water cooled diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA). Then, the non-demineralized slabs were fixed, dehydrated and embedded with epoxy resin. Representative ultrathin sections were obtained using an ultramicrotome (Sorvall Porter-Blum MT-2B, Newtown, CT, USA). The specimens were observed in the TEM without additional staining.

## Results

### 1. Microtensile bond strength

After 24 hours, the μTBS of the SBMP-24 (61.5 MPa) and SE-24 (55.5 MPa) were significantly higher than the PB (50.3 MPa) values (Table 3). No significant difference was found between SBMP-24 and SE-24. Both variables (type of adhesive and storage time) significantly influenced the μTBS values to dentin. Three months water-storage negatively affected the μTBS values for SBMP-3 (52.9 MPa), while for SE-3 the μTBS values remained stable (55.6 MPa). For PB-3, the fluoride-containing adhesive, the μTBS values increased significantly after 3 months water-storage (61.0 MPa).

After 24 hours, most failures of SBMP-24 and SE-24 were mixed failures between the hybrid layer and partial failure in the adhesive resin (type 4) (Figure 2). In contrast for PB-24, most failures were mixed failure between the adhesive resin and partial failure in the adhesive resin (type 5).

After 3 months, SBMP-3 and SE-3 showed mostly mixed failures of type 4 and type 5. For PB-3, 100% of the failures were type 5.

Table 3

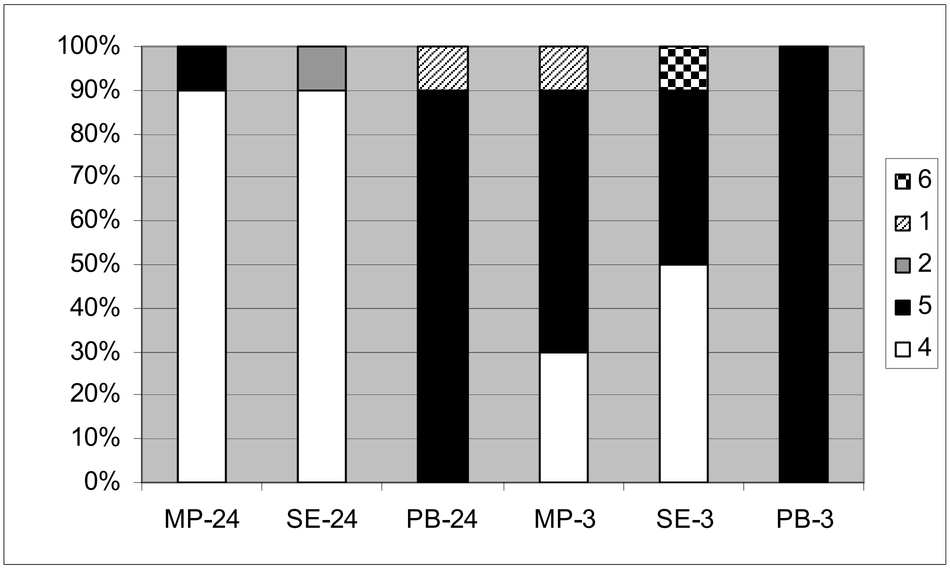
Mean microtensile bond strengths and standard deviations (MPa).

| Adhesive | Storage time<br>24h | <br>3 months |
|---|---|---|
| SBMP | 61.5 (10.5) a A | 52.9 (8.9) b B |
| SE | 55.5 (11.8) a b A | 55.6 (13.0) a b A |
| PB | 50.3 (9.9) b B | 61.0 (13.6) a A |

Same lowercase letters indicate (p>0.05) in columns, and same uppercase letters indicate (p>0.05) in rows.
Two-way ANOVA and Tukey's test (α=0.05)

Figure 2

Incidence (%) of failure patterns according to SEM analysis.



## 2. Polarized Light Microscopy

Representative PLM images from each group are shown on Figure 3. Outer lesions were observed in all specimens. The shape and size of the outer lesions produced by demineralizing with the acidic solution were similar for each material. Inhibition zone formation could only be observed for the fluoride-containing adhesive (PB) after both 24 hours and 3 months water-storage. The observed inhibition zone slopes from the top up to the end of the outer lesion.

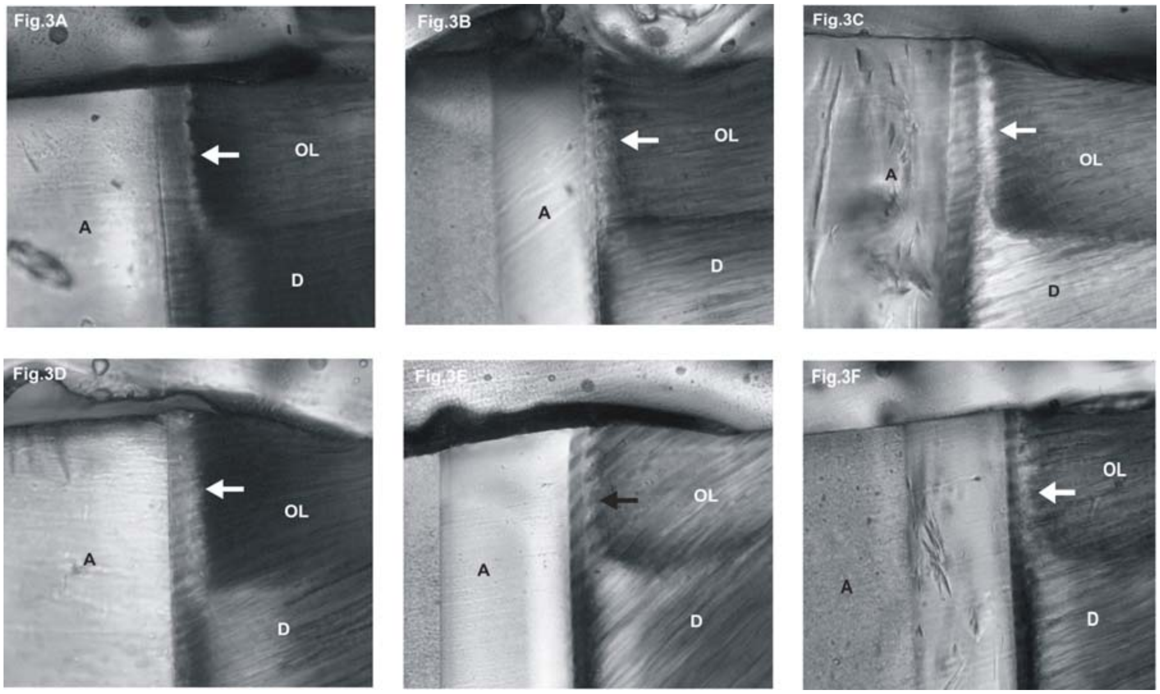


Figure 3. Polarized light photomicrographs of the ultra-structure of the interfaces after artificial caries challenge. All the samples showed a similar outer lesion. (a) SBMP-24h (right upper), note adjacent to adhesive (arrow) an area alike to the hybrid layer, since the hybrid layer is acid resistant, that zone is homogeneous and parallel to the adhesive. (b) SE-24h (left center upper), similar to the adhesive-interface of SBMP-24, a thick acid-resistant zone (arrow) was observed. (c) PB-24h (left upper), note adjacent to acid-resistant zone an inhibition zone (arrow) that was observed when a fluoride-containing adhesive was used. (d) SBMP-3m (right lower), similar ultra-structure of SBMP-24h. (e) SE-3m (left center lower), similar to SE-24h. (f) PB-3m (left lower), could be clearly observed a thicker inhibition zone (arrow) after 3 months water-storage. A=adhesive resin; D=dentin; OL=outer lesion.

## 3. Degree of conversion

*After 24 hours, SE (69.6%) showed the highest DC followed by SBMP (60.5%), and PB (53.1%) (Table 4). After 1 week, SE (68.7%) and SBMP (65.5%) did not show a difference in their DC values, while PB again had the lowest DC (56.6%). At 1 month, similar to 24 hours, SE (70.7%) had the highest values of DC, followed by SBMP (61.3%), and PB (58.3%).*

*Despite the fact that PB, the fluoride-containing adhesive showed a significant increase in DC after 1 month, the DC of PB was still lower compared with SE and SBMP.*

Table 4

Degree of conversion (%).

| | SBMP | SE | PB |
|---|---|---|---|
| 24 hrs | 60,5 (2,8) Bb | 69,6 (1,3) Aa | 53,1 (0,4) Cb |
| One week | 65,5 (1,5) Aa | 68,7 (1,8) Aa | 56,6 (2,1) Bab |
| One month | 61,3 (0,6) Bab | 70,7 (0,05) Aa | 58,3 (1,6) Ca |

*Uppercase letters identify equal values within rows and lower case within columns.*

## 4. Transmission Electron Microscopy

Representative images are shown on Figure 4.

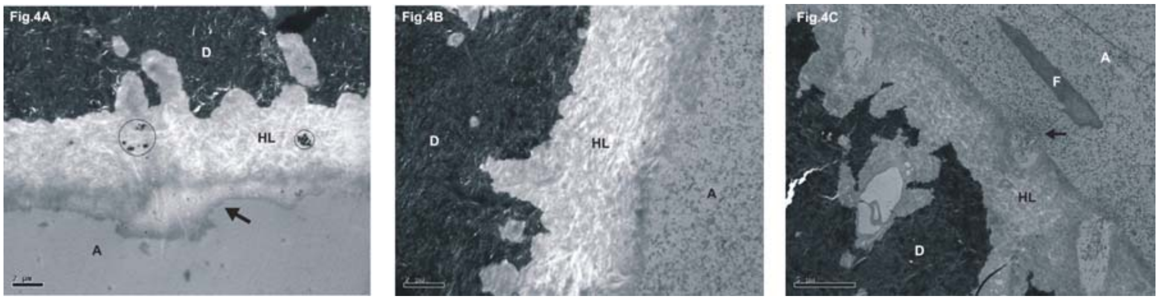


Figure 4. Transmission electron microscopy photomicrographs of resin-dentin interfaces of SBMP-24 (a), SE-24 (b), and PB-24 (c). All non-demineralized sections were immersed in ammoniacal silver nitrate. In (a) represents the SBMP-24 resin-interface, nanoleakage could be observed in the form of silver deposits (circumference) within the hybrid layer. A possible phase separation between HEMA, polyalkenoic-acid copolymer, and other components of the adhesive could be seen under the hybrid layer (arrow). In (b) represents the SE-24 interface. A filled adhesive could be identified; despite of homogeneous hybrid layer, in high magnification silver deposits points could be found within the hybrid layer. In (c) represents the fluoride-containing adhesive (PB-24), note a filler of fluoride within the filled adhesive. It could be observed the microfiller get in the tags.

## Discussion

One of the major goals of adhesive dentistry is to create a strong and durable bond between the resin composite restoration and the tooth substrate. Basically, the durability of the bond depends upon the complete monomer penetration into the demineralized dentin to achieve a quality hybrid layer. Many researchers have investigated methods to enhance the potential diffusion of monomers into conditioned dentin, as well as how to improve substrate monomer permeability [7,9,21-24]

Based on the fact that fluoride-releasing restorative materials have been widely used for caries prevention [25-28], fluoride-containing adhesive systems have been introduced to the market. Fluoride-containing adhesives should bond effectively to tooth structure, contribute to a reduction in microleakage, and possibly prevent recurrent caries due to their fluoride release. One such material is Clearfil Protect Bond (Kuraray Medical Inc.), a two-step self-etching primer adhesive system that contains an antibacterial monomer (MDPB) in the primer and sodium fluoride in the adhesive. Although many studies have shown the significant effect of fluoride in inhibiting caries [25-28], very little evidence exists about the performance of fluoride containing adhesive systems.

Based on our previous study [8], self-etching primer adhesive systems containing in composition an antibacterial monomer (MDPB) and a fluoride releasing component must be considered advantageous. These materials obtain significant bond strength and have a potential effect against secondary caries formation and lesion progression. Therefore, the present study complemented our earlier experiment [8], demonstrating the positive effect of fluoride-containing adhesive on microtensile bond strength of dentin restorations after 3 months water-storage. To identify the unique influence of the fluoride component in the adhesive, we used the same adhesive system application technique (i.e. the wet bonding technique: 35% phosphoric acid + primer of SBMP + bond) for all groups and varied only the adhesive resin component. SBMP applied according to manufacturers' instructions served as the control group. From the results after 3 months water-storage, the specimens of the fluoride-containing adhesive (PB) demonstrated a significant increase in bond strength values, while SE, a non-fluoride containing adhesive remained stable, and SBMP (control group) values decreased significantly. These findings corroborate with those of Nakajima et al. [29], who found that the durability of dentin bonding created by a fluoride-releasing adhesive improved after six-month storage compared with a fluoride-free adhesive. They hypothesized that the fluoride somehow prevented the degradation of dentin, resulting in an improvement in the long-term stability of the dentin interface.

The results of the present study suggest that the fluoride contained in the adhesive had a positive effect on the resin-dentin interface. One hypothesis is that fluoride might interact with components of dentin underneath the hybrid layer, allowing the remineralization of the dentin substrate. Fluoride-sodium filler is a pretreated inorganic compound which breaks into pieces, a characteristic of sodium that makes it easily soluble in water. Perhaps this characteristic explains the efficient release of fluoride ions from this fluoridated adhesive resin [11]. Carvalho et al. [30] demonstrated the existence of partially demineralized, uninfiltrated zones of dentin beneath the hybrid layer formed with some self-etch adhesive systems. They speculated that the acidic monomers lose their potential to further etch dentin, and may create spaces containing products formed by dissolved calcium and phosphate ions during self-etching. Hence, when the fluoride-releasing adhesive is used, the fluoride could be released to those spaces and a possible reaction of the fluoride and other products prevent the future demineralization of the dental hard tissues.

According to Itthagarun et al. [31], the fluoride release from bonded fluoride-releasing restorative materials and the potential benefit of artificial caries inhibition are indirect indications of the permeability of dentin adhesives and hybrid layers to water and ion movement. Ferracane et al. [22] reported that a fluoride-containing adhesive can release fluoride into the microspaces of a restored cavity, and conjectured that this may offer some level of protection from demineralization and recurrent caries. Furthermore, Ferracane et al. [22] stated that fluoride-containing adhesive demonstrated continuous fluoride release into water for more than 4 months and that the fluoride concentrated at the base of hybrid layer might continue to diffuse into the deeper dentin over time. These data are in agreement the current results, since after 3 months water-storage, the samples restored with fluoride-containing adhesive showed significant increase in the µTBS values.

Recent studies have stated that a single-step self-etch adhesive is permeable probably because of the high concentration of hydrophilic and acidic resin components, and the absence of a comparatively more hydrophobic surface resin layer [32-35]. In the current study, the wet bonding technique was used, and as a result, an uninfiltrated demineralized dentin should be present. Therefore, probably more dissolved calcium and phosphate ions are presented to react with fluoride. The primer used in our study contains HEMA, polyalkenoic-acid copolymer, and water, which represents a composition that may be more susceptible to form a permeable membrane after polymerization [1,6,35]. HEMA is polymerized into linear poly-HEMA chains, undoubtedly containing residual water that cannot be sufficiently removed since HEMA is hygroscopic and may absorb moisture [1,6,35]. In addition, an excess of water may also prevent optimal polymerization of the adhesive monomers. The polyalkenoic-acid copolymer has a high-molecular-weight, so a phase separation may occur, with the copolymer being filtered out by the collagen network and deposited as a distinct gel on the surface of the collagen network (Figure 4A) [1]. Consequently, the rather poorly infiltrated and polymerized hybrid layer is more susceptible to degradation. These characteristics would correlate with the decrease of the µTBS values for SBMP after storage. Several factors undoubtedly contribute to this loss of bond strength including plasticization and breakdown of hydrolytically unstable monomers or oligomers. Moreover, Spencer et al. [6] indicated a physical separation of the adhesive as it mixes with water in the demineralized dentin matrix, leading to partitioning of the adhesive into hydrophobic BisGMA and hydrophilic HEMA-rich phases. That phase separation might inhibit not only the formation of an integrated collagen/polymer network but suppresses adhesive infiltration throughout the width of the demineralized and subjacent dentin [6].

Conversely, SE bond showed stable µTBS values after 3 months water-storage. A possible explanation may be related to the composition of SE bond, which contains a functional monomer 10-MDP, that effectively interacts chemically with hydroxyapatite [23,36]. This interaction produces a calcium salt that is hardly soluble. The less soluble the calcium salt of the acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite substrate. Also, the microfillers contained in SE may have a positive effect on bond strength as the microfillers may be able to penetrate inside the resin tags and have the potential to improve the bond strength (Figure 4B). Probably, SE bond was able to infiltrate better than SBMP bond and the functional monomer 10-MDP allowed a stable interaction with the remaining hydroxyapatite around the collagen fibrils.

Even though PB contains 10-MDP and microfiller in its composition, the bond strength was significantly lower compared with SBMP and SE bond values after 24 hours. Incomplete polymerization of adhesive monomers has been speculated as one of the reasons for the occurrence of nanoleakage and a decrease in bond strength [24]. The compromise in the degree of conversion of adhesive monomers may be caused by the entrapment of residual water within adhesive-dentin interfaces [24]. The analysis of degree of conversion indicated that the addition of NaF somewhat interfered with the polymerization of the adhesive. The degree of conversion of the three adhesives used in this study was evaluated after 24 hours, 1 week and 1 month. We do not evaluated DC after 3 months, since previous studies showed that there is little possibility that the DC will increase after 1 month. The DC of the SBMP and SE remained constant after 1 month, while the DC of PB increased. Nevertheless, the DC of PB remained significantly lower than SE and SBMP. Although the increase of the degree of conversion of PB was significant after 1 month, we speculate that the difference is not substantial enough to affect the bond strength. It is also important to point out that the DC samples were prepared in an ideal condition, without water and oxygen interference. Interestingly, the DC data demonstrated that NaF can hamper the polymerization of the adhesive; however we could not extrapolate these results to the bond strength performance of PB. Another possible explanation is the amount of water in the PB. Perhaps this adhesive has a high concentration of water in its composition, which might compromise the polymerization. Finally, the manufacturer does not provide the exact composition and concentration of each component in the adhesive, so other factors could be involved in producing the lower degree of conversion of PB. According to Malacarne et al. [37] the structural and topological features of polymers are fundamental in determining the extent to which polymers will be affected by an aqueous environment. Since the water sorption, solubility and water diffusion coefficient of methacrylate-based resins are dependently on adhesives' composition and hydrophilicity.

Regarding failure modes, PB showed almost all mixed failures between the adhesive resin and composite resin (type 5) after both 24 hours and 3 months water-storage. This suggests that the fluoride-containing adhesive was weaker than the non-fluoride containing adhesive. This would be in agreement with the degree of conversion results, which showed PB being the lowest. In contrast, SBMP and SE demonstrated mostly mixed failures between the hybrid layer and the adhesive resin (type 4), after 24 hours, with mixed failures of type 4 and type 5 after 3 months. Therefore, one may speculate a possible degradation in the adhesive during storage, leading to an increase in the number of type 5 failures. In accordance with the failures modes, a potential degradation of the adhesive would have a negative influence on the µTBS values.

The results of this study suggest that the fluoride-containing adhesive had a positive effect on bond strength and after artificial caries challenge. Demineralized lesions and inhibition zones have been previously evaluated using polarized light microscopy [31,38-40]. PLM clarity showed the formation of an inhibition zone when the fluoride-containing adhesive was used, both after 24 hours and 3 months water-storage (Figure 3). Formation of inhibition zones appeared to be associated with the amount of fluoride released from the materials and different components included in each material [41]. Therefore, the formation of an inhibition zone was material dependent [41]. Itota et al. [15] suggested that the fluoride-releasing adhesive systems may enhance mineralization of decalcified dentin underneath composite resin and contribute to the longevity of the restorations.

As fluoride-releasing adhesives directly contact the cavity wall, fluoride ions released from them easily penetrate and diffuse into the dentin at the cavity wall. The fluoride ions penetrating into the dentin enhance mineralization of the dentin and reduce the demineralization of the dentin. Therefore, the dentin penetrated by fluoride ions offers greater resistance against secondary caries as compared to dentin that has not contacted fluoride [27]. The result suggested that the fluoride-releasing adhesive enhanced mineralization of decalcified dentin.

Considering the present results, fluoride may be considered an important component in the adhesive to ensure long-term success of the restoration. Since the instability of resin-dentin bonds is still a strong challenge to adhesive dentistry, it is suggested that fluoride-containing adhesives may shown promise in resisting the degradation of the dentin-resin interface and secondary caries formation and its progression.

## Conclusions

The fluoride-containing adhesive demonstrated significant effects on formation of inhibition zone after artificial caries challenge. Regardless of the fact that the degree of conversion of the fluoride-containing adhesive was lower than SBMP and SE (non-fluoride containing adhesives), the μTBS values increased after 3 months in water-storage.

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# CONSIDERAÇÕES GERAIS

O trabalho do capítulo 2 foi realizado em conseqüência dos resultados obtidos no capítulo 1. Visto que o primeiro estudo mostrou que o flúor presente no adesivo do sistema autocondicionante - Clearfil Protect Bond – apresentou um efeito positivo na formação de uma zona ácido-base resistente mais espessa adjacente à camada híbrida quando comparado à zona ácido-base resistente formada quando se usou o adesivo que não contém flúor – Clearfil SE Bond, dúvidas quanto ao efeito real do flúor deram continuidade aos estudos.

Pelos resultados obtidos no capítulo 1, a presença do flúor no adesivo, assim como a presença de um monômero antibacteriano – MDPB – no primer, não interferiram nos valores de resistência de união quando comparado ao sistema adesivo sem flúor e monômero antibacteriano. Dessa forma, com o objetivo de verificar se, realmente, o flúor foi responsável pela formação de uma zona ácido-base resistente mais espessa, os experimentos do capítulo 2 foram realizados.

Nos experimentos do capítulo 2, algumas mudanças na metodologia de trabalho foram realizadas:

(1) Foi verificado no trabalho inicial, por meio de microscopia eletrônica de varredura, a presença ou não da zona ácido-base resistante após um desafio ácido de 90 minutos, seguido da imersão dos espécimes por 20 minutos em hipoclorito de sódio a 5%, considerado como desafio base, por isso chamada zona ácido-base resistente. Como a literatura mostra que o desafio ácido para formação de uma lesão de cárie artificial maior ocorre após 5 dias imersos nessa solução ácida, conforme o protocolo mostrado no capítulo 2, optou-se por induzir a formação de uma região desmineralizada maior (aproximadamente 100µm). Já que o interessante seria verificar se o flúor teria uma ação positiva mesmo após um desafio ácido prolongado. Portanto, no capítulo 2, as amostras foram submetidas somente ao desafio ácido, por isso a zona foi chamada de ácido-resistente apenas.

(2) Para verificar se realmente o adesivo que contém flúor tem um efeito positivo, optou-se por variar somente os adesivos na aplicação dos sistemas adesivos. Ou seja, foi utilizado para todos os grupos a mesma técnica – técnica do condicionamento ácido total – ácido fosfórico 35% + primer do SBMP + adesivo (SBMP-controle-não contém flúor; SE Bond-não contém flúor ou Protect Bond-contém flúor). Tais grupos foram utilizados para realização do teste de microtração, microscopia de luz polarizada e microscopia eletrônica de transmissão.

Os resultados de microtração do capítulo 2, interessantemente, mostraram um aumento significativo na resistência de união após 3 meses de estocagem dos espécimes em água, quando se utilizou o adesivo que contém flúor. Enquanto com o SBMP, houve uma queda significativa nos valores de união e quando se utilizou o adesivo do SE Bond, os valores se mantiveram estáveis após 3 meses de estocagem em água. Diante desses resultados, especulações quanto à ação positiva do flúor aumentaram. Visto que se usou a técnica do condicionamento ácido total, ou seja, nessa técnica estudos prévios comprovam a presença de uma zona de dentina desmineralizada não infiltrada pelos monômeros abaixo da camada híbrida, a qual seria suceptível a degradação comprometendo a longevidade das restaurações. Dessa forma, uma hipótese seria que o flúor agiu positivamente, de alguma forma, nessa região desmineralizada. Talvez o flúor tenha se ligado aos íons cálcio e fosfato que ficaram livres nessa região, possibilitando uma remineralização dessa dentina e conseqüentemente reforçando essa região retardando a degradação dessa interface antes desprotegida.

Outra hipótese foi uma possível polimerização tardia do adesivo na interface ao longo do tempo. Para se esclarecer essa dúvida, optou-se por avaliar o grau de conversão de cada adesivo após 24 horas, 1 semana e 1 mês. Os resultados mostraram que o adesivo com flúor apresentou o menor grau de conversão comparado ao SBMP e SE, embora tenha tido um aumento significativo após 1 mês. Deve-se ressaltar que a composição entre os 3 adesivos são diferentes, portanto essa diferença no grau de conversão não pode ser especulada ao fato somente da presença ou não do flúor no adesivo.

Quanto às imagens de microscopia de luz polarizada, pode-se observar e confirmar que o flúor de alguma forma agi positivamente na interface de união após ao desafio ácido, sendo sua ação contínua mesmo após 3 meses de estocagem do espécime.

Diante dos resultados obtidos nos capítulos 1 e 2, pode se especular indiretamente que a região adjacente à camada híbrida é permeável, visto que se temos a ação positiva do flúor próxima a camada híbrida, hipoteticamente existe a presença da água, já que o flúor necessita da água para se movimentar. Conforme estudos anteriores, a interface dente-restauração é suceptível a degradação, o que compromete a longevidade das restaurações de resina composta. Visto que ainda não se tem um sistema adesivo capaz de manter uma união estável e duradoura dessa interface, a incorporação de componentes que atuem de forma positiva, aumentando a longevidade dessa união devem ser estudadas e avaliadas tanto em pesquisas *in vitro* e *in vivo*.

# CONCLUSÕES

Diante dos dois capítulos deste trabalho, pode-se concluir:

(1) O monômero antibacteriano MDPB presente no primer e o flúor no adesivo do sistema Clearfil Protect Bond não interferiram nos valores de resistência de união, quando comparado ao sistema adesivo Clearfil SE Bond, que não contém MDPB e flúor.

(2) Por meio de microscopia eletrônica de varredura, observou-se a presença de uma zona ácido-base resistente mais espessa somente quando se usou o adesivo que contém fluor.

(3) A aplicação do ácido fosfórico a 35% + primer do SBMP + adesivo com flúor (Clearfil Protect Bond), após 3 meses de estocagem, mostrou um aumento significativo nos valores de resistência de união, enquanto que a aplicação do Scotchbond Multi Purpose Plus-controle demonstrou uma queda significativa nos valores de resistência de união. Para a aplicação do ácido fosfórico a 35% + primer do SBMP + adesivo sem flúor do Clearfil SE Bond, os valores se mantiveram estáveis após 3 meses de estocagem em água.

(4) Por meio de microscopia de luz polarizada, a presença da zona ácido-resistente, após desafio ácido, foi observada somente quando se usou o adesivo contendo flúor, tanto após 24 horas como 3 meses de estocagem em água.

(5) O grau de conversão do adesivo contendo flúor foi o menor quando comparado aos adesivos SBMP e SE após 24 horas, 1 semana e 1 mês, embora seu grau de conversão tenha aumentado significativamente após 1 mês.

(6) Apesar do adesivo com flúor apresentar o menor grau de conversão, apresentou um efeito positivo na resistência de união após 3 meses de estocagem de água, assim como foi capaz de formar uma zona ácido-resistente após desafio ácido, mesmo 3 meses após armazenagem em água.

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# ANEXOS

## 1. REPRINT - ARTIGO DENTAL MATERIALS JOURNAL

Dental Materials Journal 25(3): ●–●, 2006

# Evaluation of Antibacterial and Fluoride-releasing Adhesive System on Dentin — Microtensile Bond Strength and Acid-base Challenge

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This study evaluated the influence of a fluoride-containing adhesive on microtensile bond strength ($\mu$TBS) to dentin, as well as analyzed the dentin-adhesive interface after acid-base challenge. Experimental groups were: G1 - Clearfil SE Bond control (SE); G2 - Clearfil Protect Bond control (PB); G3 - Primer[SE]/Adhesive[PB]; G4 - Primer[PB]/Adhesive[SE]. For $\mu$TBS evaluation, dentin surfaces were ground, bonded, and composite resin crowns were built up to obtain beams to be tested. For interfacial analysis, adhesive system was applied on dentin surface and a low-viscosity resin was placed between two dentin disks. Then, the specimens were subjected to acid-base challenge, sectioned, and polished to be observed by SEM. $\mu$TBS data showed no statistical differences among the groups (G1: 51.3, G2: 47.6, G3: 55.0, G4: 53.9; mean in MPa). Through SEM, it was observed that a thick acid-base resistant zone adjacent to the hybrid layer was created only when the fluoride-releasing adhesive was used. In conclusion, the presence of fluoride in an adhesive contributed significantly to preventing secondary caries, and did not interfere with dentin-adhesive bond strength.

Key words: Adhesive system, Dentin, Fluoride-releasing material

## INTRODUCTION

The success of restorations hinges on the adhesion stability between composite resin and tooth structure. To maintain this adhesion stability, the presence of a hybrid layer is essential in that it forms a resistant structure against bacterial invasion. Currently, simplified systems such as self-etching primer/adhesive systems have demonstrated good clinical performance to dentin. This approach is less technique-sensitive and reduces the time required for the bonding procedure. Although advances in adhesive dentistry have brought about improvements in bonding systems and techniques, bond failures at the tooth-restoration interface remain a challenge in the dental field.

Microgaps between restorative materials and the cavity wall permit invasion of fluids and bacteria, leading to secondary caries. This occurs probably due to inadequate marginal adaptation of composite restorations[1)]. According to previous reports[2,3)], secondary caries is the commonly cited reason for failure and replacement of restorations. Hence, antibacterial activity is considered to be an important beneficial property of dentin bonding systems for successful restorative treatments.

From this point of view, new versions of adhesive system containing fluoride in composition have been introduced in order to inhibit the action of secondary caries arising from enamel cracks or microleakage at the tooth-restoration interface. With regard to these fluoride-releasing restorative materials, some researchers have extensively demonstrated their significant cariostatic and antibacterial effects[4,5)].

An experimental two-step self-etching primer/adhesive system, ABF (former name of Clearfil Protect Bond) — composed of an antibacterial primer containing MDPB (12-methacryloyloxydodecylpyridinium bromide) and a fluoride-releasing adhesive — has shown the potential in artificial secondary caries inhibition around restorations[6–8)]. However, there is little information about the influence of antibacterial components on bonding strength. Likewise, information is scarce concerning antibacterial primer performance and fluoride-releasing adhesive effect against artificial caries challenge.

Tsuchiya *et al.*[9)] described the formation of an acid-base resistant zone adjacent to the hybrid layer after acid-base challenge. Results of the study[9)] clearly showed the influence of adhesive material composition in the formation of acid-base resistant zone. Although the characteristics of that resistant zone are still unclear, their results suggested the potential effect of self-etching primer adhesive systems in inhibiting secondary caries.

Therefore, the purpose of the present study was to evaluate the influence of primer and bonding agent — by interchanging between a self-etching primer adhesive system with antibacterial properties (Clearfil Protect Bond) and a non-fluoride-containing self-etching primer adhesive system (Clearfil SE Bond) — on microtensile bond strength. In addition, *in vitro* inhibitory effect on artificial secondary caries around adhesive restorations was observed using a SEM.

## MATERIALS AND METHODS

Table 1 lists the compositions, manufacturers, and batch numbers of the materials employed in this study.

Sixteen caries-free extracted human third molars were obtained under a protocol (#023/2004) approved by the Ethical Committee at the Piracicaba School of Dentistry, State University of Campinas. Before use, the teeth were cleaned of debris and stored in physiological saline containing 0.1% thymol.

### *Experiment 1 — Microtensile bond test*

1) Tooth specimen preparation

For each tooth, the coronal portion was removed to expose a flat, midcoronal dentin surface using a low-speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) under water refrigeration (Fig. 1A). The exposed dentin surface was ground using 600-grit silicon carbide abrasive paper under water stream for 60 seconds to produce a standardized smear layer (Fig. 1B).

2) Bonding and restorative procedures

Prior to adhesive application, the teeth were randomly assigned into four groups, according to the bonding procedures shown in Table 2. After applying the bonding resin, a composite resin, Clearfil AP-X, was built up using increments approximately 1 mm thick. Each increment was light-activated for 40 seconds (Optilux 500/Demetron-Kerr, Danbury, CT,

Table 1 Materials used in this study

| Material | Components | Batch Number | Manufacturer |
|---|---|---|---|
| *Adhesive System* | | | |
| Clearfil SE Bond (SE) | Primer: MDP, HEMA, water, PI, accelerators, CA<br>Adhesive: MDP, HEMA, dimethacrylates, PI, CA, microfiller | Primer: 00507A<br>Adhesive: 00712A | Kuraray Medical Inc., Tokyo, Japan |
| Clearfil Protect Bond (PB) | Primer: MDPB, MDP, HEMA, MFM, PI, water<br>Adhesive: MDP, HEMA, MFM, PI, microfiller, NaF | Primer: 000010<br>Adhesive: 000017 | Kuraray Medical Inc., Tokyo, Japan |
| *Restorative Material* | | | |
| Clearfil AP-X | Bis-GMA, TEGDMA, barium glass filler (85 wt%), PI, accelerators | 01063A | Kuraray Medical Inc., Tokyo, Japan |
| Metafil flo | 2,2-Bis[4-(methacryloxy-polyethoxy)phenyl]propane, bifunctional methacrylate monomers, silica, 3-(trimethoxysilyl)propyl methacrylate | GS1 | Sun Medical, Moriyama, Japan |

MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; PI: photoinitiator; CA: catalyst; MDPB: 12-methacryloyloxydodecylpyridinium bromide; MFM: multifunctional methacrylate; NaF: sodium fluorite; Bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane; TEGDMA: triethylene glycol dimethacrylate

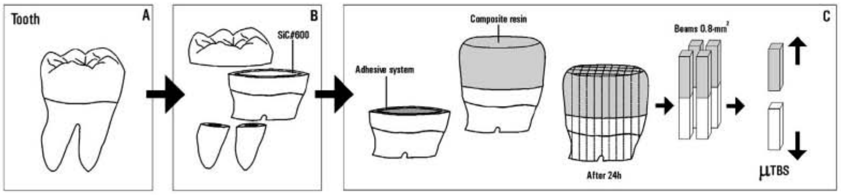

Fig. 1 Specimen preparation (µTBS).

USA).

3) Microtensile test

After the specimens had been stored in distilled water at 37℃ for 24 hours, the bonded samples were sectioned perpendicular to the adhesive interface into serial slabs with a diamond saw under water lubrication. Each slab was further sectioned to obtain beams with an adhesive area of approximately 0.8 mm$^2$.

Then, the specimens were fixed to a testing apparatus with a cyanoacrylate adhesive (Zapit, Dental Ventures of American, Anaheim, USA) and subjected to microtensile testing at a crosshead speed of 1 mm/min (EZ Test, Shimadzu Co., Kyoto, Japan) (Fig. 1C). The mean bond strengths obtained were analyzed using the Kruskal-Wallis one-way analysis of variance on Ranks test. The fractured beams of debonded specimens were sputter-coated with gold and observed under a SEM (JSM-5310LV, JEOL, Tokyo, Japan) to determine the mode of failure. Failure modes were classified into four groups: (1) failure in adhesive resin; (2) failure at hybrid layer; (3) failure in dentin; and (4) mixed failure between hybrid layer and partial failure in adhesive resin.

*Experiment 2 — SEM interfacial observation after acid-base challenge*

Approximately 1.5 mm-thick dentin disks were obtained from the midcoronal portion of each tooth with the use of a diamond saw under running water (Fig. 2A). Two disks were obtained from each tooth (Fig. 2B). Each surface of the dentin disk was ground with 600-grit silicon carbide paper under running water for 60 seconds (Fig. 2C), and the adhesive system was applied. Bonding procedures were conducted in the same manner as previously described.

After applying the bonding resin, a thin layer of a low-viscosity composite resin, Metafil flo (Sun Medical, Moriyama, Japan), was placed between two disks and light-activated to produce a dentin disk sandwich. The resin-tooth bonded specimens were stored in distilled water at 37℃ for 24 hours. Subsequently, the dentin disk sandwich was vertically sectioned at the dentin-adhesive interface (Fig. 2D), and the blocks of dentin disk sandwich were embedded in epoxy resin.

Specimens were subjected to an acid challenge by being immersed in a buffered demineralizing solution (2.2 mmol/L $CaCl_2$, 2.2 mmol/L $NaH_2PO_4$, and 50 mmol/L acetic acid) adjusted to pH 4.5 for 90

Table 2 Application procedures of adhesive systems

| Group | Application procedure |
|---|---|
| Clearfil SE Bond (SE) | Primer: apply 20s, air-dry<br>Adhesive: apply and light-cure 10s |
| Clearfil Protect Bond (PB) | Primer: apply 20s, air-dry<br>Adhesive: apply and light-cure 10s |
| Primer: SE<br>Adhesive: PB | Primer: apply 20s, air-dry<br>Adhesive: apply and light-cure 10s |
| Primer: PB<br>Adhesive: SE | Primer: apply 20s, air-dry<br>Adhesive: apply and light-cure 10s |

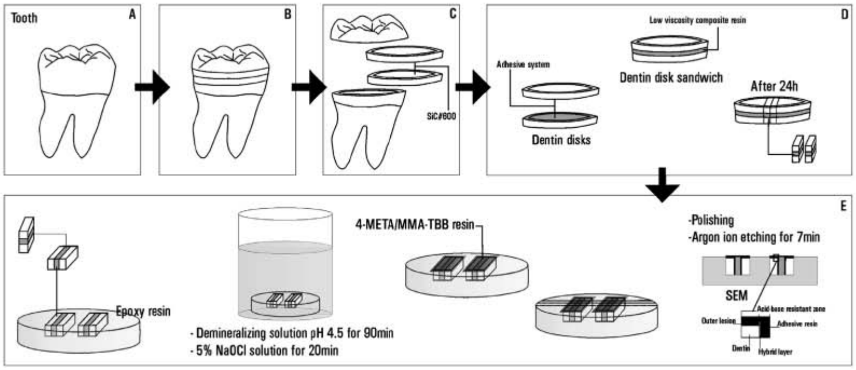

Fig. 2 Specimen preparation (SEM).

minutes. In a pilot study, the time for acid challenge was determined by SEM observation of the artificial caries lesion being of approximately 10 μm depth. After the acid challenge, specimens were immersed in 5% sodium hypochlorite solution for 20 minutes to remove any demineralized dentin collagen fibrils, and then rinsed with running water for 60 seconds.

After which, a 4-META/MMA-TBB resin (Super Bond C&B, Sun Medical, Moriyama, Japan) was applied to protect the demineralized surface from the polishing procedure. The samples were then vertically sectioned at the dentin-adhesive interface and polished with diamond pastes down to 0.25 μm. The polished samples were etched with an argon ion beam (EIS-IE, Elionix, Tokyo, Japan) for seven minutes for distinct ultrastructural identification of the dentin-adhesive interface (Fig. 2E). Following which, the samples were sputter-coated with gold, and the morphological changes of the dentin-adhesive interface produced by acid-base challenge were observed under a SEM.

## RESULTS

### *Microtensile bond strength results*

Mean bond strengths and standard deviations are shown in Table 3. There were no significant differences among the groups ($p=0.538$). The results suggested that the incorporation of an antibacterial monomer in the primer and a fluoride-releasing material in the adhesive did not compromise the microtensile bond strength values.

Table 3 Results of microtensile bond strength test

| Group | MPa (SD) |
|---|---|
| Clearfil SE Bond (SE) | 51.30 (19.6)* |
| Clearfil Protect Bond (PB) | 47.64 (10.7)* |
| Primer: SE/Adhesive: PB | 54.99 (10.0)* |
| Primer: PB/Adhesive: SE | 53.85 (12.6)* |

*Indicate no statistically significant differences ($p=0.538$) according to Kruskal-Wallis one-way analysis of variance on Ranks

Failure mode proportions of the debonded specimens are shown in Fig. 3. Most of the failures were adhesive or mixed failures regardless of the group.

### *Scanning electron microscopic analysis*

Fig. 4 shows the representative SEM images from each group analyzed in this study. Outer lesions and acid-base resistant zones were observed in all specimens. Depth of the outer lesion ranged from 7 to 10 μm after acid-base challenge. In parallel, a thin hybrid layer — approximately 0.5 μm thick — was also observed in all groups. SEM analysis showed that the structures of both adhesive and hybrid layer were not damaged after acid-base challenge, regardless of the material used.

Fig. 4a of SE/SE shows the interface of Clearfil SE Bond adhesive system and dentin. The presence of the acid-base resistant zone (approximately 0.5 μm thick) beneath the hybrid layer was clearly observed. Similarly, the acid-base resistant zone was observed when PB primer and SE adhesive were applied (Fig. 4d). The observed acid-base resistant zone was homogeneous and parallel to the hybrid layer. On the other hand, the interfaces of Clearfil Protect Bond group and SE primer/PB adhesive group (Figs. 4b and 4c) showed sharp formation of a thick acid-base resistant zone (over 1.0 μm thick) adjacent to the hybrid layer. Further, the acid-base resistant zone was formed from the upper slope to the end of outer lesion. Therefore, the thick acid-base resistant zone adjacent to the hybrid layer could be observed only when the fluoride-releasing adhesive was used.

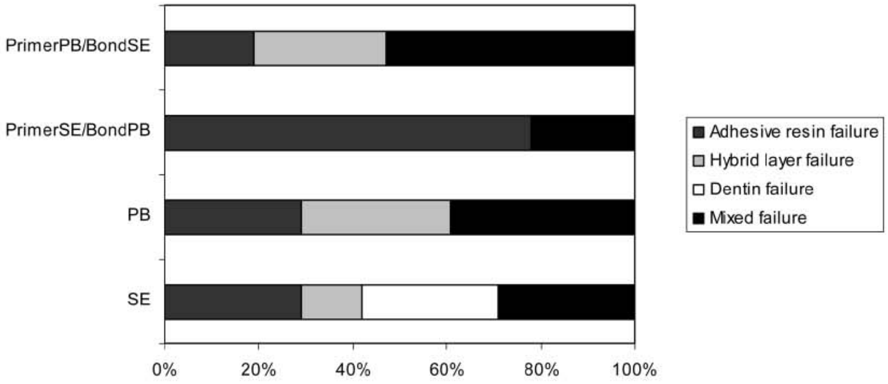

Fig. 3 Incidence (%) of failure patterns according to SEM analysis.

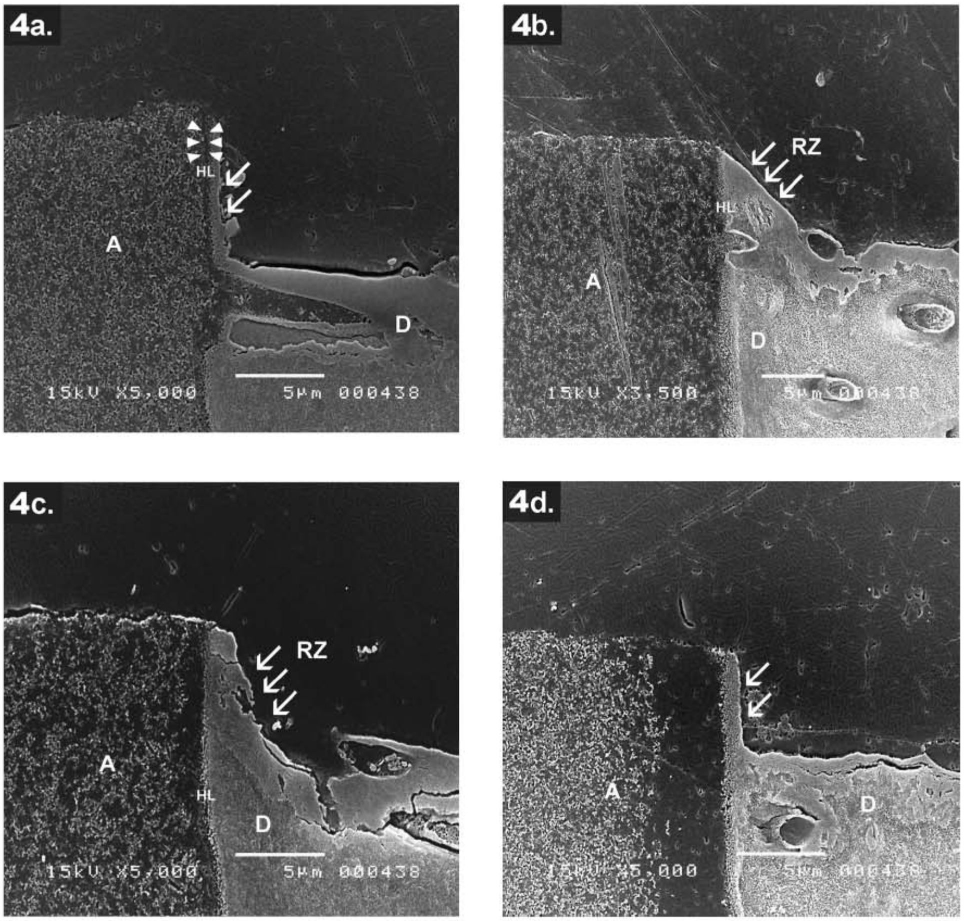

Fig. 4 SEM images of the ultrastructure of the interfaces after acid challenge.
(a) SE/SE Clearfil Se Bond: Acid resistant zone of approximately 0.50 μm width (arrows) could be clearly observed beneath the hybrid layer (center of arrowheads); it is a thin and homogeneous zone parallel to the hybrid layer;
(b) PB/PB Clearfil Protect Bond: Thick acid-base resistant zone could be observed close to the hybrid layer, and which slopes from the top of the outer lesion;
(c) SE/PB Primer of CSE and Adhesive of CPB: Similar to the adhesive interface of CPB, a thick acid-base resistant zone over 1.0 μm wide (arrows) was observed;
(d) PB/SE Primer of CPB and Adhesive of CSE: Thin acid-base resistant zone of approximately 0.50 μm width could be observed (center of arrowheads).
A=adhesive resin; D=dentin; HL=hybrid layer; and RZ=acid-base resistant zone.

## DISCUSSION

Fluoride release from restorative materials has been extensively researched for many years[5,10,11]. This is because fluoride has been shown to exhibit anticariogenic activity by increasing enamel and dentin resistance to subsequent acid attack as well as inhibit carbohydrate metabolism in dental plaque. It has been widely accepted that fluoride could facilitate remineralization or prevent demineralization of the dental structure[10,12].

Currently, composite resins have been selected as the major direct restorative material in clinical dental practice. Against this background, manufacturers have been trying to develop various fluoride-releasing adhesive systems and composite resins[13]. Studies have reported that fluoride-containing dentin adhesives may release fluoride into marginal gaps, and thereby exert a beneficial effect on adjacent demineralized enamel and dentin[8,14)].

In addition, the presence of a fluoride-releasing component in dentin bonding is advantageous in that it imparts its inherent antimicrobial properties[6)]. For this reason, antibacterial monomers have been developed and incorporated in dental resins[6,15)]. In particular, Imazato *et al.*[16)] demonstrated that the antibacterial monomer, MDPB, synthesized from quaternary ammonium dodecylpyridinium, could be considered as the most promising candidate to be accepted as a true non-agent-releasing antibacterial monomer[13,17,18)].

Several reports[19−21)] have established that self-etching primer/adhesive systems could certainly be used in restorative dentistry because of their ability to provide efficient marginal sealing. However, even if an adhesive system shows high bond strength, secondary caries is still found in clinical resin restorations after long-term use.

The primer of PB has an antibacterial monomer (MDPB), and the adhesive has a fluoride-releasing component (treated sodium fluoride). On the other hand, Clearfil SE Bond is an antibacterial-free adhesive system. The objective of this study, therefore, was to verify the influence of interchanging both self-etching primer and adhesive resin (SE and PB) on microtensile bond strength, as well as in the morphology of dentin-adhesive interface after acid-base challenge. In this way, the independent effectiveness and interference from the primer and adhesive of these adhesive systems could be observed.

From the microtensile bond strength test results, there were no statistically significant differences among the groups[7,22)]. These results corroborated those of Imazato *et al.*[23,24)], whereby it was found that the incorporation of MDPB into dentin primer did not demonstrate any adverse effect on the bond strength or curing behavior[25)] of the adhesive system. Regarding the presence of a fluoride-releasing component in the adhesive resin, several studies[6,24,26−29)] have established the ability of fluoride ions to inhibit secondary caries by the remineralization of dentin around the restoration. According to Han *et al.*[8)], although the mechanism of fluoride action on caries reduction is not fully understood for clinical use, fluoride-releasing adhesive resins and luting cements are useful in preventing the initiation of the caries process and the development of secondary caries in restored teeth.

Nakajima *et al.*[30)] reported that the durability of dentin bonding created by a fluoride-releasing adhesive did not change after six-month storage, as compared to the significant decrease in bond strength with a fluoride-free adhesive. They hypothesized that the fluoride component somehow prevented the degradation of dentin, resulting in the improvement of long-term stability at the dentin interface.

Although this study did not specifically evaluate the influence of each component of the adhesive systems (Table 1) on microtensile bond strength, we could conclude that the presence of fluoride-releasing component in the adhesive system did not alter the bond strength values when compared with a non-fluoride adhesive. Therefore, based on the microtensile bond strength test results, PB could be considered a reliable adhesive system for clinical use — with due consideration to its antibacterial activity too.

Besides the high bond strength values, PB exhibited a significant behavior in the acid-base challenge. A demineralizing solution was used to induce the acid attack, and 5% NaOCl solution was used to remove demineralized dentin collagen fibrils[9)]. An acid-base resistant zone formation was clearly observed through the SEM images. This zone was formed beneath the hybrid layer with or without fluoride-releasing component. Therefore, the SEM method was useful for analyzing the ultrastructural morphology of the dentin-adhesive interface after acid-base challenge[9)]. Moreover, the argon ion etching technique allowed the hybrid layer to be visibly distinguished from the dentin-resin interface[31)].

Toba *et al.*[32)] stated that by means of confocal laser scanning microscopy, experimental ABF was found to be effective in inhibiting artificial secondary caries around restorations[29)]. Nevertheless, thickness of the inhibition zone was relatively thinner than those created with conventional glass-ionomer cements[4,32)].

We speculated that the formation of a thick acid-base resistant zone was related to the presence of fluoride-releasing component in the adhesive resin[26,27,33)]. This was because the formation of a thicker acid-base resistant zone took place only when the fluoride-containing adhesive was used (Figs. 4b and 4c). Therefore, the formation of acid-base resistant zone was material-dependent[34)]. Further, Torii *et al.*[4)] suggested that fluoride-releasing adhesive systems may enhance the mineralization of decalcified dentin beneath composite resins and thereby contribute to the longevity of restorations.

With the fluoride-free adhesive system, a thin and homogeneous acid-base resistant zone (approximately 0.5 $\mu$m thick) was detected along the interface between dentin and adhesive. According to Tsuchiya *et al.*[9)], the existence of the acid-base resistant zone suggested that the monomer had penetrated deeper than the hybrid layer — which was revealed by argon ion etching. Moreover, the micromechanical attachment of adhesive to dentin is acid-base resistant. This could be observed even after acid-base challenge.

Carvalho *et al.*[35)] demonstrated that with some mild self-etch adhesive systems, a zone of partially demineralized but uninfiltrated dentin was formed beneath the hybrid layer. They speculated that due to the reduced etching potential of acidic monomers toward the base of hybrid layers, spaces containing products formed by dissolved calcium and phosphate ions during self-etching were created. Hence, a possible explanation for the presence of a thick acid-base resistant zone adjacent to the hybrid layer after acid-base attack, when the fluoride-releasing adhesive was used, was that fluoride was released to those spaces beneath the hybrid layer. As a result, the reaction of fluoride and other products prevented the demineralization of dental structure.

According to Itthagarun *et al.*(1), the fluoride released from bonded fluoride-releasing restorative materials and the potential benefit of artificial caries inhibition are indirect indications of the permeability of dentin adhesives and hybrid layers to water and ion movement. In addition, Ferracane *et al.*(14) reported that a fluoride-containing adhesive released fluoride into the microspaces of a restored cavity, and thus offered some degree of protection from demineralization and recurrent caries.

Although the characteristics of the acid-base resistant zone are still unclear, analysis from the current study adequately suggested that the acid-base resistant zone formation was due to monomer penetration and fluoride release. This was especially observed when the fluoride-containing adhesive of Clearfil Protect Bond was used, since its acid-base resistant zone (over 1.0 $\mu$m thick) was thicker than that formed with Clearfil SE Bond (approximately 0.5 $\mu$m thick).

Previous experiments(36−38) have shown the evident nanoleakage within the hybrid layer from non-fluoride-releasing self-etching primer/adhesive systems. Through this study, it was shown that the use of self-etching primer/adhesive systems containing an antibacterial monomer (MDPB) and a fluoride-releasing component was beneficial. Apart from rendering protection against secondary caries formation and progression(32), these materials allowed significant bond strength to be yielded. As fluoride-releasing adhesives are in direct contact with the cavity wall, fluoride ions released from them easily penetrate and diffuse into the dentin at the cavity wall(32).

The progression rate of secondary caries is an important factor that determines the longevity of restorations. As such, further work should be carried out with regard to long-term antibacterial efficacy, as well as the quality and stability, of the acid-base resistant zone beneath the hybrid layer against caries progression.

## ACKNOWLEDGEMENTS

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We certify that the research project "Microtensile bond strength and ultrastructural analyses on enamel and dentin surfaces of self-etching primer adhesives systems", register number **023/2004**, of ***Mirela Sanae Shinohara*** and ***Mário Fernando de Goes***, is in agreement with the recommendations of 196/96 Resolution of the National Health Committee - Brazilian Health Department and was approved by the Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas - UNICAMP.

Piracicaba - SP, Brasil, 07/04/2004

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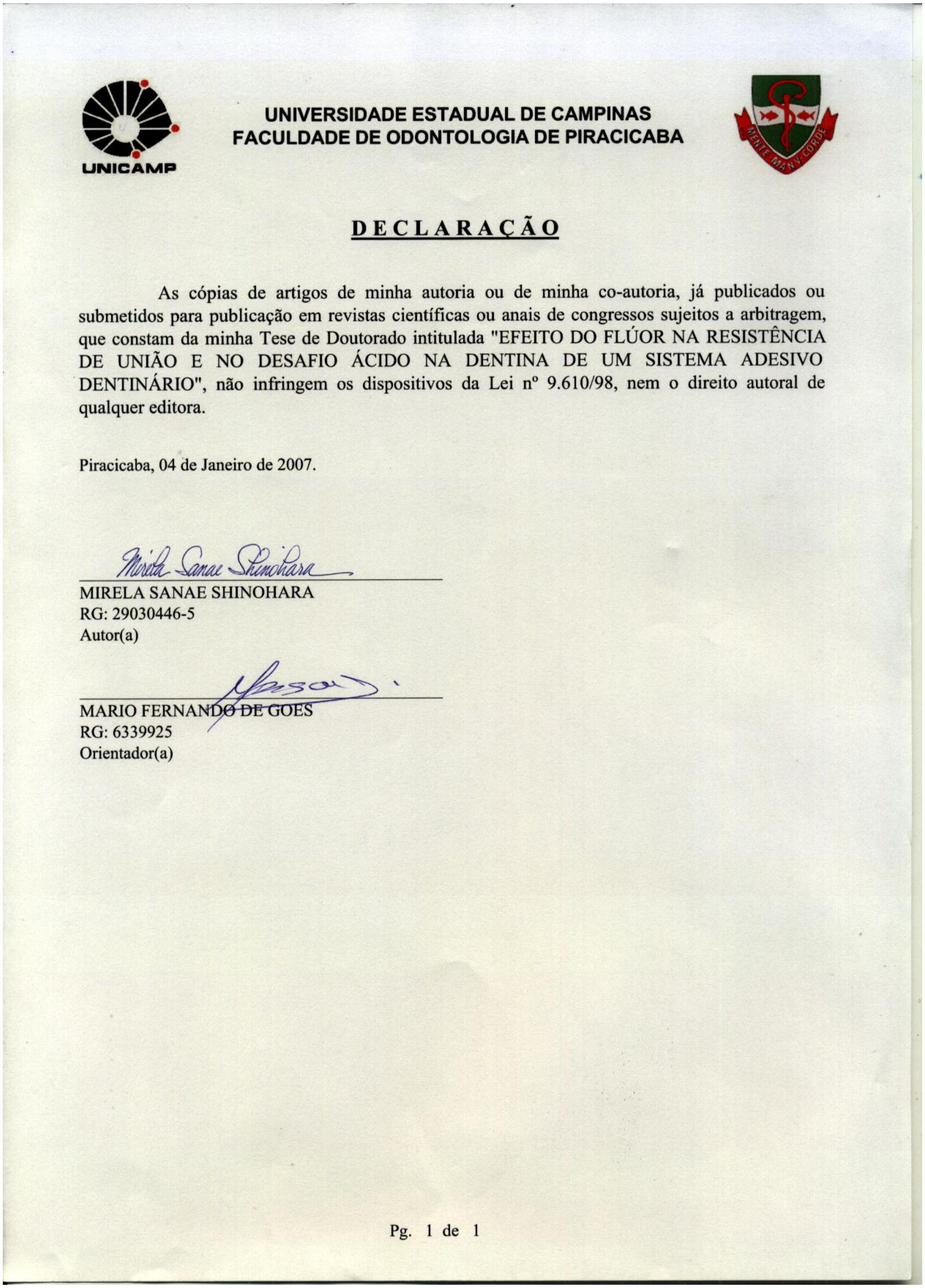

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