Marcos Antonio Japiassú Resende Montes

TENDÊNCIAS DA UNIÃO E ADAPTAÇÃO MARGINAL ENTRE DENTINA E COMPÓSITO ODONTOLÓGICO

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

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EPÍGRAFE

Não há outra felicidade para o homem senão alegrar-se...

E é uma dádiva de Deus o homem comer e beber e, mediante seu trabalho, desfrutar da felicidade...

Assim, come teu pão com alegria,

E bebe contente teu vinho,

Porque Deus se agrada das tuas obras.

Usa sempre vestes brancas e não falte óleo perfumado sobre a tua cabeça.

Goza a tua vida com quem tu amas

Todos os dias da tua vida

Que logo passa...

Sagradas Escrituras, Livro do Eclesiastes
3.12-13; 9.7-9

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SUMÁRIO

RESUMO

A união ao esmalte é tida como um procedimento estabelecido, já a união à dentina permanece um desafio. Os maiores obstáculos para uma união à dentina são, além da contração de polimerização rígida dos compósitos restauradores, que desafia a interface, a própria dentina como substrato, devido ao seu alto conteúdo orgânico e umidade. Este estudo, buscou avaliar e discutir o conceito de "parede cavitária resiliente" bem como a morfologia das interfaces de união à dentina. Avaliar morfologicamente os efeitos de pré-tratamentos de superfície para união à dentina, relacionando-os a implicações clínicas. Avaliar a qualidade marginal de cavidades com alto fator-C, com uso de resinas de baixa viscosidade e remoção do colágeno. Avaliar a adaptação interna de cavidades com alto fator-C e margens em dentina, e avaliar e propor um novo método para observação da região desmineralizada na base da camada híbrida. Com base nos resultados, pôde-se concluir que o uso de uma camada intermediária de baixo módulo de elasticidade entre o adesivo e o compósito restaurador pode absorver tensões, melhorando o selamento marginal e preservando a união. Os pré-tratamentos de superfície promovem alterações diferenciadas na dentina, e devem influir nos procedimentos de união. A qualidade marginal foi melhorada com a remoção do colágeno, para o sistema adesivo com acetona, e o uso de uma camada intermediária de baixo módulo de elasticidade para o sistema auto-condicionante. Os sistemas adesivos testados não foram capazes de prevenir a nanoinfiltração quando usados de acordo com as recomendações dos fabricantes. A remoção do colágeno melhorou a adaptação interna com o sistema adesivo com acetona, e preveniu a ocorrência da nanoinfiltração. A metenamina de prata mostrou-se um método apropriado para detecção e estudo da nanoinfiltração.

ABSTRACT

While bonding to enamel has been considered a reliable procedure, bonding to dentin remains a challenge. There are two major problems related to dentin bonding: the rigid polymerization shrinkage of the restorative composites that challenges the bonded interface; and dentin itself as a bonding substrate, due to its high organic content and its intrinsic wetness. This study, composed by four independent and sequenced articles, had the following objectives. To evaluate and to discuss the "elastic cavity wall" concept and the morphological aspects of the bonded interfaces. To evaluate the morphological effects of surface pre-treatments recommended to dentin bonding, and relate them to clinical implications; To evaluate the marginal quality of restorations in cavities with high C-factor by using low-viscosity resins and after collagen depletion. To evaluate the internal adaptation in cavities with high C-factor and margins in dentin, and to evaluate and to propose a new method for observations of the demineralized zone at the base of the hybrid layer. The results demonstrated that the use of a low elastic modulus intermediate layer between the adhesive and the composite resin may absorb stress, thereby may enhance the marginal seal and preserve the bond. The surface pre-treatments promoted distinct alterations on dentin, and must influence considerably the bonding procedures. The marginal quality was improved with collagen depletion, after acid-etching, for the acetone-based adhesive system, and the use of a low elastic modulus intermediate layer for the self-etching primer. The bonding systems tested were unable to prevent nanoleakage when used according to manufacturer's instructions. Collagen depletion enhanced internal adaptation of restorations for the acetone-based system and prevented nanoleakage occurrence. Silver methenamine staining is a useful method to detect nanoleakage.

1. INTRODUÇÃO

Desde a última década tem-se observado, numa tendência mundial crescente, uma ampliação dos limites do uso de materiais restauradores estéticos. O declínio nos índices de cárie, as técnicas de união à dentina que têm permitido preparos mais conservadores com mínima intervenção operatória, o melhor desempenho quanto às propriedades mecânicas, aliado a uma maior resistência ao desgaste e uma menor contração de polimerização, estenderam definitivamente a aplicação dos compósitos aos dentes posteriores.

Se por um lado, o surgimento de compósitos restauradores com menor contração de polimerização e melhor desempenho mecânico, obtidos através de um aumento na relação carga/matriz orgânica, possibilitou a ampliação desses limites de uso, a magnitude da tensão gerada pela contração de polimerização tornou-se maior, devido à maior rigidez dos materiais e, conseqüentemente, mais danosa às interfaces de união (CONDON & PERRACANE, 2000). Assim, um dos aspectos mais relevantes e desafiadores para a união seria, em um primeiro momento, suportar a contração de polimerização dos compósitos restauradores. A interposição de uma camada com baixo módulo de elasticidade, estabelecendo um gradiente elástico, entre a resina adesiva e o compósito restaurador tem sido sugerida como uma alternativa a esse desafio (KEMP-SCHOLTE & DAVIDSON, 1990a,b; VAN MEERBEEK *et al.*, 1993)

A união ao substrato dentário baseia-se na sua preparação com agentes condicionantes, que aumentam sua energia de superfície, de forma que o torna receptivo aos agentes de união resinosos. Enquanto a união ao esmalte, um tecido uniforme e mormente mineral, tem sido considerado um procedimento estabelecido e seguro, uma união à dentina confiável a longo prazo, ainda permanece um desafio. A dentina é um tecido heterogêneo, com diferenças regionais, composto por uma maior percentagem de material orgânico, além de ser atravessada por uma rede de túbulos que lhe conferem umidade intrínseca, e que a faz um substrato menos receptivo aos monômeros hidrófilos e hidrófobos (PERDIGÃO & LOPES, 1999).

Os princípios para união à dentina foram estabelecidos por NAKABAYASHI *et al.* (1982), e baseiam-se na infiltração de monômeros resinosos na rede de colágeno exposta pela desmineralização, criando uma estrutura mista resina/colágeno, a "camada híbrida". Duas estratégias para união à dentina são aceitas atualmente: a primeira, remove a "smear layer" e desmineraliza a dentina subjacente com ácidos orgânicos ou minerais, deixando, como resultado, uma superfície rica em colágeno onde as resinas devem-se infiltrar; a segunda, baseia-se na desmineralização/infiltração simultânea de monômeros resinosos ácidos no substrato dentinário (PASHLEY & CARVALHO, 1997). Não obstante os avanços, a união à dentina ainda não apresenta a confiabilidade de um procedimento estabelecido. Tem sido demonstrado que os monômeros resinosos não preenchem toda a rede de colágeno exposta pelo condicionamento, e que esse colágeno desprotegido estaria sujeito à hidrólise e degradação, levando a um insucesso do procedimento restaurador a longo prazo (SANO *et al.*, 1994). Ainda que os efeitos desse padrão de infiltração não estejam claros (PIOCH *et al.*, 2001), o conhecimento do fenômeno em si, carrega com uma certa dose de desconfiança e preocupação os procedimentos de união à dentina e, conseqüentemente, o uso dos compósitos como materiais restauradores dentários.

2. REVISÃO DA LITERATURA

Substrato dentinário e estratégias de união

A união de materiais restauradores à estrutura dental tem sido o principal objetivo da Odontologia restauradora, desde que BUONOCORE (1955) estabeleceu as bases para a união ao esmalte.

Assim, enquanto a união ao esmalte é tida na Odontologia como algo confiável e estabelecido, a união à dentina ainda é pouco previsível. Mesmo com a utilização dos recentes sistemas de união, a microinfiltração não foi eliminada, embora tenha sido reduzida. Vários fatores podem ser relacionados para explicar esta diferença. Um deles é o esmalte, um tecido altamente mineralizado e homogêneo. Por outro lado, a dentina é um tecido intrinsecamente úmido, atravessada por uma rede tubular contendo prolongamentos celulares e alto conteúdo de material orgânico. A área mineralizada da dentina é reduzida à medida que se aprofunda no complexo dentino-pulpar (GARBEROGLIO & BRÃNNSTRÖM, 1976). Essa heterogeneidade do tecido dentinário o torna um substrato complexo para o procedimento de união. Um outro obstáculo para um íntimo contato resina/dentina é a presença de uma camada de detritos formada na superfície da dentina após o preparo da cavidade, composta por hidroxiapatita, colágeno e restos celulares (ISHIOKA & CAPUTO, 1989). Esta camada, denominada "smear layer", obstrui os túbulos e diminui a permeabilidade da dentina em cerca de 86% (PASHLEY *et al.*, 1978; ISHIOKA & CAPUTO, 1989; PASHLEY *et al.*, 1993; PERDIGÃO & LOPES, 1999; HALLER, 2000). Nos estudos iniciais sobre a união resina/dentina, a remoção da "smear layer" através do condicionamento com ácido fosfórico, à semelhança do esmalte, foi realizada como uma tentativa para criar retenções micromecânicas. Entretanto, o resultado foi a dissolução da hidroxiapatita com abertura dos túbulos dentinários, exposição da rede colágena e aumento da umidade (PASHLEY *et al.*, 1981), tendo como conseqüência sensibilidade pós-operatória, agressão pulpar e insucesso clínico. Os maiores avanços no processo de união à dentina foram alcançados com a introdução dos sistemas de união com monômeros hidrófilos, que têm a capacidade de se difundir na rede de colágeno exposta (KANCA III, 1992; PERDIGÃO *et al.*, 1996).

Assim, o conceito atual de união do material restaurador à dentina está baseado na difusão dos monômeros através dos espaços por entre a rede de colágeno, formando uma estrutura mista constituída pelas fibras colágenas envolvidas por resina e cristais residuais de hidroxiapatita (VAN MEERBEEK *et al.*, 1992). Esta interação física foi denominada "camada híbrida" (NAKABAYASHI *et al.*, 1982, 1991). Atualmente, duas estratégias são aceitas para a obtenção desta interação física entre resina e dentina. A primeira, chamada de técnica do condicionamento ácido total ou de adesão úmida, baseia-se na remoção completa da "smear layer" e conseqüente desmineralização da dentina subjacente, por meio do condicionamento da superfície dentinária com ácidos orgânicos ou minerais, resultando em uma superfície colagenosa úmida, onde as resinas devem-se difundir para formar a "camada híbrida". A segunda estratégia utiliza monômeros ácidos, chamados de "primers" auto-condicionantes, que desmineralizam parcialmente a "smear layer" e a dentina subjacente, incorporando-as e usando-as como substrato para união (PASHLEY & CARVALHO, 1997; VAN MEERBEEK *et al.*, 1998).

Portanto, pode-se concluir que uma boa união à dentina, depende de uma preparação da superfície com agentes condicionantes que a tornem receptiva aos agentes de união. Esta preparação de superfície deve ser capaz de alterar a capacidade de umedecimento da dentina, de forma a permitir a difusão da resina por toda a extensão da superfície desmineralizada, envolvendo toda a rede de colágeno exposta pelo tratamento.

Embora a técnica do condicionamento ácido total ou adesão úmida venha demonstrando eficácia, o procedimento em si é muito vulnerável e sensível a alterações, uma vez que é difícil produzir um estado de umidade adequado, uniforme e satisfatório na superfície condicionada (TAY *et al.*, 1996). A introdução dos sistemas auto-condicionantes, que utilizam monômeros ácidos para tratamento do esmalte e dentina simultaneamente, trouxe simplicidade à técnica de aplicação e, além disso, como esses sistemas não expõem totalmente a rede de colágeno, aumentaram a segurança do procedimento e diminuíram a influência das diferenças regionais apresentadas pela dentina na resistência da união (PEREIRA *et al.*, 1999). A camada híbrida formada por estes sistemas é significativamente menor em espessura, cerca de 1 µm, quando comparada com a espessura da camada híbrida formada pelos sistemas que dependem do condicionamento ácido total, cerca de 4 µm (VARGAS *et al.*, 1997; PRATI *et al.*, 2000).

A "camada híbrida"

O grande desafio da interface de união resina/dentina, em um primeiro momento, seria suportar as tensões geradas pela contração de polimerização da resina restauradora. Nesta situação é preciso considerar o módulo de elasticidade do material restaurador. Quanto maior o módulo de elasticidade da resina composta, maior a tensão gerada na interface de união (DAVIDSON & DE GEE, 1984; CONDON & FERRACANE, 1998, 2000). Além disso, a magnitude da tensão gerada pela contração de polimerização inclui a relação entre superfícies unidas e livres (fator – C) e a velocidade da reação de polimerização (FEILZER *et al.*, 1987; CARVALHO *et al.*, 1996).

Com isso, o papel desempenhado pela camada híbrida no processo de união tem sido observado com bastante interesse, e alguma controvérsia. Alguns estudos indicam que a infiltração de monômeros ou "hibridização" dos túbulos dentinários e da dentina intertubular é responsável por uma proporção substancial da resistência de união resina/dentina (GWINNETT, 1993; SANO *et al.*, 1999). Por outro lado, outros estudos sugerem que a presença do colágeno não oferece nenhuma contribuição quantitativa na resistência de união da interface (GWINNETT, 1994). A camada híbrida apresenta um módulo de elasticidade relativamente baixo (VAN MEERBEEK *et al.*, 1993) e, teoricamente, poderia funcionar como uma área de absorção das tensões geradas pela contração de polimerização, estabelecendo uma região resiliente entre a resina restauradora e a dentina. Entretanto é provável que sua pequena espessura seja a responsável pela não absorção das tensões, como sua natureza sugere (VAN MEERBEEK *et al.*, 1993; GWINNETT *et al.*, 1996).

Dessa forma, o efeito da remoção do colágeno e, por conseqüência, a não formação da camada híbrida, tem sido objeto de consideração nos procedimentos operatórios restauradores. Como existe uma dificuldade natural em se obter uma completa penetração da resina adesiva em toda extensão da rede de colágeno exposta (SANO *et al.*, 1994), especula-se que uma união à dentina mais previsível poderia ser obtida diretamente com a hidroxiapatita da dentina parcialmente desmineralizada após a remoção do colágeno com hipoclorito de sódio (NaOCl), que alteraria sua composição superficial, tornando-a semelhante ao esmalte condicionado, ou seja, um substrato hidrófilo e, portanto, mais receptivo aos agentes de união resinosos (SAKAE *et al.*, 1988; TANAKA & NAKAI, 1993; INABA *et al.*, 1995). O NaOCl tem sido usado como um agente proteolítico inespecífico que remove efetivamente componentes orgânicos em temperatura ambiente. Estudos mostram que, para alguns sistemas adesivos, a remoção do colágeno com NaOCl pode, de fato, aumentar a resistência de união (WAKABAYASHI *et al.*, 1994; CHERSONI *et al.*, 1997; BOSCHIAN *et al.*, 1997; VARGAS *et al.*, 1997; INAI *et al.*, 1998; SABOIA *et al.*, 2000), enquanto para outros sistemas adesivos a remoção do colágeno não altera ou diminui a resistência de união (GWINNETT, 1994; INAI *et al.*, 1998; SABOIA *et al.*, 2000; FRANKENBERGER *et al.*, 2000) e não altera ou aumenta a infiltração marginal (UNO & FINGER, 1995; VICHI *et al.*, 1997; FRANKENBERGER *et al.*, 2000; TOLEDANO *et al.*, 2000). Assim, durante o procedimento restaurador, existe a indicação da aplicação do NaOCl com a finalidade de aumentar a capacidade de umedecimento da superfície e facilitar o contato do adesivo com um substrato dentinário (TOLEDANO *et al.*, 1999).

O conceito de "parede cavitária resiliente"

O conceito de "parede cavitária resiliente" (KEMP-SCHOLTE & DAVIDSON, 1990a,b; VAN MEERBEEK *et al.*, 1993) tem sido sugerido como uma alternativa para minimizar os efeitos da contração de polimerização rígida das resinas restauradoras. A base disto está na interposição de um material com baixo módulo de elasticidade entre a camada de adesivo e a resina restauradora com a capacidade de deformar-se e absorver as tensões, diminuindo assim o efeito de uma contração rígida na interface (DAVIDSON *et al.*, 1984; LABELLA *et al*, 1999). Neste sentido, as resinas de baixa viscosidade, introduzidas em 1996, passaram a ter uma indicação dentro deste conceito (BAYNE *et al.*, 1998). Embora apresentem maior contração de polimerização do que os compósitos tradicionais, seu módulo de elasticidade é menor e isto a torna mais resiliente e menos critica à interface de união (SWIFT *et al.*, 1996; BAYNE *et al.*, 1998; LABELLA *et al.*, 1999; UNTERBRINK & LIEBENBERG, 1999; CHOI *et al.*, 2000).

Nanoinfiltração

Acredita-se que a eficácia dos sistemas adesivos atuais esteja baseada na completa difusão através da rede de colágeno exposta pelo condicionamento, de maneira que toda rede de colágeno seja envolvida e a dentina parcialmente desmineralizada subjacente seja alcançada. A falha neste processo leva à formação de uma camada de colágeno exposto na base da camada híbrida, não protegido pela hidroxiapatita ou recoberto pela resina. Sugeriu-se que esta camada porosa de colágeno exposto poderia levar a uma diminuição da resistência de união e aumento na microinfiltração a longo prazo (SANO *et al.*, 1994). Este padrão de infiltração foi demonstrado ocorrer em interfaces de adesivo-camada híbrida sem formação de fendas, pela exposição das restaurações a uma solução de nitrato de prata, e o termo *nanoinfiltração* foi designado para diferenciá-lo da microinfiltração típica (SANO *et al.*, 1995). O efeito da nanoinfiltração para a união à dentina ainda não está esclarecido (PIOCH *et al.*, 2001a), embora acredite-se que a existência desse espaço entre a dentina e a resina adesiva possa permitir a penetração de produtos bacterianos e de fluido dentinário, o que, a longo prazo, poderia levar à degradação hidrolítica da resina adesiva e/ou do colágeno exposto, e assim comprometer a estabilidade da união (SANO *et al.*, 1999). As questões relacionadas à real influência da nanoinfiltração para a união à dentina a longo prazo têm movido diversos pesquisadores, na busca do estabelecimento das relações entre os padrões da nanoinfiltração e as mais diversas variáveis envolvidas no processo de união, tais como: sistemas adesivos (SANO *et al.*, 1995; DORFER *et al.*, 2000); tempo de armazenamento (OKUDA *et al.*, 2001); diferenças nos padrões *in vivo/in vitro* (SCHNEIDER *et al.*, 2000); ciclagem mecânica (LI *et al.*, 2002a); ciclagem térmica (LI *et al.*, 2002b); resistência de união (PEREIRA *et al.*, 2001) e remoção do colágeno (PIOCH *et al.*, 2001b). Nenhuma das variáveis até então estudadas pôde ser, por si, direta ou firmemente relacionada à ocorrência da nanoinfiltração, exceto a ausência do fenômeno após a remoção do colágeno.

Objetivos gerais

Os objetivos deste estudo *in vitro* foram, de forma geral, discutir e contribuir com o estabelecimento dos conceitos a respeito da união à dentina.

1. Discutir o conceito de parede cavitária resiliente e morfologia das interfaces de união à dentina;
2. Avaliar morfologicamente os efeitos dos pré-tratamentos de superfície atualmente recomendados para união à dentina, e relacioná-los a possíveis implicações clínicas;
3. Avaliar da qualidade marginal de cavidades com alto fator-C e margens em dentina, usando as duas estratégias de união, aplicando o conceito de parede cavitária resiliente associado a diversos pré-tratamentos de superfície.

4. Avaliar a adaptação interna de cavidades com alto fator-C e margens em dentina, com as duas estratégias de união, além de avaliar e propor um novo método para observação e estudo da nanoinfiltração.

Nota

Os artigos incluídos nesta tese foram publicados ou enviados para publicação.

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3.1 CAPÍTULO 1

A morphological and tensile bond strength evaluation of an unfilled adhesive with low-viscosity composites and a filled adhesive in one and two coats

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Abstract

Objective: The purpose of this study was to measure the tensile bond strength (TBS) testing of resin composite to dentin of three low-viscosity composites, in association with an unfilled adhesive, and a filled adhesive one and two coats respectively, and to evaluate and compare the SEM morphological observations.

Methods: The labial surface of 120 bovine lower incisors were ground to obtain a flat dentin surface allowing demarcation of a 4 mm diameter area with adhesive tape. The teeth were randomly divided in 6 Groups of 20 each. The dentin of each ground surface was etched with 35% H_3PO_4 for 15 s, followed by application of the respective adhesive: Single Bond (SB) for Groups 1, 2, 3 and 6; Optibond Solo (OS) for Groups 4 and 5. In Groups 1 and 4, a resin composite rod was luted to the surface of each specimen with Z100 resin composite. Group 2 received an intermediate layer of Flow It (FI) composite; Group 3 received an intermediate layer of Protect Liner F (PLF) composite; Group 5 received a second coat of OS; and Group 6 received an intermediate layer of an experimental low-viscosity composite (EM). A resin composite rod was luted to the surface of each specimen with Z100 resin composite. All specimens were stored in distilled water at 37° C for 24 h prior to TBS testing. Each specimen was inspected by SEM and classified according to adhesive or cohesive failure mode. One specimen of each group was cut longitudinally, polished and prepared for SEM observation.

Results: The TBS values were: Group 1 (7.86 MPa ± 2.28), Group 2 (7.62 MPa ± 1.85), Group 3 (7.60 MPa ± 2.14), Group 4 (7.96 MPa ± 2.36), Group 5 (7.50 MPa ± 2.70) and Group 6 (7.18 MPa ± 2.40). No significant statistical differences were observed among the groups. However, the analyses of the failure mode presented a considerable variation.

Significance: The use of a filled adhesive or an unfilled adhesive along with a low-viscosity composite as an intermediate layer may provide a stress absorbing layer, whereby improving the preservation of the bonded interface area.

Keywords: Low-viscosity composite; Filled adhesive; Polymerisation shrinkage; Hybrid layer; Elastic modulus, Elastic cavity wall.

1- Introduction

The initial effectiveness and durability of the interface between the resin composite and the tooth tissues may be described as a simple relationship between the bond strength and the stress generated by polymerisation shrinkage. With time, stresses caused by other factors such as occlusal loading and thermal changes will add to the process. To ensure a successful restorative procedure, the bonded interface area must be capable of withstanding those stresses. The elastic modulus of the restorative composite has been reported to be an important factor for the generation of shrinkage stresses: as the elastic modulus increases, more shrinkage stress is transmitted to the interface with less relief [1]. The hybrid layer has a relatively low elastic modulus to relieve polymerisation shrinkage stresses that work as a stress absorbing layer. However, it is not as thick as the bonding resin lying adjacent;—it does not appear to play as important role in relieving stresses as its nature suggests [2,3].

Any material with a high elastic modulus will frequently destroy the bond, leading to postoperative sensitivity and poor marginal quality [4]. Consequently, any material that reduces polymerisation shrinkage and interfacial stress would possess a desirable ability to withstand "plastic flow" on its initial polymerisation phase;—allowing the material to absorb and support the strain, and diminish the effect of a rigid contraction at the interface [5,6].

The first generation of low-viscosity composites was introduced in 1996, developed in response to requests for special handling characteristics, rather than for any clinical performance criteria. As such, their limitations were unknown [7]. These so-called flowable composites were created by retaining the same small particle size of traditional hybrid composites, but reducing the filler content and, consequently, reducing their viscosity. This low filler content brought some concern regarding inferior mechanical properties and higher polymerisation shrinkage when compared to traditional hybrid composites [7,8]. These inferior mechanical properties would restrict their use to low-stress applications in restorative dentistry. The higher polymerisation shrinkage could potentially create more stress on interface areas than with traditional composites during composite curing. However, their lower elastic modulus would, in-turn, be less detrimental and, thus, allow its flow—according to Hooke's law.

Thus, the main benefit of any low-viscosity composites could be to act as a stress-absorbing layer between the hybrid layer, the adhesive resin and the shrinkage of the resin composite, by partially relieving the polymerisation contraction stress [2-5,7-11]. Recommendations for filled adhesives follow the same principles of low-viscosity composites, that is, once the stress absorption is related to the thickness and elastic modulus, thicker layers will absorb greater stress [4,5]. The purpose of this study was to measure the tensile bond strength (TBS) of resin composite to dentin of three low-viscosity composites, in association with an unfilled adhesive, and a filled adhesive one and two coats, respectively, and to evaluate and compare the SEM morphological characteristics.

2- Materials and methods

The materials, manufacturers, composition and batch numbers for this study are listed in Table 1. One hundred and twenty freshly extracted bovine lower incisors were stored frozen, and then used to evaluate TBS. The teeth were ground on their labial surfaces, using a model trimmer to obtain a flat dentin surface. Each was finished with 600-grit silicone carbide paper under water to produce a standardised smear layer. The bonding area was demarcated by positioning an adhesive tape on the prepared surface, in which a 4 mm diameter hole had been punched.

The teeth were randomly divided into six groups of 20 each. All dentin surfaces were etched with 35% phosphoric acid (Scotchbond Etching Gel, 3M Dental Products) for 15 s, rinsed with running water for 20 s and the excess water removed by blotting with tissue paper, leaving the dentin visibly moist. Adhesives were applied using a saturated disposable brush, gently air dried with oil-free compressed air from a syringe for 5 s, keeping the tip 2 cm from the surface and light-cured according to manufacturer's directions. In Group 1, two coats of SB adhesive were applied to the etched dentin surface and placed as described and light cured for 10 s.

In Group 2, following SB application the adhesive surface was covered with FI composite, pressed with a matrix strip and glass slide and light-cured for 40 s. In Group 3, SB was applied and a layer of PLF composite pressed with a matrix strip and glass slide and light-cured for 20 s.

In Group 4, one coat of OS adhesive was brushed for 15 s to the etched dentin surface according to manufacturer's directions, and light-cured for 20 s. In Group 5, two coats of OS were applied, the first being light cured for 20 s and the second then applied and pressed with a matrix strip and glass slide and light-cured for 20 s.

In Group 6, SB adhesive was applied, the surface covered with a layer of EM, pressed with a matrix strip and a glass slide and light-cured for 40 s.

For TBS testing, a resin composite rod of Z100, approximately 15 mm long and 4 mm in diameter with a wire loop was fabricated. The rod surface for adhesion was ground with 220-grit silicone carbide paper under water, ultrasonically cleaned for 5-min, silanised and luted perpendicular to the pretreated (Group) surface with resin Z100. Excess material was removed with an explorer, and the composite light-cured from 3 directions for 30 s each. All light-curing procedures used a 3M XL1500 (3M Dental Products) curing unit. The specimens were stored in distilled water at 37° C for 24 h before TBS testing.

TBS were performed using the same device described by Fusayama et al. [12] and others [13-16], using an Instron Universal Testing Machine, model 4411 (Instron Co., Canton, MA, USA) with a cross-head speed of 0.5 mm/min.

After testing, TBS values were recorded, each specimen was gold-sputter coated (SCD 050 Sputter Coater, BAL-TEC) and observed by SEM (JEOL, JSM-5600LV, Scanning Electron Microscope, Japan) for evaluation of the fracture pattern. Fracture modes were adapted as classified into one of four types: as described by Tanumiharja et al. [17].

Type 1, adhesive failure between the adhesive resin and dentin, and partial cohesive failure in adhesive resin.

Type 2, total cohesive failure in the adhesive resin

Type 3, partial cohesive failure in dentin

Type 4, cohesive failure in low-viscosity composite layer.

The results of TBS were submitted to analysis of variance ANOVA ($p<0.05$).

2.1. Interface micromorphology:

Six specimens prepared as described above, one from each Group, were observed by SEM for interfacial micromorphology. The six specimens were sectioned perpendicular to the bonded interface with a low speed wheel saw under water, obtaining 12 interface sections. Each interface was finished with a 1000-grit silicone carbide paper under water and then polished with 6, 3, 1 and 0.25 μm diamond paste using a polish cloth under water. The interface sections were rinsed between the polishing with water and debris and paste ultrasonically removed for 5 min. Six of 12 sections were demineralised, one from each Group, with a 50% (w/v) H_3PO_4 acid solution for 3 s and then immersed in a 1% NaOCl solution for 10 min to remove the non-encapsulated collagen fibrils. Each section was gold-sputter coated and observed by SEM. The thickness of the hybrid, adhesive and low-viscosity layers of each section were measured directly on the microscope monitor using a multi-point measuring device.

3- Results

TBS values and standard deviations for all treatments are listed in Table 2. Mean TBS ranged from 7.96 ± 2.36 MPa for Group 4 (OS) to 7.18 ± 2.40 MPa for Group 6 (SB + EM). Using one-way ANOVA differences in TBS values among all the tested groups were not significant.

Failure mode observations showed considerable variation among the six groups. For Group 1 with SB, *n*=12 showed a Type 3 partial cohesive failure in dentin that was the most observed mode (Fig. 1), followed by (*n*=8) for Type 1 as an adhesive failure between the bonding resin and dentin.

In Group 2 with SB + FI, we observed almost equal failure modes, Type 1 (*n*=9, Fig. 2) and Type 4 (*n*=8) cohesive failure in low-viscosity composite layer, *n*=3 showed a Type 3 partial cohesive failure in dentin.

In Group 3 using SB + PLF, there were (*n*=16) Type 4 cohesive failure in the low-viscosity composite layer (Fig. 3), *n*=3 showed a Type 1 failure, and *n*=1 showed a Type 3 failure.

In Group 4 using OS, the most frequent (*n*=15) failure mode was Type 1, followed by Type 2 failure seen as a cohesive failure (*n*=5) in the resin bonding layer (Fig. 4).

In Group 5 using OS with two-coats demonstrated a failure mode of Type 4 in (*n*=10), *n*=6 showed Type 2 failure, and *n*=4 showed Type 1 failure.

In Group 6 using SB + EM, a high failure mode of Type 1 was seen in *n*=14 and *n*=6 presented a Type 4 failure mode.

Generally, we note that in Group 5 with OS, the second coat of OS was considered the low-viscosity composite layer. A Type 2 cohesive failure mode in the resin bonding layer, was only present in Groups 4 (OS) (*n*=5) and 5 (OS-2c) (*n*=6), where the filled OS adhesive was applied (Fig. 4), while a Type 3 failure mode of partial cohesive failure in dentin was observed in three of the four Groups where the non-filled SB adhesive was used (Fig. 1). The SB Group 1 showed the highest prevalence (*n*=12) of Type 3 failure mode, followed by SB+FI Group 2 (*n*=3) and SB + PLF Group 3 of (*n*=1). These failure mode data are summarised in Table 3.

3.1. Interface micromorphology:

A hybrid layer was clearly observed for both bonding systems used in this study. For SB, the hybrid layer was about 4-5 µm thick with the presence of long resin tags. The bonding resin layer thickness was about 8-14 µm (Fig. 5). For OS, the hybrid layer was about 6-8 µm thick (Fig. 6); with no measurable difference in resin tags between either system. The bonding resin layer thickness was greater, about 30-50 µm being non-uniform (Control Group 4), with presence of some air bubbles (Control Group 4 and Group 5). The low-viscosity resin layers showed a thickness ranging from approx. 200 µm for PLF to approx. 250 µm for both FI and EM (Figs. 7 and 8).

4. Discussion

Polymerisation shrinkage of composites is the most relevant problem for a successful restorative procedure, once the stress generated by this contraction challenges the bonded-interface area. One possible answer relies on the theory that a low elastic modulus intermediate layer may work as a stress absorber minimising this detrimental contraction—creating the concept of an "elastic stress breaker along the cavity wall" [2-5,7-11]. A low-viscosity composite resin appears to be the most adequate material for this situation, as it has a lower elastic modulus than conventional hybrid composites, and since it has a lower filler content, even with higher polymerisation shrinkage, it would be less harmful to the long-term bond because it is less rigid.

The bonding systems used in this study are single component dentin bonding agents, combining the primer and adhesive resin into one solution. Both SB and OS bonding systems utilise ethanol and water as solvents, and are Bis-GMA/HEMA based. The basic difference is that while SB is an unfilled resin, OS contains a barium glass filler with approx. 0.6 µm sized particles in its composition (Table 1).

The thickness of the hybrid layer for the two bonding systems was approx. 4-5 µm for SB and 6-8 µm for OS (Figs. 5 and 6). Since both systems have a similar chemical base (Bis-GMA/HEMA), the presence of a barium glass filler in OS is the only difference for the bonding procedures. The barium glass filler gives the bond a gel consistency, which, by the theory of "elastic cavity wall", improves the adhesive strength and reduces the polymerisation shrinkage of the adhesive resin. Furthermore, the brushing motion during the application of OS may damage parts of the demineralised dentin surfaces [18], which was probably responsible for the higher thickness of hybrid layer to OS and the air bubbles inclusion. However, as the hybrid layer was visualised under SEM, which is only made possible by sectioning the resin-dentin interfaces, it is possible that even a light inclination of the cut may have made the hybrid layer appear thicker [19]. Differences were also seen in the thickness between the adhesive layers, ranging from 8-14 µm for SB (Fig. 5) to 30 µm for OS (Control Group 4). Even having a relatively low elastic modulus, the hybrid layer may not be considered to serve as a stress absorber, due to its limited thickness [2,3,19], but the adjacent adhesive layer showed a distinct behaviour. The values obtained from TBS did not show any statistically significant differences between the two control Groups 1 and 4 (Table 2) when evaluated for bonding only. However, they did show considerable differences regarding their failure mode (table 3).

When the two adhesive control systems Groups 1 and 4, were compared for their failure mode, a noticeable difference in the prevalence of failure mode was observed (Table 3). SB presented a high prevalence of Type 3 mode, with a partial cohesive failure in dentin (*n*=12). On the other hand, OS showed a remarkable predominance of Type 1 mode, adhesive failure and partial cohesive failure in adhesive resin (*n*=15). One explanation may be that the thicker adhesive layer in OS specimens simply absorbed part of the tensile stress, thereby reducing the stress at the resin-dentin hybrid layer. The SB adhesive layer is considerably thinner, and, thus, its thickness would not support or absorb the tensile stress. Another contribution may be due to inclusion of air bubbles during the mixing of OS, as data show that porosities incorporated during mixing, effectively increase the elasticity of the materials [3]. Cohesive failure in dentin does not require a superior quality of bond strength [20-23]. The tendency for dentin failure may be explained by the brittle characteristics of the materials involved, allowing a deflection of forces into dentin. This was seen when we applied the low-viscosity composites FI, PLF and EM onto the SB surface in Groups 2, 3 and 6 and a second coat of OS was applied (Group 5): bond strength values were maintained, however, failure modes were changed (Table 3).

Low-viscosity composites were created by retaining the same small particle sizes of traditional hybrid composites, but reducing the filler content, thereby reducing the material viscosity [7]. Indeed, its elastic modulus is significantly lower (30% to 50%) than that of traditional hybrid composites [10].

FI is a first-generation low-viscosity composite, with barium borosilicate glass as its filler, with an average diameter of 1.5 μm and a high filler content (70.5% by weight) when compared with the other low-viscosity composites of its generation. PLF is a low-viscosity composite with a microfiller content of 42% by weight. EM is an experimental low-viscosity composite with a zirconium/silica filler particle (average diameter of 1.5 μm) and a filler load of approx. 68% by weight. According to the manufacturer's information EM also contains a dimethacrylate polymer (patent pending) that modifies the rheology of the material allowing it to flow under pressure, but holds its shape and stays in place until light cured, a thixotropic behaviour. This handling characteristic was observed in this study.

The use of low-viscosity composites in this study did not alter the mean TBS of the unfilled adhesive SB to dentin (Table 2), but influenced considerably the failure mode (table 3). When low-viscosity composites were used, we observed a drastic reduction in Type 3 failure, partial cohesive failure in dentin, suggesting the use of an intermediate low elastic modulus layer will work as a shock absorber or a "stress-breaker". Even having a lower elastic modulus than Z100, when FI was used, we still observed some Type 3 failure (*n*=3) when compared to PLF (*n*=1) and EM (*n*=0). Its high filler content as cited above, and its mechanical properties, lower but comparable to conventional composites [7], could be responsible for these results.

The use of EM is quite interesting as it and SB and Z100 belong to the same manufacturer and present the same zirconium/silica filler particle (EM and Z100), giving the idea of a unique body specimen configuration with a decreasing elastic modulus from the traditional composite resin, passing by the low-viscosity composite layer, to the adhesive resin layer. Although it did not present any Type 3 failure, the high prevalence of Type 1 failure (*n*=14) could have occurred, due to a mechanical behaviour close to that presented by FI, as both have almost the same filler loading (68%/70.5% by weight) and the same mean particle size.

PLF displayed a distinct performance when compared to FI and EM in this study, presenting the highest prevalence of Type 4 failure (*n*=16), partial cohesive in low-viscosity composite layer. One possible explanation for this behaviour is due to its different profile, as a microfilled composite with 42% by weight of colloidal silica and prepolymerised filler, by itself, making the difference when compared to the previous two regarding the elastic modulus, as traditional microfilled composites differ from traditional hybrid composites. In this study, even though PLF demonstrated a thinner layer (200 μm, Fig. 8), and FI and EM (Fig. 7) a thicker layer (250 μm), its lower elastic modulus must have been the reason for such a prevalence of Type 4 failures (*n*=16), and, thus, absorbing part of the stress and failing cohesively, reinforcing the elastic cavity wall concept.

This study indicates that the use of low-viscosity intermediate resins and a filler adhesive had no effect in TBS of composite resin to dentin, but influenced remarkably the failure modes. Although these specimens did not undergo thermal and occlusion stresses, or polymerisation shrinkage stress in a cavity condition, it is possible to conclude that low-viscosity intermediate resins will act as a stress absorbing layer due to its lower elastic modulus, that allows its deflection between the rigid traditional composites and dentin substrate, improving the marginal seal and increasing the long-term durability of the dentin bond.

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Table 1. Materials used in this study

Materials	Composition*	Batch number	Manufacturer
Single Bond (SB)	HEMA, Bis-GMA, dimethacrylates, ethanol, water, polyalkenoic acid, copolymer, initiator	8004BS	3M Dental Products, St. Paul, MN, USA
Optibond Solo (OS)	Bis-GMA, GPDM, HEMA, silica, barium glass, sodium hexaflurosilicate, ethanol, water	905268	Kerr Corp. Orange, CA, USA
Flow It (FI)	Bis-GMA, TEGDMA, barium glass, silica, titanium dioxide	26656	Jeneric/Pentron, Inc., USA
Protect Liner F (PLF)	Bis-GMA, TEGDMA, fluoride-methyl methacrylate, camphorquinone, silanised colloidal silica, prepolymerised organic filler	0039AY	Kuraray Co., LTD. Japan
Z100	Bis-GMA, TEGDMA, zirconium/silica filler	8004A3	3M Dental Products, St. Paul, MN, USA
Experimental low - viscosity resin (EM)	Bis-GMA, TEGDMA, dimethacrylate polymer**, zirconium/silica filler	n.a.**	3M Dental Products, St. Paul, MN, USA

* According to manufacturer's informations.

** Patent pending (according to manufacturer's information); n.a. = not available.

Table 2. Tensile bond strength of the Groups evaluated (MPa).

Groups	Mean*	SD
Group 1 (SB)	7.86	± 2.28
Group 2 (SB + FI)	7.62	± 1.85
Group 3 (SB + PLF)	7.60	± 2.14
Group 4 (OS)	7.96	± 2.36
Group 5 (OS-2c)	7.50	± 2.70
Group 6 (SB + EM)	7.18	± 2.40

* No significant difference by ANOVA ($p<0.05$)

Table 3. Failure modes

Groups	Type 1[a]	Type 2[b]	Type 3[c]	Type 4[d]
Group 1 (SB)	8	0	12	n.a.[e]
Group 2 (SB + FI)	9	0	3	8
Group 3 (SB + PLF)	3	0	1	16
Group 4 (OS)	15	5	0	n.a.[e]
Group 5 (OS-2c)	4	6	0	10
Group 6 (SB + EM)	14	0	0	6

[a] Type 1: Adhesive failure between adhesive resin and dentin, and partial cohesive failure in adhesive resin.
[b] Type 2: Total cohesive failure in adhesive resin.
[c] Type 3: Partial cohesive failure in dentin.
[d] Type 4: Partial cohesive failure in low-viscosity composite layer.
[e] n.a. = not available.

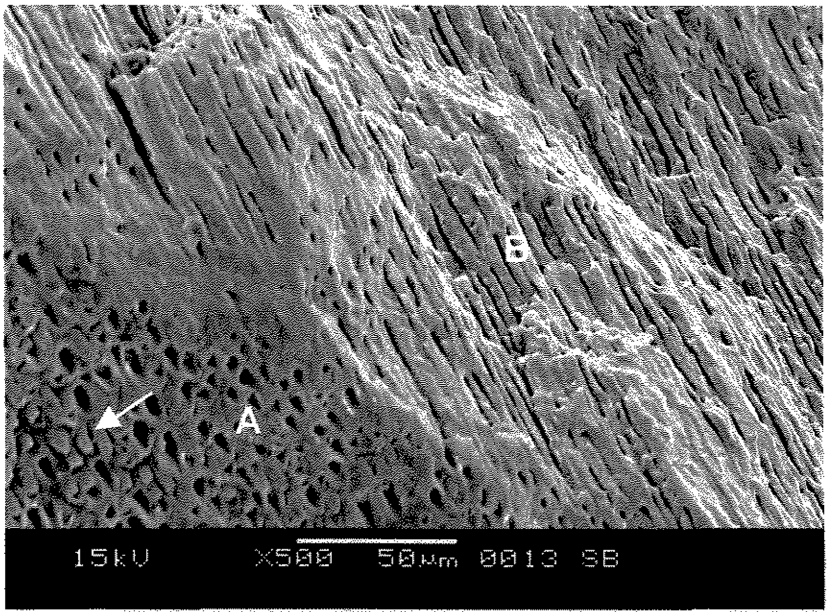

Figure 1: SEM photomicrograph illustrating a Type 3 failure, showing the bonding resin remnants (A) with some dentin tubules filled with resin (white arrow) and the dentin cohesive failure area (B).

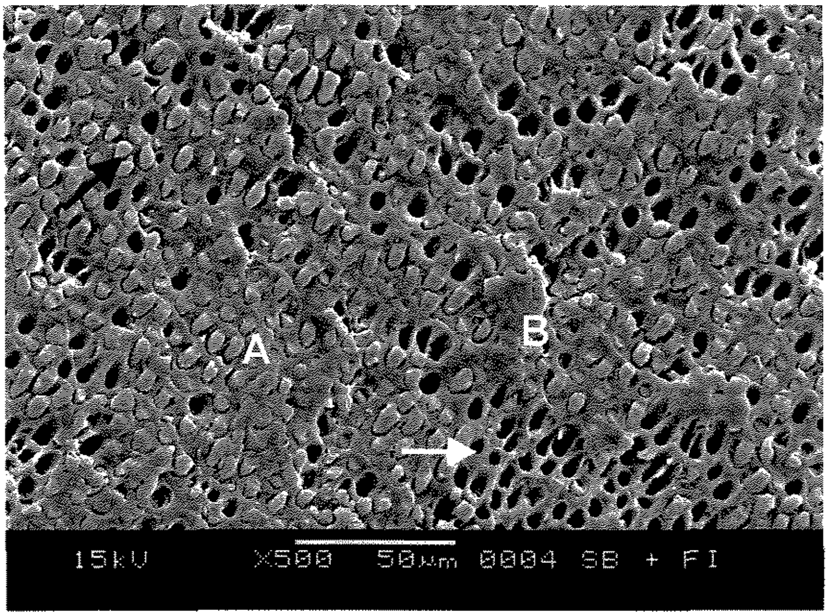

Figure 2: SEM photomicrograph illustrating a fractured surface (Type 1 failure: Adhesive failure between adhesive resin and dentin) (A), and partial cohesive failure in adhesive resin (B). Some dentin tubules are opened (white arrow) while in some areas the tubules remain filled by the bonding resin (black arrow).

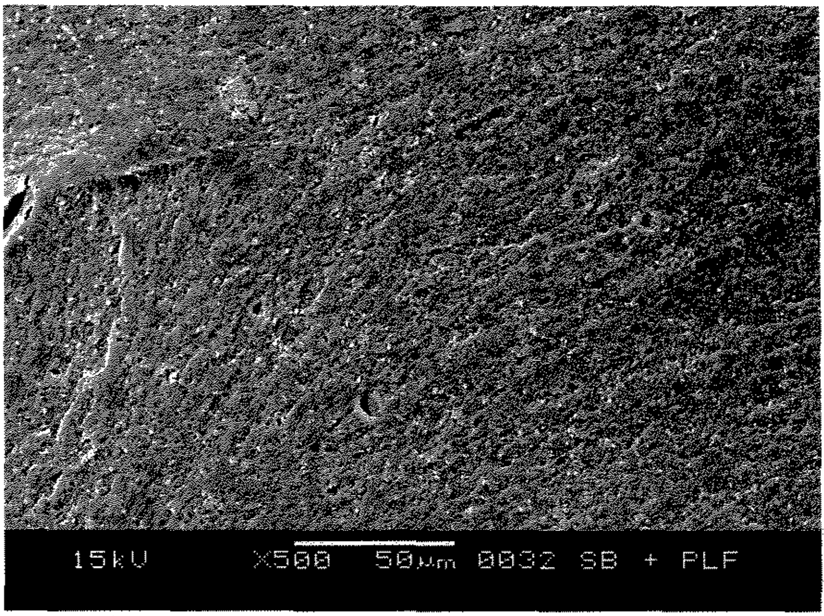

Figure 3: SEM photomicrograph illustrating fractured surface where a cohesive failure in low-viscosity composite layer is observed, Type 4, prevalent when the low-viscosity composite Protect Liner F was applied.

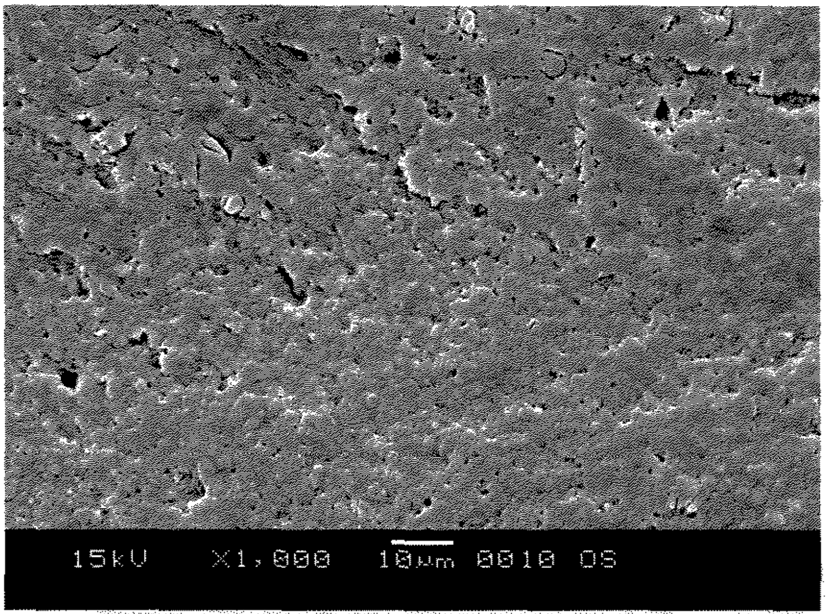

Figure 4: SEM photomicrograph illustrating a fractured surface, where a total cohesive failure in adhesive resin layer is observed, Type 2, occurred when the bonding resin Optibond Solo was applied.

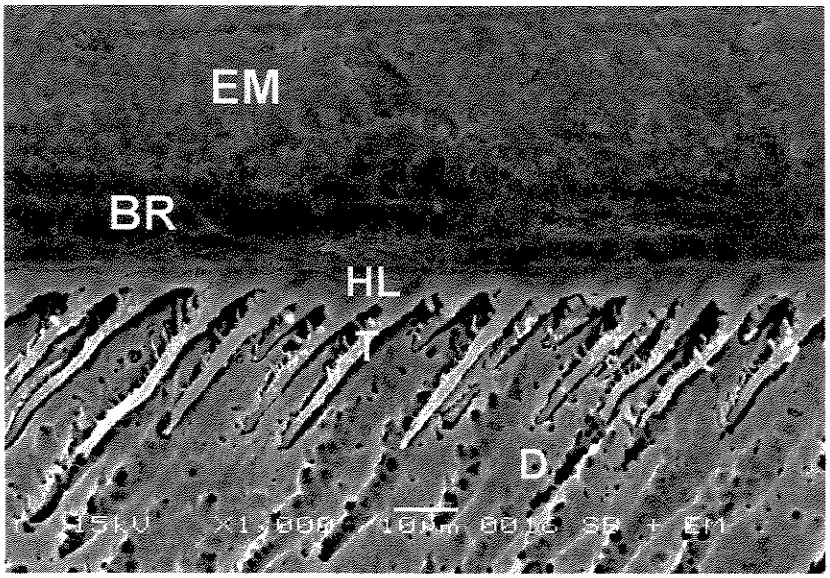

Figure 5: SEM photomicrograph illustrating the resin/dentin interface produced by Single Bond, after treatment with H_3PO_4 and NaOCl. After Single Bond was light cured, a low-viscosity composite (EM) was applied. A uniform resin tags formation (T) can be noticed. Hybrid Layer , HL; bonding resin layer, BR; Dentin, D.

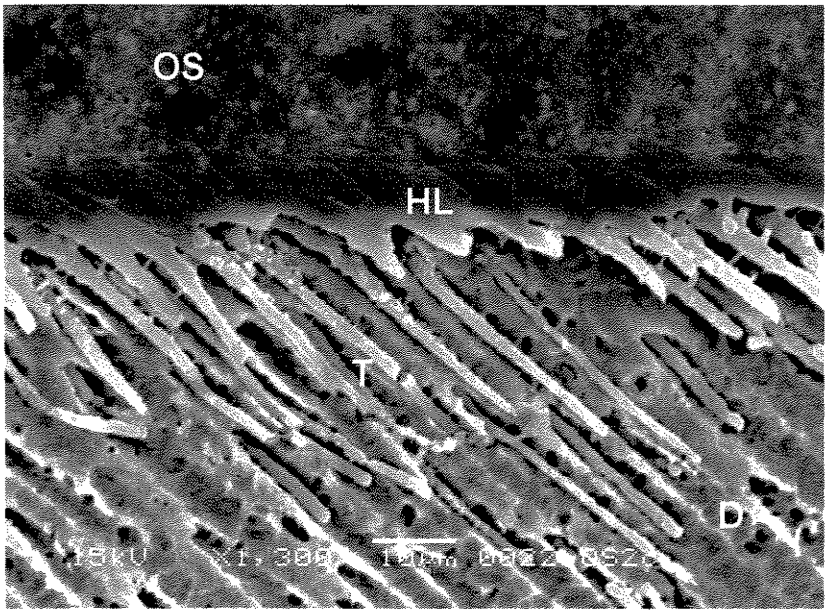

Figure 6: SEM photomicrograph illustrating the resin/dentin interface produced by Optibond Solo, after treatment with H_3PO_4 and NaOCl. After a first application of Optibond Solo was light cured, a second layer was applied. Long resin tags formation can be noticed (T). Hybrid Layer, HL; bonding resin layer, OS; Dentin, D.

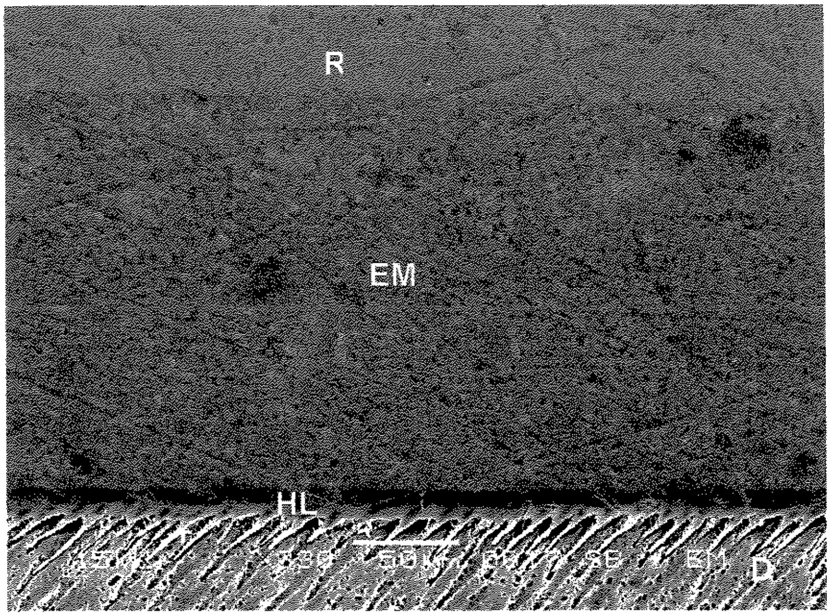

Figure 7: SEM photomicrograph illustrating the resin/dentin interface produced by Single Bond, after treatment with H_3PO_4 and NaOCl. After Single Bond was light cured, a layer of the experimental low-viscosity composite (EM) was applied. Notice the low-viscosity composite layer thickness. Hybrid Layer and bonding resin layer = HL; Resin tags = T; Dentin = D; Composite resin Z 100 = R.

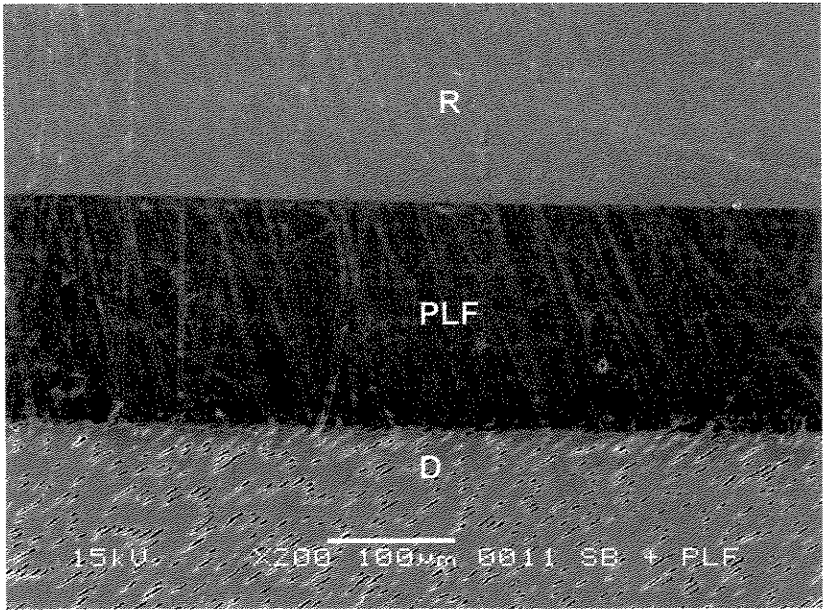

Figure 8: SEM photomicrograph illustrating the resin/dentin interface produced by Single Bond. It can be observed the intermediate layer of low-viscosity composite Protect Liner F (PLF). This specimen was not submitted to H_3PO_4 and NaOCl treatment. Dentin = D; Composite resin Z 100 = R.

3.2 CAPÍTULO 2

The "in vitro" morphological effects of current pre-treatments on dentin surface: a SEM evaluation

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Abstract

Objective: The purpose of this study was to evaluate morphologically *in vitro*, using scanning electron microscopy, the effect of some current surface pre-treatments on dentin and to relate these morphological alterations to clinical implications.

Methods: The labial surfaces of 30 bovine lower incisors were ground to obtain a flat dentin surface and finished with 600-grit SiC paper to produce standardised smear layers. The teeth were randomly divided in six groups of 5 each. Group 1 was the control group, smear layer-covered dentin; Group 2 was etched with 37% PA for 15 s; Group 3, 37% PA for 15s, followed by 10% NaOCl for 60 s; Group 4, 10% NaOCl for 60 s; Group 5, CSEB-primer was applied for 20 s; Group 6, CSEB-primer for 20 s, followed by NaOCl for 60 s. The specimens were fixed, dehydrated, dried and analysed by SEM.

Results: Treatment with 37% PA removed the smear layer, funnelled the tubules and resulted in a collagen-rich surface which appeared to have collapsed in its outermost part producing a dense surface layer with silica particles. When 37% PA treatment was followed by 10% NaOCl, the collagen network was removed and revealed an eroded and rough mineral surface with numerous lateral branches and larger tubular orifices. The action of 10% NaOCl on smear layer-covered dentin did not show significant alteration in surface morphology. The treatment with CSEB-primer dissolved the smear layer but only partially dissolved the smear plugs. The tubules did not present the typical funnel shape of PA treatment. When NaOCl was applied following CSEB-primer, major alterations were noticed in the smear plugs. From the tubular orifices, a very well defined peritubular collagen matrix could be observed.

Significance: The first step for a successful adhesive restorative procedure is the pre-treatment of the substrate for bonding. The total-etch technique has been proved to be very sensitive and aggressive to dentin surface. The role of the collagen network on dentin bonding has also been questioned, and the self-etching primers have been suggested as a reliable technique for bonding to dentin; they are less aggressive and promote a more uniform surface after treatment. The morphological alterations on smear layer-covered dentin promoted by these agents, evaluated by this study, are important to understand better the bonding techniques.

Keywords: Smear layer, phosphoric acid, Total-etch technique, Collagen, Sodium hypochlorite, Self-etching primer.

1. **Introduction**

The current concept for bonding to tooth hard tissues is based upon the infiltration of a low-viscosity resin through enamel and dentin, a process known as hybridisation [1]. While bonding to enamel has been considered a quite reliable procedure since it is a uniform tissue, mainly composed of a mineral (hydroxyapatite), bonding to dentin remains a challenge as it contains a greater percentage of water and organic material, mainly type 1 collagen, and it is an intrinsically wet tissue penetrated by a tubular network which communicates with the pulp [2]. Furthermore, when a cutting procedure is performed on tooth structure, the dentin surface is covered by an approx. 1 μm layer of debris composed mostly by cut collagen fibres and hydroxyapatite crystallites. An effective adhesion to dentin begins with the treatment of this so-called *smear layer* [3].

There are two different ways that the current bonding systems obtain an acceptable micromechanical retention between resin and dentin. The first method is based upon the complete removal of the smear layer and demineralisation of subsurface intact dentin, using acid etching with mineral or organic acids, leaving a collagen rich and moist surface where resins must diffuse to form a collagen-resin interdiffusion zone first described as a *hybrid* *layer* [1]. The second method uses slightly acidic primers, so-called self-etching primers, to partially demineralise the smear layer and the underlying intact dentin, incorporating the dissolved smear layer remnants and using them as a bonding substrate [4,5].

Based on the methods mentioned above, a successful bonding to dentin depends upon the preparation of a surface with priming agents which render it receptive to a bonding agent. This surface preparation must be able to alter the wettability of dentin in order to permit the diffusion of resin to the complete extent of the demineralised surface, enveloping the entire collagen network exposed by such treatment.

In the first method, the most commonly used conditioning agent is phosphoric acid (PA) in concentrations that vary from 10% to 40%. A major problem with this technique is its sensitivity [6]. The removal of the smear layer and the smear plugs from the dentin tubules increases the natural surface moisture of dentin and its vulnerability [7]. The maintenance of the structural integrity of the collagen fibres and of the interfibrillar spaces to allow permeation of adhesive monomers is totally moisture dependent, since it plays a crucial role in preventing the collapse of the collagen network [6,8,9]. Furthermore, the acidic conditioners induce considerable changes on collagen conformation mostly associated with denaturation processes [10]. Consequently, without an adequate and precise post-etching treatment of this surface, it will result in a dramatic failure including severe postoperative sensitivity and pulpal irritation.

The role of the hybrid layer in dentin bonding has been constantly questioned [11-13]. It has been shown that resin monomers do not fully diffuse through the collagen network after acid etching of the dentin. This incomplete penetration into the collagen network may produce a porous layer of collagen not protected by hydroxyapatite or encapsulated by resin. This exposed collagen is subjected to hydrolysis and degradation, resulting in increased microleakage and failure over time [14]. Moreover, the theoretical benefit of avoiding the acid-exposed collagen layer is that these above mentioned problems could be prevented. The removal of collagen is suggested as a suitable method to alter the composition of dentin as it becomes similar to etched enamel, i.e., a more predictable and hydrophilic substrate for bonding [15-17].

Sodium hypochlorite (NaOCl) is a non-specific proteolytic agent that effectively removes organic compounds at room temperature [15]. Data from studies suggest that for some adhesive systems the removal of collagen fibres with NaOCl pre-treatment may actually increase bond strength [18-23], while for other adhesive systems the collagen removal did not alter or diminished bond strength [11,22-24] and did not alter or increased marginal leakage [12,24-26]. Scanning electron microscopy (SEM) studies of demineralised dentin surfaces that have been treated with NaOCl reveals an altered eroded surface appearance [22,27] and the development of a network of secondary channels [27].

The composition of a smear layer is generally similar to the originating tissue; thus, it is assumed that its mineral phase is similar to that of normal dentin (about 50% vol), and that the collagen phase (30% vol) is insoluble and cannot be rinsed away after acid etching. When the acidic conditioners remove the solid mineral content of the smear layer, this residual collagen phase of the smear layer resists, thereby enriching the surface with organic material, which may then partially occlude the spaces between demineralised collagen fibres interfering with adhesive infiltration [3]. A pre-treatment of the smear layer with NaOCl could eliminate this collagen phase and enhance the resin infiltration after acid etching. SEM studies have shown no considerable morphological changes when NaOCl was applied on a smear layer, suggesting only a superficial collagen removal [16,28].

The advent of the self-etching primers represented a very simple strategy to prevent the collagen from collapsing, avoiding its unprotected exposure. When these systems are applied, there is no need for etching, rinsing and drying, so that the risk of over-etching, over-drying or over-moistening the dentin is eliminated [29]. A recent morphological study classified these acidic primers into three classes as mild, moderate and aggressive, based on their ability to penetrate dentin smear layers and their depth of demineralisation into the subsurface dentin. The more aggressive system completely solubilised the smear layer and smear plugs and formed hybrid layers with a thickness similar to those obtained by PA conditioned dentin, while the mild system was able to diffuse through the smear layer to the subsurface intact dentin, producing a thin but authentic 0.5 μm hybrid layer and dentinal tubules obliterated with thick, hybridised smear plugs [30].

The morphological alterations promoted by these pre-treatments on dentin may play an important role in bonding to dentin, since they change the surface features considerably and must thereby influence the diffusion of the bonding resins through the substrate.

The objective of this study was to evaluate morphologically, by SEM, the effect of some current surface pre-treatments on ground dentin and to relate these morphological alterations to clinical implications.

2. **Materials and methods**

The materials, manufacturers, composition, pH and batch numbers for this study are listed in Table 1. Freshly extracted bovine lower incisors (n=30) obtained in a local *abattoir*, stored frozen for no longer than 1 month, were used in this study. The teeth were ground flat on their labial surfaces on a water irrigated grinding wheel using a 320-grit silicone carbide paper (SiC) to expose the underlying dentin. Each was finished with 600-grit SiC to produce standardised smear layer. In order to obtain rectangular shape specimens, the roots and incisal portions were removed with a low-speed diamond saw under water, which was also used to notch the incisal and gingival edges to allow a guided fracture for a longitudinal observation. The specimens were randomly divided into six groups of 5 each according to the treatment (Table 2):

Group 1. Control Group, no treatment. The specimens were rinsed with an air-water spray.

Group 2. The dentin surface was etched with 37% PA for 15 seconds and rinsed for 20 seconds with running water.

Group 3. The dentin surface was etched with 37% PA for 15 seconds, rinsed for 20 seconds with running water, and a 10% NaOCl solution was lightly scrubbed on the etched surface for 1 minute using a disposable brush and then rinsed off for 20 seconds with running water.

Group 4. A 10% NaOCl solution was lightly scrubbed on the dentin surface for 1 minute using a disposable brush and rinsed off for 20 seconds with running water.

Group 5. The self-etching primer Clearfil SE Bond (CSEB) was applied on a briefly air-dried dentin surface using a disposable brush and left undisturbed for 20 seconds, gently air thinned and then immersed in a 50% acetone solution for 30 seconds to remove the primer, and then rinsed for 20 seconds with running water.

Group 6. The same as for Group 5, but after the 50% acetone solution immersion the specimens were rinsed with running water, and a 10% NaOCl solution was applied to the dentin surface as for Groups 3 and 4.

The specimens were immediately immersed in a 2.5% glutaraldehyde in 0.1M sodium phosphate buffer at pH 7.2 for 12 hours at 4°C. After fixation, the specimens were rinsed with 15 ml of 0.2M sodium phosphate buffer at pH 7.2 for 1 hour, in 3 baths of 20 minutes each. The specimens were dehydrated in ascending grades of ethanol in the following steps: 25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 30 minutes and 100% for 60 minutes. After the final ethanol step the specimens were dried by immersion in hexamethyldisilazane (HMDS) for 10 minutes, placed on a filter paper inside a covered glass vial, and air-dried at room temperature for 24 hours [40].

After drying, the specimens were fractured using a sharp chisel placed and pressed on the notch. The specimens then provided two halves that were mounted on aluminium stubs with carbon tape, one to permit a cross-section analysis and the other for a longitudinal-section observation. The specimens were gold-sputter coated (SCD 050 Sputter Coater, BAL-TEC, Liechtenstein) and observed by SEM (JEOL, JSM-5600LV, scanning electron microscope, Japan). The specimens were observed in several magnifications and the measurements were made directly on the microscope monitor using a multi-point measuring device.

Table 1. Materials used in this study

Materials	Composition	pH	Batch number	Manufacturer
Phosphoric acid	Etching gel silica-thickened 37% H_3PO_4	0.05	66561	Dentsply Ind. Com. Ltda., Brazil
Clearfil SE Bond (primer)	MDP, HEMA, Hydrophilic dimethacrylate, Camphorquinone, N,N-Diethanol p-toluidine, water, ethanol	2.0	00157A	Kuraray Co. Ltd., Japan

Abbreviations: MDP: 10-methacryloyloxidecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate.

Table 2. Treatment Groups.

Groups	Etchant	10% NaOCl
1	-	-
2	37% H_3PO_4 (15 s)	-
3	37% H_3PO_4 (15 s)	60 s
4	-	60 s
5	CSEB primer (20 s)	-
6	CSEB primer (20 s)	60 s

3. Results

The analyses of the specimens in cross-sections or longitudinal-sections showed a varied morphology that depended on the various dentin pre-treatments. These results are shown in Figs. 1 to 13.

Group 1: The smear layer-covered dentin observed in cross-section showed a surface completely covered by a crust of cutting debris appearing to have little surface porosity; the orifices of the underlying tubules were completely obstructed (Fig. 1). The smear layer particles could not be observed individually, even in higher magnifications, since the surface appeared very compacted. The longitudinal-sections produced a thin smear layer ranging from 0.5 to 0.7 μm (Fig. 2).

Group 2: Treatment with PA resulted in a demineralised layer that appears to have collapsed in its outermost part producing a dense surface layer. The smear layer was completely removed and the dentin tubules were opened. The diameter of the tubules ranged from 3 to 4 μm (Fig 3). A higher magnification image focused on the tubular orifice and revealed details of the peritubular collagen matrix, surrounding the tubular aperture as a dense interlacement of collagen fibres (Fig. 4). Granular silica deposits proceeding from the PA thickener were also evident randomly distributed on intertubular collagen surface (Figs 3 and 4). When the dentin tubules were observed longitudinally three distinct successive layers in the intertubular demineralised dentin zone were evident: an outermost layer of compacted collagen fibres with some remnants of the smear layer and of the acid etching (this outermost layer apparently collapsed onto the tubules lumen narrowing its aperture); a second or intermediate layer of separated collagen fibres appearing a more porous network; and a third deeper layer presenting wide spaces and few collagen fibres (Figs. 5A and B). The tubules presented a funnel shape, which was attributed to a differential demineralisation pattern between intertubular and peritubular dentin. The intertubular fibres presented a random distribution while the peritubular fibres showed a circular pattern (Figs 5A and B).

Group 3: When acid-etching dentin was followed by NaOCl deproteinisation, the cross-section dentin surfaces developed an eroded or "moth-eaten" aspect. The intertubular collagen was completely removed and only a few remnant fibres could be seen in peritubular collagen matrix. The tubular orifices were larger, ranging from 3 to 5 μm and the funnel shape was more evident. The orifices of numerous lateral branches and a reduction of intertubular dentin area were clearly observed (Fig. 6). The longitudinal-sections showed funnel shaped tubules, some remnant collagen fibres from the peritubular collagen matrix and some lateral branches open in the lumen of the tubule near the dentin surface (Fig. 7).

Group 4: When NaOCl was applied to smear layer-covered dentin there was no significant alteration in surface morphology, mainly when observed in cross-sections (Fig. 8). The longitudinal-sections showed a light but noticeable reduction in smear layer thickness, ranging from 0.3 to 0.5 μm (Fig. 9)

Group 5: The observation of the cross-section dentin surfaces showed that the treatment with CSEB-primer was strong enough to dissolve the smear layer but only partially dissolved the smear plugs. The demineralisation was superficial and did not show any noticeable difference between intertubular or peritubular dentin pattern, showing the mineralised peritubular dentin collar around the tubules lumen. The tubular orifices ranged from 2 to 4 μm, and in addition to the partially demineralised smear plugs some exposed collagen fibres from the peritubular collagen matrix were also observed (Fig. 10). The longitudinal-sections did not show the typical funnel shape as observed for PA specimens with the tubules appearing partially occluded by the smear plugs (Fig. 11).

Group 6: When the treatment with CSEB-primer was followed by NaOCl deproteinisation the major alterations were noticed in the partial dissolution of the smear plugs, clearly observed in cross-sections. A very well defined peritubular collagen matrix could be observed through the tubular orifices (Fig. 12). The longitudinal-sections observations confirmed the alterations found in the cross-sections (Fig. 13).

4. **Discussion**

There are two major problems related to dentin bonding; one is related to the rigid polymerisation shrinkage of the restorative composites that challenges the bonded interface, and the other concerns dentin itself as a bonding substrate. The high organic content and the intrinsic wetness due to its tubular structure and the outward flow of fluid have made dentin bonding difficult to accomplish successfully [3,4]. Moreover, after cavity preparation, the dentin is covered by a layer of cutting debris with a very limited strength, low permeability [31] and wettability [32], the so-called smear layer. Thus, the only two options to achieve an acceptable bond strength to the dentin are the removal of the smear layer or the development of agents that penetrate through the smear layer into the underlying dentin matrix [4]. It has been related that smear layer particles vary widely in their size, from 0.05 to 10 μm and that the larger particles have a plate shape, which permit them to be compacted in a layer that is rarely thicker than 1 μm [33]. In our study the smear layer was observed in cross-sections as a compact crust, so that the particles could not be observed individually even in higher magnifications (Fig. 1); it was thin, ranging from 0.5 to 0.7 µm, when observed longitudinally (Fig.2). This thinness was probably a result of a light pressure during the specimens finishing procedures with 600-grit SiC.

For the first method approach, i.e., removal of smear layer, also called total-etch technique, the most common agent used is PA in gel form and in concentrations that vary from 10 to 40%. The 37% silica thickened PA used in this study completely removed the smear layer, funnelled tubule orifices, increased intertubular porosity and produced a dense demineralised collagen-rich layer that appeared to have suffered a high degree of collapse and/or denaturation in its outermost part (Fig. 3 to 5). There are some possible reasons for this phenomenon: (1) some unintended air-drying after acid etching; once the water evaporates from the outer part of collagen network the forces of surface tension at the air-water interface tend to collapse it, making the spaces around the collagen fibres smaller [34,35], although care had been taken to keep the dentin visibly moist; (2) a surface shrinkage of the outermost exposed collagen during the preparation processes for microscopy [36]; (3) the presence of the residual collagen phase of the smear layer, which resists acid etching and which consists of small pieces of demineralised and/or denatured collagen that prevents the collagen network from being completely exposed. These particles could occlude the spaces of the outermost demineralised area, thereby making this surface more compacted and less permeable [3]; and, (4) acid etching has seemed to induce conformational modifications on dentin collagen, characteristics of denaturation and fragmentation processes [10] that could also be responsible for the compacted outermost layer.

Granular silica deposits from the PA thickener are also present on the collagen surface (Figs. 3 and 4). It has been explained that these silica remnants could not be removed even with vigorous rinsing and that these silica particles could influence the demineralisation depths and even the morphology of etched dentin, acting as a buffering agent [34]. It has also been proposed that these adsorbed silica particles may act as a filler reinforcing agent for the unfilled bonding resins [37]; however, the distribution of these particles was observed to be quite random and non-uniform, and it could be one more obstacle to overcome. Furthermore, as these particles would not chemically bond to the infiltrating resin, they could concentrate tensions inside the hybrid layer and lead to failure over time.

When the acid-etched dentin surfaces were observed in cross-sections, the diameter of the tubules ranged from 3 to 4 μm (Fig. 3), and in higher magnifications the circular tangle of collagen fibres of the peritubular matrix could be clearly seen (Fig. 4). It is interesting to observe that the tubule orifices appear to be narrowed when the mineral portion is lost (Figs 3 and 4). The explanation for this narrowing is that the mineral portion that recovers the collagen structure prevents its expansion, and once it is gradually removed by the acid etching, the collagen expands and bulges out laterally, narrowing the tubule orifices [38, 39].

When the tubules were observed longitudinally three successive layers could be distinguished: (1) a superficial compact layer, as mentioned above, composed of collapsed and/or denatured collagen and residual material that narrowed the tubules lumens, (2) a second or intermediate more fibrous and porous layer; and (3) a deeper layer which was first described as an "hiatus", with wide spaces and few collagen fibres, separating the intermediate fibrous layer from the mineralised dentin, as previously described [40]. It has been suggested that this deepest layer could be responsible for the leakage within the hybrid layer due to incomplete resin infiltration through the collagen network, the so-called *nanoleakage* [41]. The tubules' funnel shape were also very evident after acid etching, which could be explained by the different demineralisation pattern between the intertubular and the more mineralised peritubular dentin. Peritubular dentin is 40% more mineralised than intertubular dentin and is formed from inside the tubule with a progressive deposition that reduces the tubule lumen over time [42]. This difference in the demineralisation pattern may be explained by the structure of the two types of dentin itself, i.e., the higher organic content of intertubular dentin helps to diminish the acid diffusion through the mineral portion, while the higher mineral content of peritubular dentin allows acid diffusion from its surface into the tubules, from where it is etched. This action mechanism explains the porous matrix areas located around the tubules, suggesting they were etched from inside (Fig. 5 A and B).

Resin infiltration into intertubular dentin is totally dependent on the porosity present after acid etching. This collapse and/or denaturation of the outermost portion of the collagen matrix may reduce the permeability of the demineralised zone to as little as 10% of its theoretical maximum value in extreme cases [3]; Thus, this concept of bonding to moist dentin must be considered far from perfect as it is extremely technique sensitive, since it is completely dependent on an ideal moisture and permeability, which are quite difficult to control and achieve [6,43].

The main objective of collagen removal is to overcome the sensitivity of the total-etch technique, facilitating the access of the adhesive resins to a more permeable substrate with an increased surface area and less sensitivity to water content [15-17], since hydroxyapatite is a high-energy substrate while collagen has a low-energy surface [32]. NaOCl solutions are widely used in various dental procedures based on their non-specific deproteinising action and have been evaluated for their effects in dentin bonding procedures with various and controversial results [11,12,18-26]. The ideal NaOCl concentration and treatment time, as a dentin treatment agent, have also been determined and are considered to be 10-wt/vol% and 60 s, respectively [16]. This concentration and treatment time were used in this study.

The cross-section surfaces after acid etching followed by NaOCl deproteinisation showed a completely eroded surface with a "moth-eaten" appearance (Fig. 6), which was in agreement with previous findings [22,27]. The use of NaOCl removed the totally demineralised collagen network and completely altered the dentin surface into a porous structure with multiple irregularities, which appears to be more compatible with bonding resins than acid etched dentin. Nevertheless, the studies of bonding to collagen-depleted dentin do not confirm entirely this hypothesis [11,12,22-26]. The collagen removal increased the tubules orifices, from where few remnant collagen fibres from the peritubular matrix could be observed, and evidenced their funnel shape, once a large amount of the intertubular dentin area was removed. Numerous lateral branches were also seen opening on the peritubular area. One of the most interesting results of the collagen depletion is that it allows an evaluation of the extent and aggressiveness of the total-etch technique after the usually recommended 15 s of etching time (Figs. 6 and 7).

The effects of NaOCl on dentin composition have also been focused by some studies. Sakae et al [15] reported that from a crystallographic viewpoint the crystals in NaOCl-treated dentin were similar to enamel crystals. Inaba et al [17] found that dentin treatment with NaOCl solution promotes a mineral redistribution on the outermost surface and Marshall et al [44] using nanomechanical measurements showed a 75% reduction of the elastic modulus and hardness in dentin surface after collagen depletion with NaOCl. These results indicate that further studies are necessary to evaluate the clinical efficacy and security of this procedure.

The smear layer is considered the first obstacle to be surpassed for a reliable bonding to dentin. The acid etching has shown to be quite effective, but this procedure removes mostly its mineral content. If the smear layer composition is similar to that of normal dentin, i.e. 50% mineral phase and 30% collagen phase, collagen network resulting from acid etching would be enriched with these collagen remnants from the smear layer and would act as a barrier to the infiltration of bonding resins in the outermost collagen surface [3]. A pre-treatment with NaOCl could remove this organic phase and enhance the diffusion of bonding resins. When observed in cross-sections the specimens' surfaces (Fig. 8) showed no significant changes when compared to smear layer-covered dentin (Fig. 1), and confirmed previous findings [16,28]. However, in longitudinal-sections a light reduction in smear layer thickness was observed, which was probably due to the surface collagen removal and primarily to the scrubbing action during NaOCl application (Fig. 9). The application of NaOCl on smear layer could also be advantageous due to its bacteriolytic effect, since bacteria in the smear layer on the ground dentin surface is one of the problems encountered clinically [45]. The ground dentin surface pre-treatment with NaOCl may be a useful method to improve bonding procedures with minimal risks of damage. Further studies could confirm this hypothesis.

The second strategy for bonding to dentin is based upon the use of non-rinsing acidic monomers, the so-called self-etching primers. It has been suggested that they do not remove the smear layer completely, impregnating the smear plugs and fixing it at the entrance of the tubule [37]. This strategy for adhesion is very attractive because it constitutes a very simple method to prevent the collapse of the collagen network, avoiding its unprotected exposure. Another advantage is that they are recommended to be used in dry dentin and require only one primer application with subsequent air-thinning [46]; Thus, this simplification in the bonding technique reduces moisture dependence by avoiding overwetting or overdrying, which can negatively influence adhesion and diminishes the influence of regional differences in the substrate [47]. The first self-etching system was developed in Japan (Clearfil Liner Bond II: LBII, Kuraray, Japan) [48] and recently several self-etching systems have been introduced by many manufacturers. In this study, a further simplified system recently introduced by the same manufacturer was used, the Clearfil SE Bond (CSEB), which consists of one-bottle self-etching primer and one-bottle adhesive resin.

The CSEB-primer contains an unsaturated methacrylated phosphate ester, 10-methacryloyloxydecyl-dihydrogen phosphate (MDP), as the acidic monomer, combined with other resin monomers, photoiniciator and accelerator into a single bottle. When this primer was applied on the dentin surface for the recommended 20 s and observed by SEM in cross-sections, it could be clearly observed that it was strong enough to dissolve the smear layer, but that it only partially dissolved the smear plugs. In addition to the partially dissolved smear plugs, the tangle of fibres that form the peritubular collagen matrix could be observed inside the tubules, which confirms the demineralisation through the smear layer to the intact dentin (Fig. 10). In longitudinal-sections there was no difference in demineralisation pattern between intertubular and peritubular dentin, confirmed by the absence of the typical funnel shape observed in PA-etched specimens (Fig. 11). In a recent study [30] the CSEB-primer was not able to dissolve smear layer in specimens that, as in our study, were polished with 600-grit SiC paper to produce standardised smear layers, but etched through it to demineralise the subsurface intact dentin to a depth of 0.5 μm. Another study related that the CSEB system produced a hybrid layer of approx. 1-1.5 μm thick with the same standardised smear layer produced with 600-grit SiC paper [49]. These light differences we found in our study were probably due to the small thickness of the smear layer produced during the polishing of the specimens. Indeed, the smear layer thickness has been demonstrated to have a negative influence on bonding with self-etching systems [50].

The surface treatment with CSEB-primer followed by NaOCl deproteinisation did not show any drastic surface alterations as observed with PA-etched and deproteinised specimens, what confirms the mild acid etching pattern of this primer. The most noticeable changes occurred in the partially dissolved smear plugs (Fig. 12). The possible explanation for this phenomenon is twofold: the scrubbing motion during the NaOCl application or the removal of organic compounds from the smear plugs, and it is probably a result of both. The presence of a very well defined peritubular collagen matrix, clearly observed through the tubules orifices, leads to the conclusion that the NaOCl action was superficial (Fig. 12).

When the two current strategies for bonding to dentin were compared in terms of the dentin surfaces after pre-treatments, the differences were remarkable. The dentin surface provided by PA (total-etch technique) appeared to be less permeable due to its outermost collapsed collagen layer. Moreover, the demineralisation pattern appeared to be quite irregular, which must make the complete infiltration of the bonding resin through the demineralised collagen to the underlying mineralised dentin more difficult. When the collagen was removed by NaOCl deproteinisation, the aggressiveness of PA was shown in the resultant mineralised dentin surface, which appeared to be very vulnerable. The surface provided by the self-etching primer (CSEB-primer) rendered a more uniform and apparently less vulnerable surface, which was confirmed when the collagen was depleted by NaOCl.

The pre-treatment of dentin surfaces for bonding still remains as the initial obstacle to be surpassed to accomplish a safe and successful composite restoration technique. The pre-treatments evaluated by this study showed the remarkable differences in the dentin substrate that must influence the results in bonding procedures and tried to highlight their possible advantages and weaker points. Further *in vitro* and *in vivo* studies should be carried out to evaluate the effects of these dentin pre-treatments for dentin bonding.

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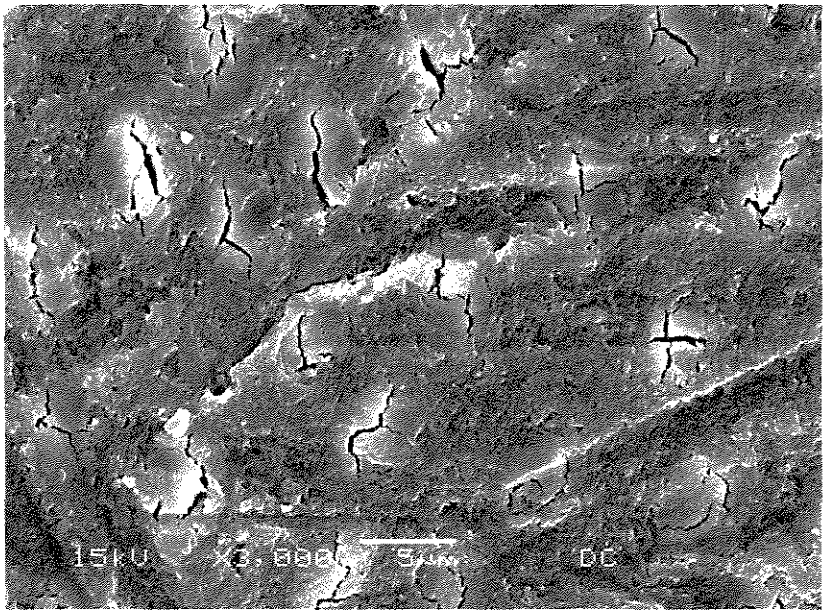

Figure 1. SEM photomicrograph illustrating the smear layer-covered dentin. The orifices of the underlying tubules are covered by a compact crust of cutting debris.

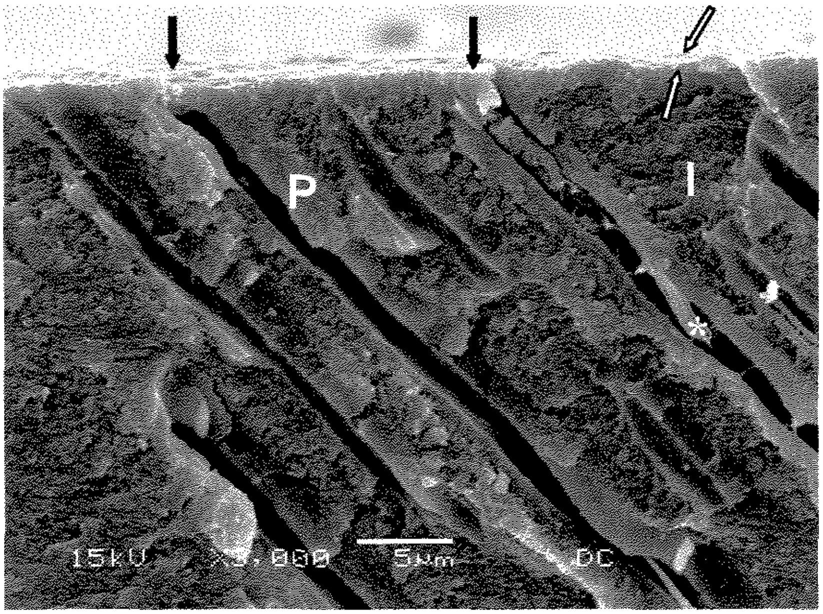

Figure 2. SEM photomicrograph illustrating the smear layer-covered dentin in longitudinal-section. Note the small thickness of smear layer, approx. 0.7 µm (white arrows) and the tubules occluded by smear plugs (black arrow). Note also some remnants of lamina limitans (*) preserved by the fixative procedures. Peritubular dentin (P); intertubular dentin (I).

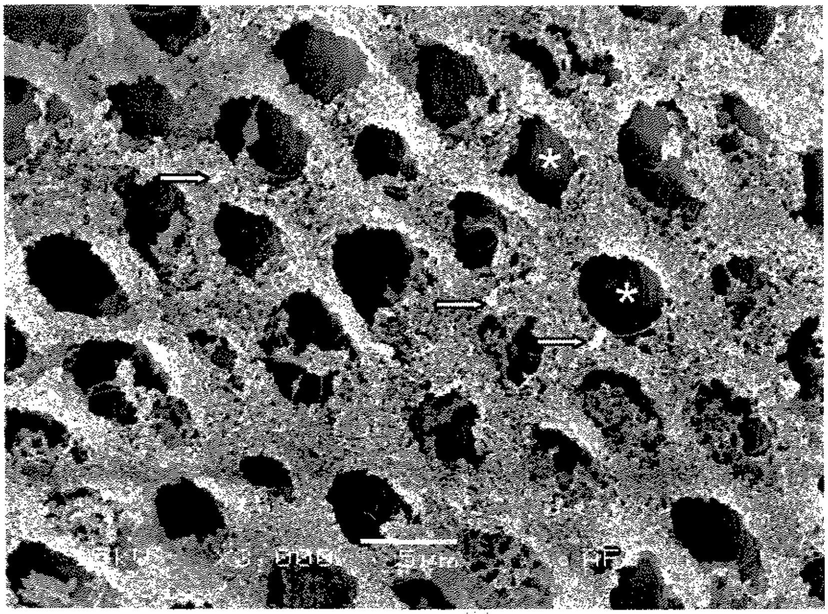

Figure 3. SEM photomicrograph of the dentin surface treated with PA showing a complete removal of smear layer, a dense collagen-rich surface and opened tubules. Observe the outer exposed peritubular fibres (*) and the silica deposits (arrows).

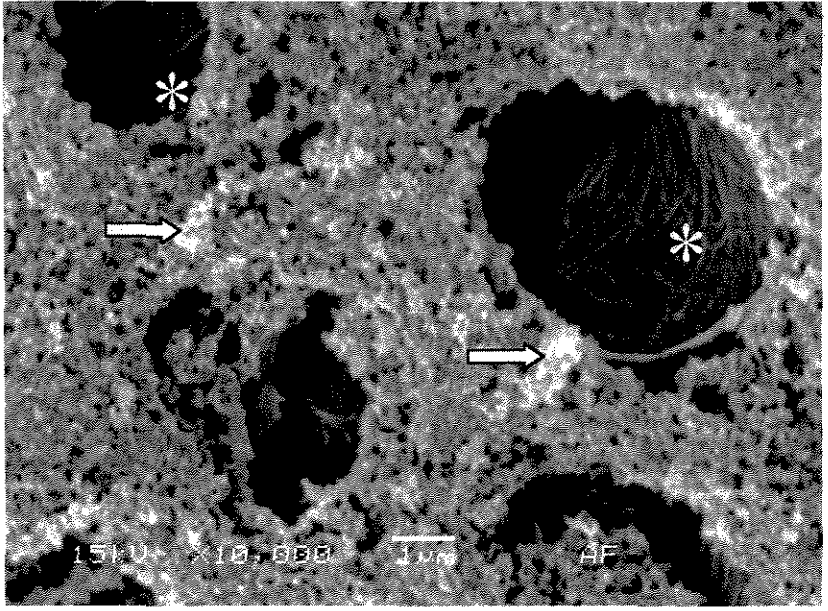

Figure 4. higher magnification SEM photomicrograph (x 10,000) of the dentin surface treated with PA. Note the dense and appearing collapsed intertubular collagen-rich surface; silica deposits (arrows). Note also inside the tubule orifice the peritubular collagen matrix as a dense tangle of fibres (*).

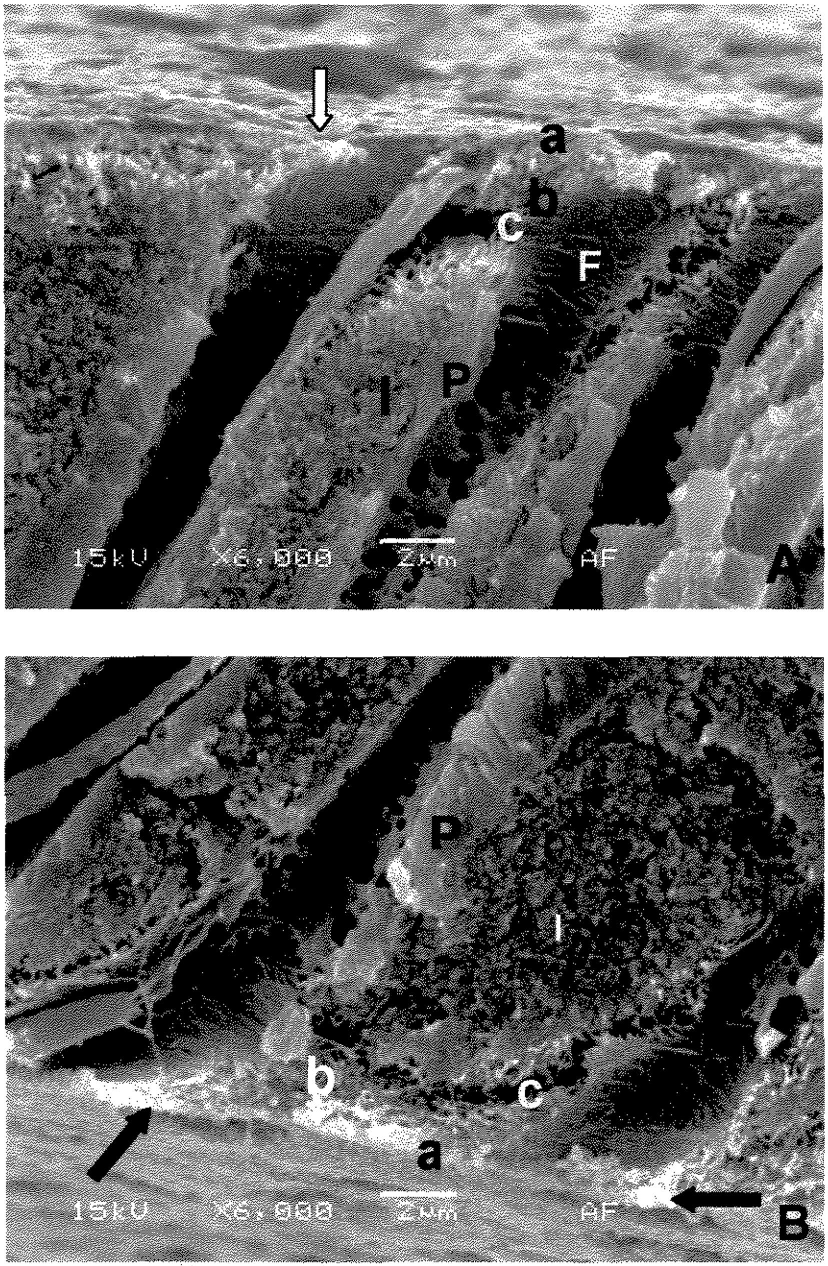

Figure 5A and B. SEM photomicrographs illustrating the dentin surface treated with PA in longitudinal-section views. Note the three distinct layers in the intertubular demineralised dentin zone: (a) the outmost layer of compacted fibres appearing to collapse onto the tubules lumen (arrow); (b) the more porous intermediate layer of separated collagen fibres, and (c) a zone of few collagen fibres and a hiatus observed close to unaffected intertubular dentin (I). note also the tubules typical funnel shape and the circular arrangement of peritubular collagen fibres (F). Peritubular dentin (P).

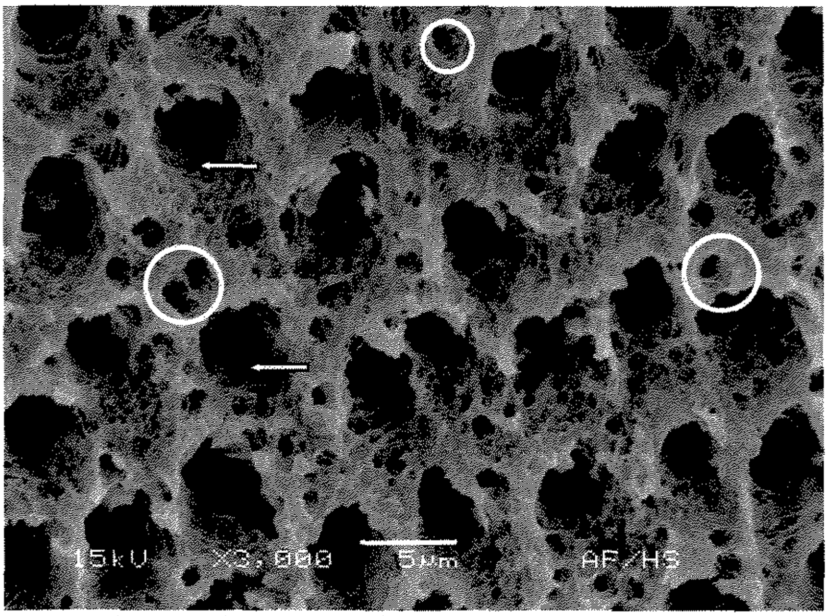

Figure 6. SEM photomicrograph illustrating the dentin surface after acid etching with PA and deproteinisation with NaOCl, in cross-section. Note the "moth-eaten" aspect and the lateral branches (circles). The tubules apertures are wider than for PA etched dentin in Fig. 3. Some remnants collagen fibres from the peritubular dentin matrix could be observed (arrows).

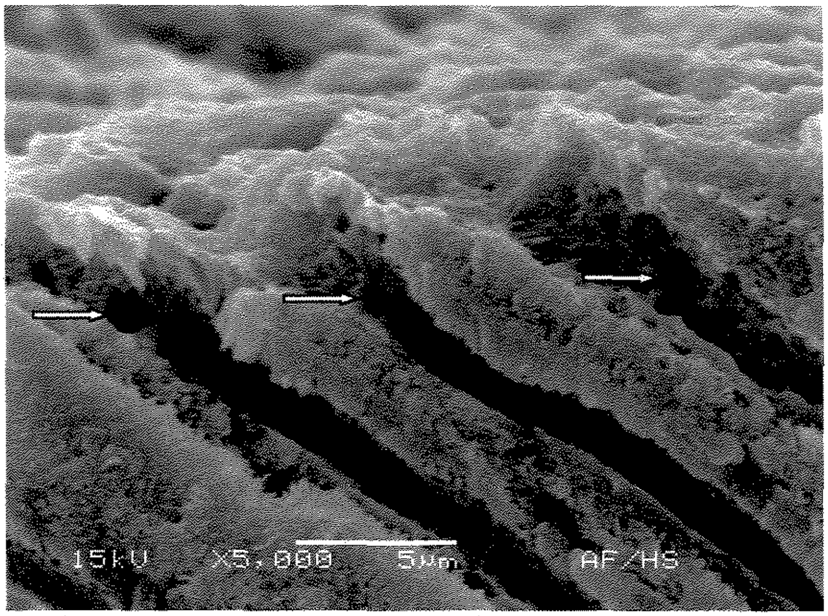

Figure 7. SEM photomicrograph in longitudinal-section of etched and deproteinised dentin. Note the eroded surface and the funnel shape of the tubules. Several lateral branches are also evident in this image (arrows).

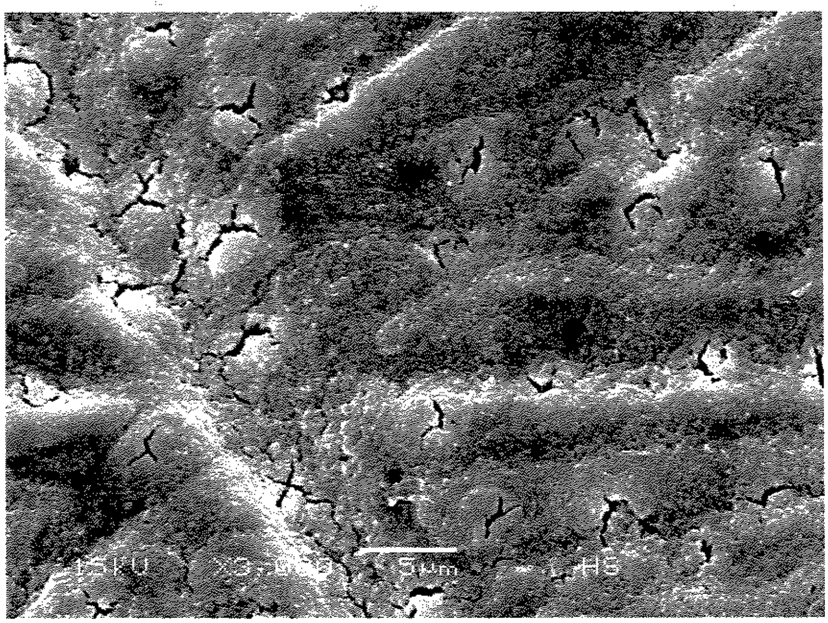

Figure 8. SEM photomicrograph illustrating the smear layer-covered dentin surface after treatment with NaOCl. There is no significant change in surface morphology when compared to Control Group (Fig. 1).

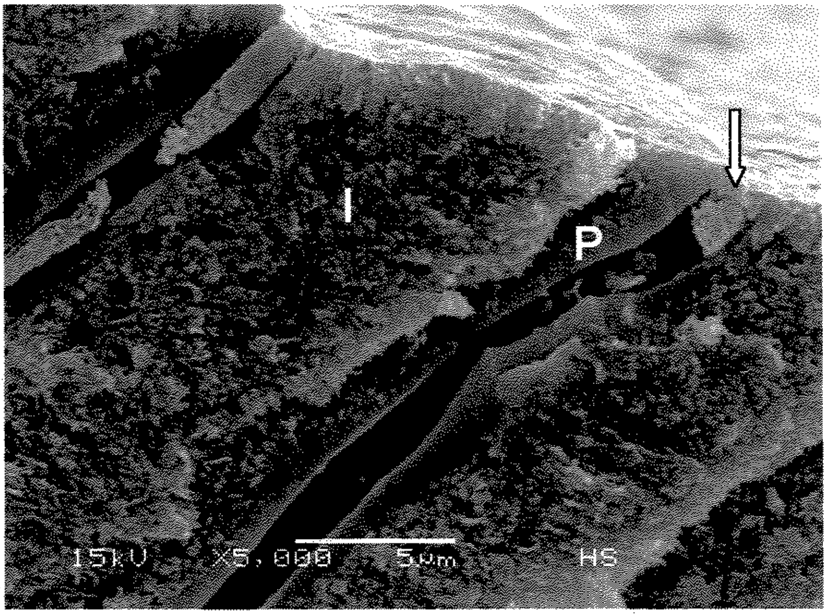

Figure 9. Longitudinal-section SEM photomicrograph illustrating the smear layer-covered dentin after NaOCl treatment. A light reduction in smear layer thickness was observed. Peritubular dentin (P); Intertubular dentin (I); Smear plug (arrow).

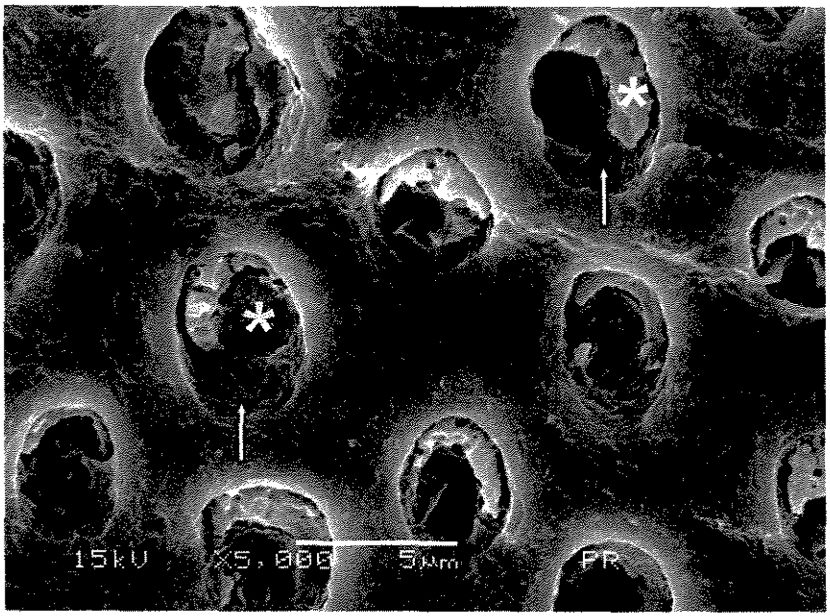

Figure 10. SEM photomicrograph illustrating the cross-section dentin surface after treatment with CSEB-primer. Note the absence of smear layer debris and the partially dissolved smear plugs (*). Note also some exposed collagen fibres from the peritubular collagen matrix (arrows).

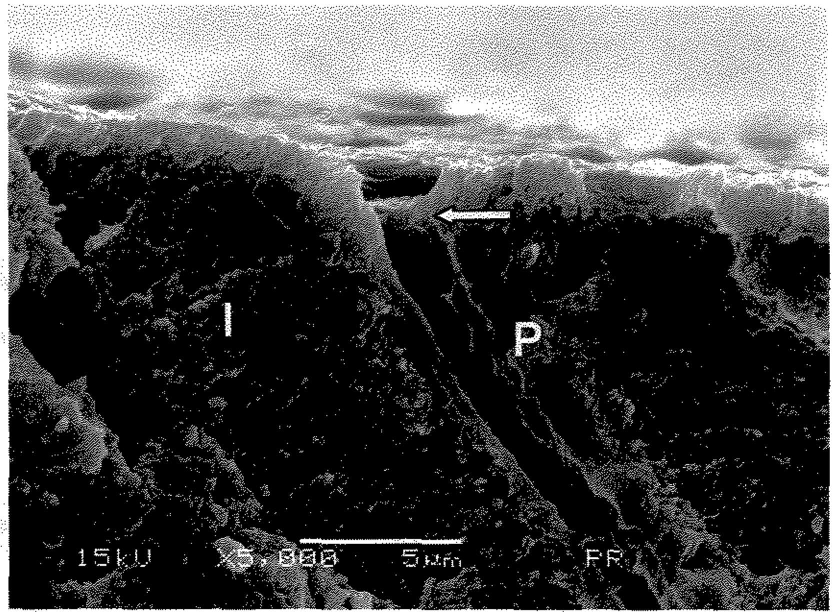

Figure 11. SEM photomicrograph illustrating the dentin after treatment with CSEB- primer in longitudinal-section. The tubule does not present the typical funnel shape observed after PA treatment and is partially occluded by a smear plug (arrow). Peritubular dentin (P); Intertubular dentin (I).

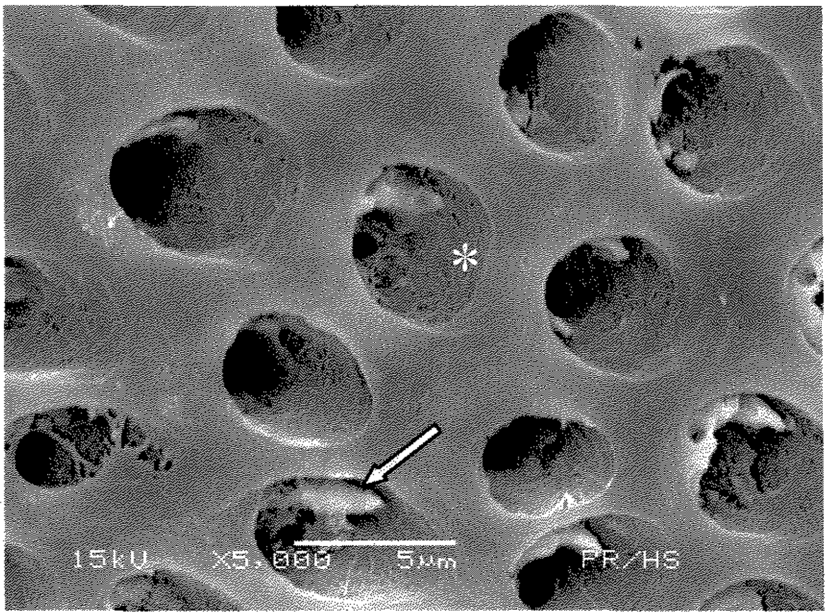

Figure 12. SEM photomicrograph illustrating a cross-section of the dentin surface after treatment with CSEB-primer followed NaOCl deproteinisation. Note that the partial dissolution of smear plugs (arrow) which is more pronounced than for dentin only treated with CSEB-primer (Fig. 10) and the very well defined tangle of peritubular collagen fibres (*).

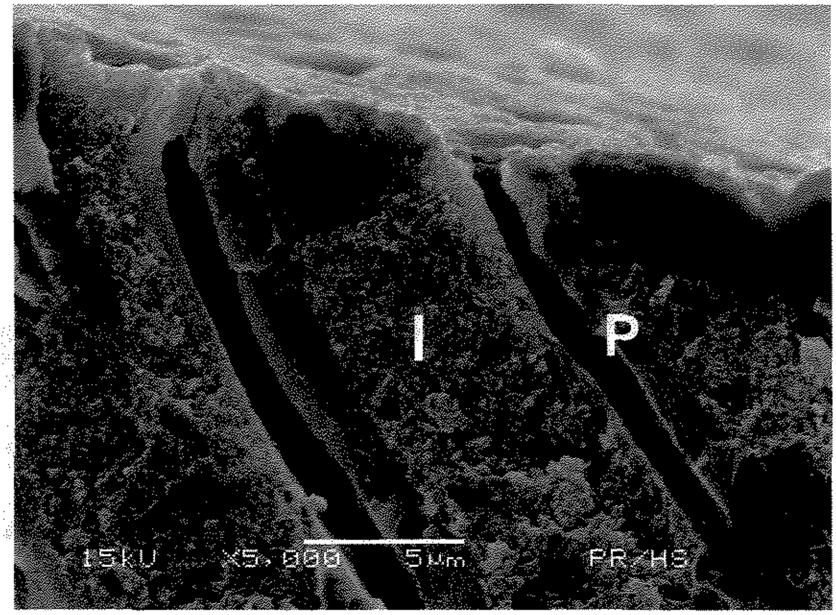

Figure 13. SEM photomicrograph illustrating a longitudinal section of dentin surface after treatment with CSEB-primer followed NaOCl deproteinisation. Smear plugs were less frequent than for dentin only treated with CSEB-primer (Fig. 11). Peritubular dentin (P); Intertubular dentin (I).

3.3 CAPÍTULO 3

Effect of collagen removal and use of a low-viscosity resin liner on marginal adaptation of composite resin restorations with margins in dentin

Enviado para publicação no ***Operative Dentistry***

Clinical Relevance

Marginal quality was significantly enhanced by collagen depletion with 10% sodium hypochlorite in an acetone-based adhesive system, and by the use of an intermediate layer of low-viscosity composite in a self-etching adhesive system.

SUMMARY

This study evaluated the influence of collagen removal and the use of a low-viscosity liner on marginal quality of composite restorations for the total-etch system, Prime & Bond 2.1 (PB) and the self-etching primer system, Clearfil SE Bond (CSEB), in high C-factor cavities with margins in dentin. High C-factor cavities were made on dentin exposed from ground labial surfaces of 100 bovine lower incisors, randomly assigned to 10 treatment groups, and restored with composite Z 250, placed in bulk. Group 1 (PB), control group, PB was applied according to manufacturer's directions; Group 2 (PB/PLF), an intermediate layer of low-viscosity composite Protect Liner F (PLF) was applied on the bonding resin surface; Group 3 (PB/SH) following acid-etching, the surfaces were treated with 10% sodium hypochlorite (SH) for 1 minute; Group 4 (PB/SH/PLF) the same procedure as for Group 3 and an intermediate layer of PLF was applied as for Group 2. Group 5 (CSEB), control group, CSEB was applied according to manufacturer's directions; Group 6 (CSEB/PLF), an intermediate layer of PLF was applied; Group 7 (SH/CSEB), the cavity surface was pre-treated with SH; Group 8 (SH/CSEB/PLF), SH pre-treatment as for Group 7, and then an intermediate layer of PLF was applied; Group 9 (CSEB/SH), after CSEB-primer application, the surface was treated with SH, followed by CSEB-adhesive application; Group 10 (CSEB/SH/PLF), the same as for Group 9, then an intermediate layer of PLF was applied. The specimens were stored at 37° C for 24 h, polished, molded and replicas were obtained in epoxy resin. The replicas were gold-sputter coated and observed by SEM (x300) for marginal quality classification. The Kruskal-Wallis non-parametrical multi-comparison Test ($p<0.05$) was used to statistical analysis of the data. Results demonstrated that both adhesive systems, in the control Groups, presented low marginal quality and a high variability. The use of an intermediate layer of PLF significantly improved the marginal quality with the CSEB system, but had no effect with the PB system. Collagen depletion with SH enhanced marginal quality for the PB system and did not influence CSEB system results.

INTRODUCTION

A primary concern of composite restorations is the shrinkage that follows polymerization. This shrinkage can create forces that may disrupt the bond to cavity walls and form marginal gaps, leaving the tooth more susceptible to post-operative sensitivity and recurrent caries. In order to restore a tooth in such a way that it is leakproof, there must be no dimensional mismatch at the tooth-restoration interface. Unfortunately, resin composites do not meet these requirements (Davidson & Feilzer, 1997). As polymerization proceeds, the amount of stress generated within the composite depends on the extent of the reaction, the stiffness of the composite, and its ability to flow (Davidson & De Gee, 1984). The correlation between stiffness and filler volume of composites has been demonstrated, and more heavily filled materials have consequently higher elastic modulus and produce more polymerization stress (Braem & others, 1987); this occurrence is in accordance to Hooke's law, which states that stress is equal to dimensional change times the stiffness. Less rigid composites may be able to resolve this problem, however, lower inorganic filler content in composites has been found to result in lower mechanical properties, which are critical to their performance (Ferracane & Berge, 1995; Condon & Ferracane, 1998/2000).

Another important point is the ratio between the bounded to unbounded surfaces, termed the "C-factor", related to the shape of the prepared cavity (Feilzer, De Gee & Davidson, 1987; Carvalho & others, 1996). The relative amount of unbounded resin surface determines the ability of the composite to relieve the developing stresses. The combination of the two factors cited above, a high elastic modulus shrinking restorative material confined in a high "C-factor" cavity condition, will challenge and frequently destroy the bond leading to post-operative sensitivity and poor marginal quality (Unterbrink & Liebenberg, 1999). Many efforts have been made to overcome this problem and the concept of an "elastic cavity wall" (Kemp-Scholte & Davidson, 1990a,b; Van Meerbeek & others, 1993) has been suggested as an interesting solution. This concept is based on an interposition of a lower elastic modulus intermediate layer between the restorative composite and the adhesive-dentin interface, with the ability to withstand "plastic flow" on its initial polymerization phase, allowing the material to absorb the strain and diminish the effect of a rigid contraction at the interface (Davidson, De Gee & Feilzer, 1984; Labella & others, 1999). Low-viscosity composites could be the most appropriate materials for this concept, acting as a stress-absorbing layer between the adhesive-dentin interface and the shrinkage of the composite, by partially relieving the polymerization contraction stress

(Swift & others, 1996; Bayne & others, 1998; Unterbrink & Liebenberg, 1999; Labella & others, 1999; Choi, Condon & Ferracane, 2000; Montes & others, 2001).

The first generation of low-viscosity composites was introduced in 1996, retaining the same small particle size of traditional hybrid composites, but reducing the filler content and, consequently, its viscosity (Bayne & others, 1998). Although these so-called flowable composites present a higher polymerization shrinkage than the traditional hybrid composites, their significantly lower elastic modulus (30 – 50%) may, in turn, be less detrimental to the bond interface area, since they are less rigid (Swift & others, 1996; Bayne & others, 1998; Unterbrink & Liebenberg, 1999; Labella & others, 1999; Choi, Condon & Ferracane, 2000; Montes & others, 2001).

A current concept of bonding to dentin is based on diffusion, polymerization and consequently micromechanical retention of monomers within the network of collagen fibers exposed after acid application. This mixed structure formed by collagen fibers enveloped by resin and residual hydroxyapatite crystals was first described as the "hybrid layer" in 1982 (Nakabayashi, Kojima & Masuhara, 1982). The role performed by the hybrid layer in dentin bonding has been evaluated by many investigations and has been the subject of much controversy, since it has been shown that the resin infiltration in collagen network may be incomplete (Sano & others, 1995). Therefore, many attempts have been developed to simplify the bonding process and reduce the technique sensitivity in the last decade (Van Meerbeek & others, 1998; Harada & others, 2000). A simple method to prevent the incomplete resin infiltration into the collagen network is to avoid its unprotected exposure. This can be achieved by treating the enamel and dentin with acidic self-conditioning monomer solutions instead of employing a conventional total-etch procedure in which the acid and the primer are applied separately (Watanabe, Nakabayashi & Pashley, 1994). Thus, the self-etching primer systems are considered to be an easy and reliable procedure to achieve the best conditioned surface for bonding (Harada & others, 2000). The hybrid layers formed by these self-etching primer systems are thinner (about 1μm thick) and more uniform when compared to the hybrid layers formed by the total etch systems, which are about 4 μm thick (Vargas, Cobb & Denehy, 1997; Prati & others, 2000; Montes & others, 2001). For some authors, the hybrid layer does not contribute significantly to dentin bonding (Finger, Inoue & Asmussen, 1994; Nakajima & others, 1995; Gwinnett & others, 1996; Yoshiyama & others, 1996). For other authors the collagen removal had a beneficial effect in dentin bonding for acetone-based adhesives and an adverse effect in water and ethanol-based adhesives (Inai & others, 1998; Pioch & others, 1999; Prati, Chersoni & Pashley, 1999; Saboia, Rodrigues & Pimenta, 2000).

Dentin has an elastic modulus in the range of 11-19.3 GPa (Van Meerbeek & others, 1993; Armstrong & others, 1998). The hybrid layer has a relatively low elastic modulus, in the range of 4.8-9.6 GPa, significantly lower than that of unaltered dentin (Van Meerbeek & others, 1993). The presence of the collagen layer could possibly allow for the establishment of an elastic gradient at the interface, that could absorb part of the stress generated by the shrinking restorative composite. However, this layer is thought not to be of the necessary thickness to play an important role in relieving stresses as its nature suggests (Van Meerbeek & others, 1993). Theoretically, in order to compensate this small hybrid layer thickness, the interposition of a resilient layer provided by a material with an elastic modulus similar to that presented by the hybrid layer itself would be appropriate (Kemp-Scholte & Davidson, 1990a,b; Van Meerbeek & others, 1993). On the other hand, how the collagen removal, and consequently the absence of a hybrid layer, influences the marginal adaptation in dentin still remains uncertain, since it would put two rigid surfaces in direct contact with one of them suffering a rigid contraction. Moreover, if the hybrid layer has any role in relieving stresses generated by polymerization contraction, the differences in thickness between the hybrid layers formed by a total-etch and a self-etching primer adhesive systems should be considered.

The purpose of this study was to measure and evaluate the influence of collagen removal in a total-etch bonding technique system, in a self-etching primer system and the use of an intermediate layer of a low-viscosity composite in marginal integrity of composite restorations, in cavities with a high C-factor and margins in dentin.

METHODS AND MATERIALS

The materials, manufacturers, composition and batch numbers for this study are listed in Table 1. Freshly extracted bovine lower incisors (n=100), obtained from a local *abattoir*, stored frozen for no longer than 1 month, were used to evaluate the marginal integrity of the composite resin restorations. The teeth were ground on their labial surfaces with a water irrigated grinding wheel using a 320-grit silicone carbide paper (SiC) to obtain a flat dentin surface. Each was finished with a 600-grit SiC to produce standardized surfaces. The cavities were made using a diamond bur number 2294 (KG Sorensen Ltda. –

Brazil), especially designed for this purpose, with an active part measuring 1.8 mm in diameter and 1.5 mm deep, producing cavities with an internal area of 11.02 mm^2 and a free surface area of 2.54 mm^2, giving as result, standardized cavities with a C-factor of approx. 4.3, with margins totally located in dentin.

The teeth were randomly divided into 10 groups of 10 specimens each. For Groups 1 to 4 the dentin surfaces were etched with 37% phosphoric acid (Dentsply Caulk, Milford, DE, USA) for 15 s, rinsed with running water for 20 s and the excess water removed by blotting with tissue paper, leaving the dentin visibly moist. Adhesive Prime & Bond 2.1 (PB) was applied using a saturated disposable brush, gently air dried with oil-free compressed air from a syringe for 5 s, keeping the tip 2 cm from the surface and light cured for 20 s.

For Group 1-control (PB), the composite resin Z 250 was placed in bulk and light cured for 30 s.

For Group 2 (PB/PLF), following PB application, the adhesive surface was covered with a layer of Protect Liner F (PLF), light cured for 20 s, and the cavity was restored with Z 250, placed in bulk as for Group 1.

For Group 3 (PB/SH) after acid conditioning as for Groups 1 and 2, a 10% sodium hypochlorite (SH) solution was lightly applied for 1 minute, then the teeth were rinsed with running water for 20 seconds and the excess water removed by blotting with a tissue paper leaving the dentin visibly moist. Adhesive PB was applied, light cured for 20s and the cavity was restored with Z 250, placed in bulk as for Groups 1 and 2.

For Group 4 (PB/SH/PLF), after acid conditioning, SH and adhesive application as for Group 3, a layer of PLF was applied as for Group 2 and the cavity was restored with Z 250 as for Groups 1, 2 and 3.

For Groups 5 to 10, the adhesive system Clearfil SE Bond (CSEB) was applied. The primer was applied with a saturated disposable brush for 20 s, dried with a mild air flow, followed by application of the adhesive resin, gentle air flow, and light cured for 10 s. The cavities were restored with composite resin Z 250, placed in bulk as for Groups 1 to 4.

For Group 5-control (CSEB), the composite resin Z 250 was placed in bulk and light cured for 30s.

For Group 6 (CSEB/PLF), following CSEB application, a layer of PLF was applied as for Groups 2 and 4, and the cavity was restored.

For Group 7 (SH/CSEB), before the application of CSEB, a SH solution was applied on the dentin surface as for Groups 3 and 4, and the cavity was restored.

For Group 8 (SH/CSEB/PLF), a SH solution was lightly scrubbed on the dentin surface as for Groups 3, 4 and 7, and after the application of CSEB a layer of PLF was placed as for Groups 2, 4 and 6, and the cavity was restored.

For Group 9 (CSEB/SH), the CSEB-primer was applied for 20 s and dried with mild air flow. The specimens were then immersed in a 50% acetone solution for 30 s to remove the primer monomers, rinsed with running water, dried with an oil-free compressed air and a

SH solution was applied as for Groups 3, 4, 7 and 8. The CSEB-adhesive resin was applied, light cured for 10 s, and the cavity was restored.

For Group 10 (CSEB/SH/PLF) the procedures were the same as for Group 9, except that after CSEB-adhesive resin application, a layer of PLF was placed as for Groups 2, 4, 6 and 8 and the cavity was restored.

All light-curing procedures used a 3M XL3000 (3M dental Products) curing unit with a light intensity of 500 mW/cm^2. The light intensity was measured with a radiometer (Curing Radiometer, model 100, Demetron/Kerr, Danbury, CT 06810, USA).

The specimens were stored in distilled water at 37°C for 24 h. The restorations were then finished with 1000-grit SiC under water and polished with 6, 3, 1 and 0.25 μm diamond paste using a polish cloth under water and ultrasonically cleaned for 10 min between the grits. The restorations were molded with a low-viscosity polyvinyl siloxane material (Aquasil, Dentsply De tray, Konstanz, Germany) and the molds were poured with epoxy resin (Buehler, Lake Buff, IL, USA), gold-sputter coated (Balzers – SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL, JSM-5600LV, Scanning Electron Microscope, Japan) for evaluation, measurement and classification of the cavity margins. The measurements and classification were made with a x300 magnification directly on the microscope monitor, using a multi-point measuring device, observing all the cavity perimeter in approx. 20 sections. The measurements were recorded and classified in steps of 50–150 μm (Fig. 1), according to morphologically-defined parameters, into one of four types, as described by Hannig, Reinhardt & Bott (2001): "perfect margin" defined as a continuous, gap free transition between filling and dentin (Fig. 2); "marginal gap", observed as a gap formation and loss of interfacial adhesion (Fig. 3), "marginal irregularity", characterized as a non-continuous but yet gap free transition between filling and dentin (Fig. 4) and "overhang", that could not appear in more than 0.5% of the total perimeter sum. Marginal quality was calculated by adding each of the classified step measurements, and the total sum of each classification was expressed as a percentage of the cavity perimeter for each specimen.

The results of SEM-analysis were subjected to non-parametric multiple comparison Kruskal-Wallis test ($p<0.05$).

RESULTS

The Kruskal-Wallis test demonstrated significant differences among the Groups and the results of the non-parametric multiple comparison test are summarized in Tables 2 to 4 and the mean values are represented graphically in Fig. 5. As the occurrence of "overhang" was considered to be a mistake in specimen preparation and could not exceed 0.5% of the restorations perimeters, the values obtained in this classification, when appeared, were disregarded and consequently not considered for statistical analysis.

Group 8 (SH/CSEB/PLF) showed the highest average values (98.6%) and the lowest standard deviation (1.12%) for "perfect margin" occurrence, which were not statistically different, in decreasing order, from Group 6 (CSEB/PLF), 98.1% ± 2.03; Group

4 (PB/SH/PLF), 95.4% ± 5.14 and Group 3 (PB/HS), 91.7% ± 8.88. Two Groups from each adhesive system were used. Three among these four Groups (8, 6 and 4) had the low-viscosity composite (PLF) as an intermediate layer, and SH (8, 4 and 3) as a surface pre-treatment agent. The lowest values for "perfect margin", with the highest standard deviations, were obtained for control Group 1 (PB), 69.2% ± 16.02, which were not statistically different, in increasing order, from control Group 5 (CSEB), 78.6% ± 11.65; Group 7 (SH/CSEB), 81.1% ± 7.26; Group 9 (CSEB/SH), 83.3% ± 5.35; Group 2 (PB/PLF), 83.6% ± 15.47 and Group 10 (CSEB/SH/PLF), 86.1% ± 5.49. Thus, as observed above, control Groups 1 and 5 presented the lowest values for "perfect margin". These results are shown in Table 2 and mean values represented graphically in Fig. 5A.

Table 3 and Fig. 5B summarize the occurrence of "marginal irregularity" among the Groups. The control Groups 1 (PB) and 5 (CSEB) presented the highest values, 19.7% ± 8.53 and 14.1% ± 11.52, respectively, followed by Group 2 (PB/PLF) with 13.2% ± 12.39. The lowest values were obtained, in increasing order, by Group 8 (SH/CSEB/PLF) with 1.4% ± 1.12; Group 6 (CSEB/PLF), 1.8% ± 2.04 and Group 4 (PB/SH/PLF), 2.2% ± 2.47. For each separate adhesive system, the lowest values for "marginal irregularity" were present when the low-viscosity composite was used as an intermediate layer for CSEB and SH pre-treatment was used for PB.

Control Group 1 (PB) presented the highest values for "marginal gap", 11.1% ± 9.47, although no significant differences were noted for Group 7 (SH/CSEB), 10.8% ± 5.42; Group 9 (CSEB/SH), 10.1% ± 4.29; control Group 5 (CSEB), 7.3% ± 5.53 and Group

10 (CSEB/SH/PLF), 6.5% ± 4.87. In Group 8 (SH/CSEB/PLF) "marginal gaps" were not observed, while for Group 6 (CSEB/PLF) it was present in 0.1% ± 0.26 of the restoration margin length. For Group 3 (PB/SH), Group 4 (PB/SH/PLF) and Group 2 (PB/PLF) the values for "marginal gaps" were 2.0% ± 3.26; 2.3% ± 3.8 and 3.2% ± 4.46, respectively. These results are summarized in Table 4 and are graphically represented in Fig. 5C. When compared to the control Groups, a clear reduction in "marginal gaps" occurrence could be noted when the low-viscosity composite PLF was used as the intermediate layer for both adhesive systems. The pre-treatment with SH reduced significantly the occurrence of "marginal gaps" for PB Groups and did not influence the occurrence in the CSEB Groups.

DISCUSSION

The two major difficulties incurred in achieving a reliable technique for composite restorations are the rigid polymerization shrinkage of the restorative composites which challenges the bonded interface, and the dentin itself as a substrate for bonding.

Due to their inherent chemistry, the current composites shrink upon polymerization, introducing strain in the final restorations. The magnitude of the stress generated is dependent on the elastic modulus of the composite, i.e., at a given shrinkage value, the most rigid material will cause the highest stress (Hooke's law). Unless the surrounding structures to which the composite is bonded supply enough elastic compliance, something will fracture, in order to compensate for the reduced volume and consequently create gaps (Davidson & Feilzer, 1997). Thus, it has been suggested that if a low elastic modulus layer was interposed between the rigid shrinking composite and the adhesive layer, it would provide a decrease in this deleterious effect to the bonded walls, mainly in high C-factor cavities (Kemp-Scholte & Davidson, 1990a,b; Carvalho & others, 1996; Swift & others, 1996; Davidson & Feilzer, 1997; Rees, O'Dougherty & Pullin, 1999; Unterbrink & Liebenberg, 1999; Choi & others, 2000; Montes & others, 2001), such as the 4.3 C-factor cavities used in this study. The results of this study agree with the previous findings of Kemp-Scholte & Davidson (1990a,b) who reported an improvement in marginal quality and a reduction in the polymerization contraction stresses of between 20 and 50% when an intermediate low modulus resin layer was used. Indeed, a tendency for a better marginal quality was observed when PLF was applied, with a significant decrease in occurrence of "marginal gaps", for both adhesive systems when compared to control Groups (Table 4 and Fig. 5C). When these results are observed for each adhesive system separately, the significant effect on CSEB Groups, in which a remarkable improvement in marginal quality was obtained, is noticeable (Tables 2 to 4 and Fig. 5). Swift & others (1996) previously reported similar results, i.e., the bonding systems designed for use with low-viscosity intermediate resins, such as CSEB, generally had less microleakage than other systems, including PB, where the use of PLF had little effect on microleakage performance. PLF is a microfilled composite with 42% by weight of colloidal silica and pre-polymerized filler with a very low-viscosity that facilitates its application in thin layers. Moreover, its formulation indicates a possible and desirable elastic "buffer" behavior for use as an intermediate layer (Condon & Ferracane, 2000), thereby, reinforcing the "elastic cavity wall concept" (Montes & others, 2001).

The two adhesive systems used in this study represent the two current acceptable ways to obtain micromechanical retention between resin and dentin. PB is a single bottle adhesive system with acetone as volatile solvent, and demands a previous acid-etching procedure. Its action is based upon the complete removal of the smear layer and demineralization of subsurface intact dentin, leaving a collagen rich and moist surface where adhesive resin must diffuse to form the hybrid layer. The major problem regarding this technique is its sensitivity due to its moisture dependence, mainly to acetone based systems such as PB (Tay, Gwinnet & Wei, 1996). In fact, the high SD for "perfect margin" occurrence observed for Groups 1 and 2, 16.02 and 15.47 respectively (Table 2), directs attention to the high variability of these results, which demonstrates the difficulty of accomplishing this procedure successfully. CSEB uses a self-etching primer that partially demineralizes the smear layer and the underlying intact dentin, using them as bonding substrate (Pashley & Carvalho, 1997; Van Meerbeek & others, 1998). When the two control Groups 1 (PB) and 5 (CSEB) were compared for marginal quality, no significant difference was observed at the level of significance by the multi comparison non-parametrical test ($p<0.05$). The results indicate that under the conditions of this study, the differences between these two bonding approaches were not significant when traditionally applied, i.e., neither the thicker hybrid layer of PB nor the more uniform and thinner hybrid layer of CSEB appeared to have influenced the results. However, a slight tendency for a better performance could be noticed for CSEB. Indeed, although the hybrid layer has a low elastic modulus (Van Meerbeek & others, 1993), its thinness must have compromised the performance as a polymerization stress "breaker" in this high C-factor cavity condition, with the high elastic modulus composite Z 250 placed in bulk and a continuous and intense light-curing (500mW/cm^2) for 30 s, which is an extremely unfavorable situation for the bonding procedures.

The presence of the collagen network resulting from acid-etching has been constantly questioned as an important structure for dentin bonding, since it has been shown that the resin monomers do not fully diffuse through it to reach intact dentin. This incomplete penetration produces a porous layer of exposed collagen subject to hydrolysis and degradation, resulting in microleakage and failure over time (Sano & others, 1994). The removal of collagen with NaOCl has been suggested as a suitable method to overcome this problem, since it alters the composition of the dentin surface as it becomes similar to etched enamel, i.e., a more predictable and hydrophilic substrate for bonding (Sakae, Mishima & Kosawa, 1988; Tanaka & Nakai, 1993; Inaba & others, 1995). When NaOCl was applied after acid-etching for PB Groups 3 and 4, an improvement in marginal quality was evident and statistically significant (Tables 2 to 4 and Fig. 5). The decrease in the SD of "perfect margin" occurrence also showed the consistency of these data (Table 2), indicating that the results became more constant. Some morphological studies focusing on the resultant dentin surface after collagen depletion with NaOCl, described a rough, porous and eroded surface, which appeared more compatible with bonding resins than the collagen-rich surface produced by acid-etching (Inai & others, 1998; Perdigão & others, 1999). Indeed, after collagen depletion with NaOCl treatment, an increase in wettability may be expected due to deproteinization resulting in a hydrophilic surface, since hydroxyapatite is a high-energy substrate, whilst collagen has a low-energy surface. Thus, the interactions between adhesive resin and this rough mineral tissue are more likely to occur (Attal, Asmussen & Degrange, 1994). These results for PB are in agreement with previous findings that suggested that collagen depletion enhanced the bond strength for acetone-based adhesive systems (Inai & others, 1998; Pioch & others, 1999; Prati & others, 1999; Saboia & others, 2000). These improved results have been attributed to the higher diffusibility as well as the higher capacity to displace water of acetone when compared with other solvents such as ethanol or water (Jacobsen & Soderholm, 1995). This ability might have favored the diffusion of PB through this mineral-rich tissue and high-energy substrate, thereby enhancing marginal quality. Furthermore, Inai & others (1998) and Prati & others (1999) suggested that the adhesive monomers in PB are acidic and may be able to re-etch the mineral dentin surface. This could create a very thin hybrid layer (0.3 to 0.5 μm) not detected by SEM which may ensure higher bond strengths.

For the CSEB Groups 7 and 8, the use of NaOCl must be analyzed from another viewpoint, since these systems do not require a previous acid-etching procedure and, therefore, do not expose the unprotected collagen network as PB. When NaOCl was applied on smear layer-covered dentin, no significant difference was noticed when these Groups were compared to Groups 5 and 6, regarding neither mean values nor SD (Tables 2 to 4). The composition of the smear layer is generally similar to the originating tissue, therefore, it is assumed that its mineral and collagen phases were similar in content to that of normal dentin where the cavities were performed, i.e., about 50% vol. mineral and 30% vol. collagen (Pashley & others, 1993). Thus, the pre-treatment of the smear layer with NaOCl may have eliminated this collagen phase; however, CSEB utilize an acidic primer solution (MDP-based) which can permeate through the water-filled channels between the particles of the smear layer, enlarging them and, thereby, reaching intact dentin (Harada & others, 2000; Tay & Pashley, 2001). Thus, this collagen removal did have any influence on marginal quality, since it did not seem to represent an obstacle to CSEB-primer action. Indeed, SEM studies show no considerable morphological changes when NaOCl is applied on smear layers, suggesting only a superficial collagen removal (Tanaka & Nakai, 1993; Prati & others, 1999), however a light reduction in smear layers thickness was observed after 1 minute scrubbing action during NaOCl application (Montes & others, unpublished observations), and although this was attributed to a higher thickness of smear layers negative influence on bond strength of self-etching primers to dentin (Koibuchi, Yasuda & Nakabayashi, 2001), this light reduction, if it occurred in this study, did not influence marginal quality.

For Groups 9 and 10, the thin collagen-rich surface exposed by the action of CSEB acidic primer was depleted by NaOCl, and then, the adhesive resin was placed and polymerized. The results of these procedures indicated a significant decrease in marginal quality when compared to Groups 6 and 8, when PLF was used as an intermediate layer. However, data were not statistically different from control Group 5 and Group 7 (Tables 2 to 4). According to Tay & Pashley (2001), CSEB-primer was not strong enough to dissolve the smear layer, but etched through it to demineralize the subsurface intact dentin to a depth of 0.5 μm. Indeed, Harada & others (2000) had previously described a surface after CSEB-primer action where this mild action was observed. Theoretically, a drastic decrease would be expected in marginal quality, since the CSEB-primer had been removed and the bonding was merely based upon the interaction between the CSEB-adhesive resin and a more mineral dentin surface. The possible explanation for these results may rely on the composition of the CSEB-adhesive resin itself (Table 1), which also contains the acidic monomer MDP in its composition, and could have re-etched the dentin surface, creating a shallow hybrid layer. The higher viscosity of the CSEB-adhesive resin when compared to CSEB-primer may be attributed to the presence of the high molecular weight monomer Bis-GMA, the colloidal silica filler and the lack of ethanol and water as solvents, and must also have influenced its wettability on dentin surface. Nevertheless, the previous primer action followed by NaOCl collagen depletion must have increased the surface energy of dentin, which somehow could have partially compensated the CSEB-adhesive resin interaction for this decreased wettability and, therefore, positively influenced marginal quality.

CONCLUSIONS

Based on the results of this study, both the adhesive systems, Prime & Bond 2.1 (PB) and Clearfil SE Bond (CSEB), presented the lowest occurrence of "perfect margin" and a high occurrence of "marginal gaps" in control Groups. The high standard deviations observed for these control Groups also demonstrated the low reliability of these procedures, under the test conditions. The use of the low-viscosity resin, Protect Liner F (PLF), improved remarkably the marginal quality for CSEB Groups and diminished the standard deviations, which appeared to make the procedure more reliable. PLF also had a slight, but not statistically significant influence on marginal quality for PB Groups. Collagen depletion with NaOCl improved the marginal quality for PB Groups and reduced standard deviations, although it did not influence the results for the CSEB Groups. Thus, the hybrid layer appeared not to be the only paradigm for immediate or short-term bonding to dentin, with the products tested.

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Table 1. Materials used in this study

Materials	Composition	Batch number	Manufacturer
Prime & Bond 2.1	Etchant: 37% H_3PO_4	67510	Dentsply Caulk, Milford, DE, USA
	Adhesive: UDMA, PENTA, R5-62-1 resin, Bis-EMA, Butylated hydroxitoluene, 4-Ethyl dimethyl aminobenzoate, Cetilamine hydrofluoride, Acetone		
Clearfil SE Bond	Primer: MDP, HEMA, Hydrophilic dimethacrylate, Camphorquinone, N,N-Diethanol p-toluidine, Water, Ethanol.	51208	Kuraray Co. Ltd., Japan
	Adhesive: MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, Camphorquinone, N,N-Diethanol p-toluidine, silanated colloidal silica.		
Protect Liner F	Bis-GMA, TEGDMA, fluoride-methyl methacrylate, camphorquinone, silanised colloidal silica, prepolymerised organic filler	0039AY	Kuraray Co. Ltd., Japan
Z 250	Bis-GMA, UEDMA, Bis-EMA, zirconia/silica filler	9AM	3M Dental Products, St. Paul, MN, USA

Table 2. Occurrence of "perfect margin"

Groups	Mean (%)	SD*
Group 1 (PB)	69.2	16.02 C
Group 2 (PB/PLF)	83.6	15.47 BC
Group 3 (PB/SH)	91.7	8.88 AB
Group 4 (PB/SH/PLF)	95.4	5.14 A
Group 5 (CSEB)	78.6	11.65 C
Group 6 (CSEB/PLF)	98.1	2.03 A
Group 7 (SH/CSEB)	81.1	7.26 C
Group 8 (SH/CSEB/PLF)	98.6	1.12 A
Group 9 (CSEB/SH)	83.3	5.35 C
Group 10 (CSEB/SH/PLF)	86.1	5.49 BC

*Same letters indicate no statistical difference (p<0.05).

Table 3. Occurrence of "marginal irregularity"

Groups	Mean (%)	SD*
Group 1 (PB)	19.7	8.53 A
Group 2 (PB/PLF)	13.2	12.39 ABC
Group 3 (PB/SH)	6.4	7.14 CD
Group 4 (PB/SH/PLF)	2.2	2.47 D
Group 5 (CSEB)	14.1	11.52 AB
Group 6 (CSEB/PLF)	1.8	2.04 D
Group 7 (SH/CSEB)	8.1	5.36 BC
Group 8 (SH/CSEB/PLF)	1.4	1.12 D
Group 9 (CSEB/SH)	6.6	4.06 BC
Group 10 (CSEB/SH/PLF)	7.3	3.45 BC

* Same letters indicate no statistical difference ($p<0.05$).

Table 4. Occurrence of "marginal gap"

Groups	Mean (%)	SD*
Group 1 (PB)	11.1	9.47 A
Group 2 (PB/PLF)	3.2	4.46 BCD
Group 3 (PB/SH)	2.0	3.26 DE
Group 4 (PB/SH/PLF)	2.3	3.80 CDE
Group 5 (CSEB)	7.3	5.53 AB
Group 6 (CSEB/PLF)	0.1	0.26 DE
Group 7 (SH/CSEB)	10.8	5.42 A
Group 8 (SH/CSEB/PLF)	0.0	0.00 E
Group 9 (CSEB/SH)	10.1	4.29 A
Group 10 (CSEB/SH/PLF)	6.5	4.87 ABC

* Same letters indicate no statistical difference ($p<0.05$).

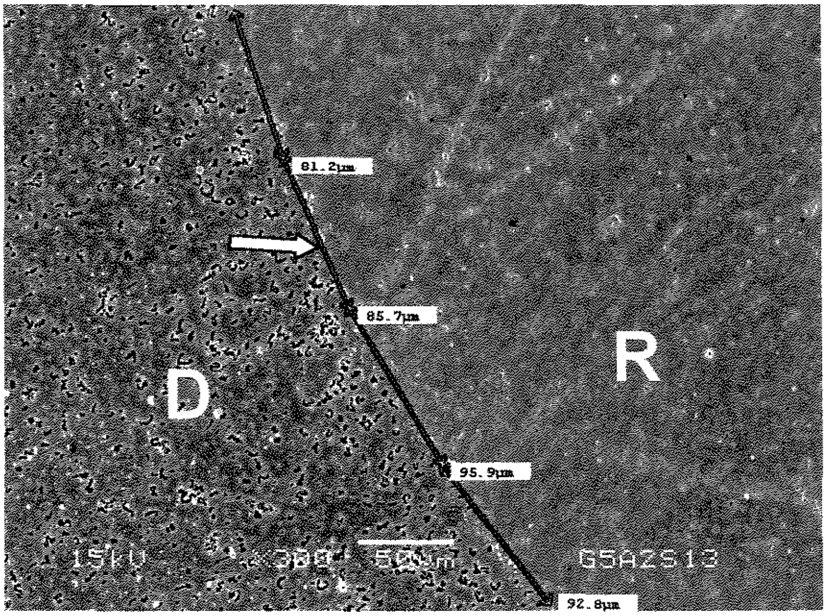

Figure 1. Photomicrograph illustrating a section of the replica in epoxy resin with the measurement steps for classification of marginal quality. **D**= dentin; **R**= composite resin; **Arrow** = margin measurement. Original magnification x300.

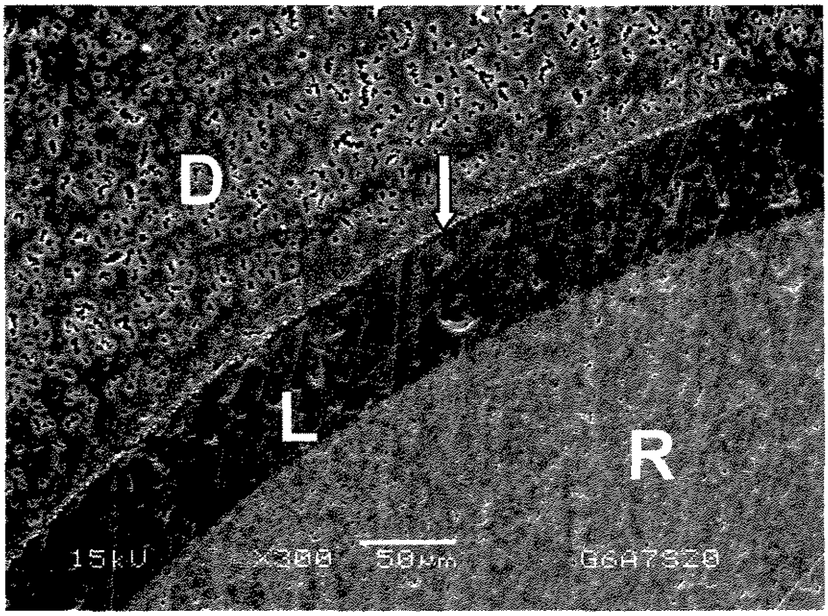

Figure 2. Photomicrograph of a replica in epoxy resin illustrating a section of a specimen of Group 6 (CSEB/PLF) classified as "perfect margin". Note the continuous transition between filling and dentin. **D**= dentin; **R**= composite resin; **L**= low-viscosity resin intermediate layer; **Arrow** = margin line. Original magnification x300.

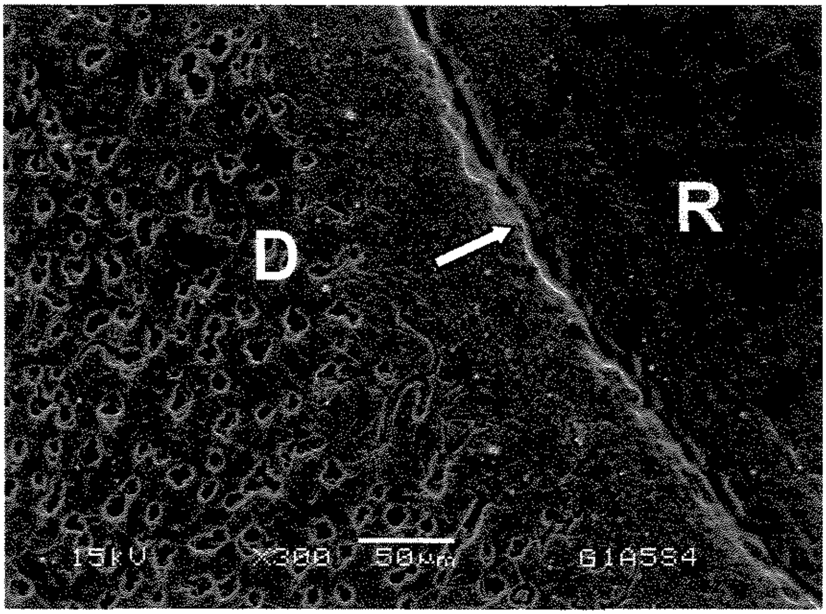

Figure 3. Photomicrograph of a replica in epoxy resin illustrating a section of a specimen of control Group 1 (PB) classified as "marginal irregularity". Note there is not a continuous transition between filling and dentin, nevertheless it is a gap free margin. **D**= dentin; **R**= composite resin; **Arrow** = margin line. Original magnification x300.

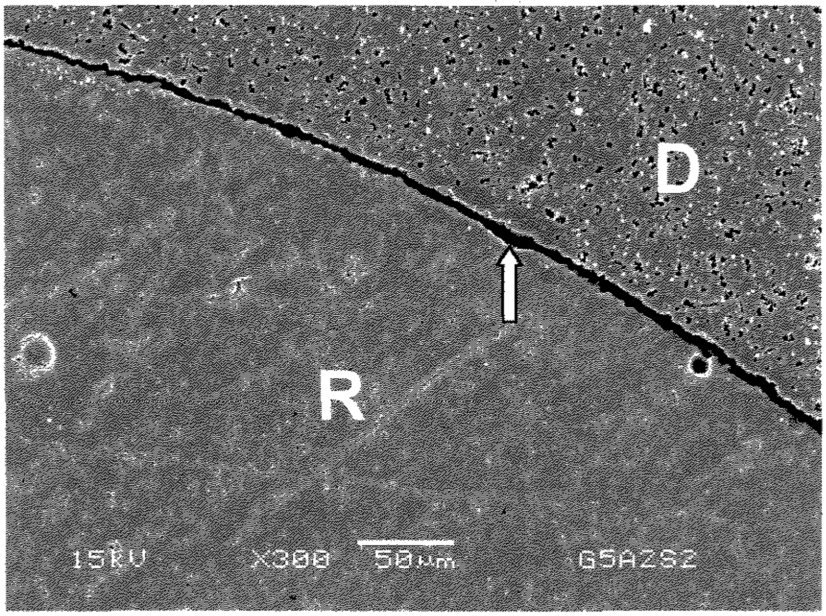

Figure 4. Photomicrograph of a replica in epoxy resin illustrating a section of a specimen of control Group 5 (CSEB) classified as "marginal gap". Note the loss of interfacial adhesion between filling and dentin. **D**= dentin; **R**= composite resin; **Arrow** = marginal gap. Original magnification x300.

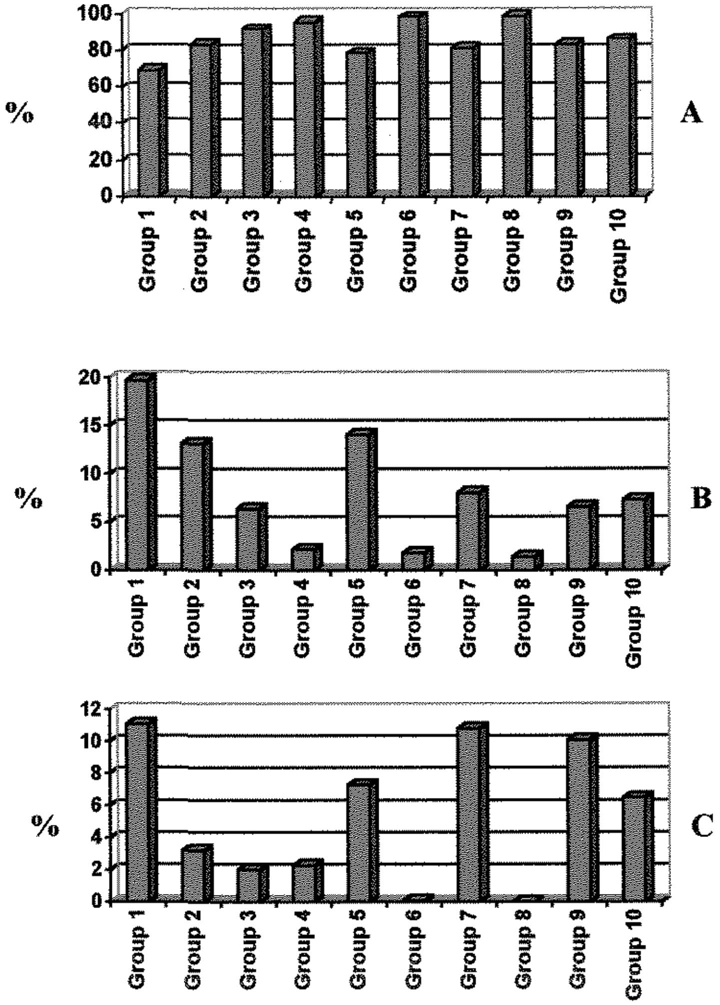

Figure 5. Comparison of mean values of marginal quality among the Groups: **(A)** perfect margin; **(B)** marginal irregularity; **(C)** marginal gap.

3.4 CAPÍTULO 4

Evaluation of internal adaptation of composite restorations and silver methenamine method for nanoleakage

Enviado para publicação no ***Dental Materials***

Abstract

Objectives: The aim of this study was to evaluate nanoleakage patterns following silver methenamine staining, and the internal adaptation of restorations bonded with a total-etch single bottle system, a self-etching primer system and a total-etch single bottle system after collagen depletion.

Methods: The dentin bonding systems used in this study were Prime & bond 2.1 (PB) and Clearfil SE Bond (CSEB). Cavities were made in grounded labial surfaces of 15 extracted bovine lower incisors, randomly divided into 3 Groups. Group 1 (PB), Group 2 (CSEB) and Group 3 (PB/SH) PB after collagen depletion. The specimens were restored, sectioned and submitted to silver methenamine staining, polished and observed with low-vacuum back-scattered SEM. One way ANOVA and multi-comparison Tukey's test were used for statistical analysis of the gap and leakage scores.

Results: No significant difference was observed in internal adaptation for PB and CSEB, but it was significantly enhanced for PB after collagen depletion. PB presented significantly greater silver deposition and a distinct pattern when compared to CSEB. No silver deposition was detected for PB after collagen depletion.

Significance: The dentin bonding systems tested were unable to prevent nanoleakage when used according to the manufacturer's instructions, however CSEB presented a lower level of silver deposition. Collagen depletion prior to PB application may enhance internal adaptation of restorations and prevent nanoleakage occurrence. Silver methenamine staining is a useful method for detection of nanoleakage.

Keywords: Nanoleakage; Silver Methenamine; Dentin bonding; Hybrid layer

1. Introduction

The current paradigm for bonding to dentin was established in 1982 and is based upon the diffusion, polymerization and consequent micromechanical retention of monomers within the network of collagen fibers, exposed by an acidic action, creating a mixed structure that was then called the "hybrid layer" [1]. It is believed that the ability of the current dentin adhesives to achieve a reliable and long-term bond to dentin is based upon a complete monomer infiltration through this exposed collagen network, in such a way that the partially demineralized underlying dentin surface is fully reached. Failure to adequately penetrate the collagen network into the partially demineralized dentin may produce a weak porous layer of exposed collagen, not protected by hydroxyapatite or encapsulated by resin, resulting in decreased bond strength and increased microleakage over time [2]. This leakage pathway was shown to exist in hybrid layer-adhesive interfaces, without gap formation, by exposing restorations to silver nitrate, and the term *nanoleakage* was suggested to distinguish this leakage from typical microleakage [3].

The technique suggested by Sano et al [3] to observe the leakage patterns of hybrid layers has been used, since it has demonstrated reliable results. Based mainly on this technique, many studies evaluating the nanoleakage for various bonding systems, and its influence on several bonding parameters, have been performed [4-15]. Silver nitrate has been accepted as a suitable method of measuring microleakage [16], however, it is a very severe test because the silver ion is very small (0.059 nm) when compared to the size of a typical bacterium (0.5-1.0 μm) [17]. This small size and high reactivity to stain [18], binding tightly to any exposed collagen fiber which are not enveloped by the adhesive resin, makes silver nitrate the most appropriate agent to detect the nanoporosities within the hybrid layer. A 50% silver nitrate solution is commonly used to immerse the specimens for 24 h [3,7-9,12-14] in total darkness, and the specimens are then immersed in a photographic developing solution for 8 h in order to reduce the silver ions to metallic silver.

Although this technique has demonstrated consistent results, some questions must be raised concerning two points; the first being the low pH of the 50% silver nitrate solution, which has been measured at between 3.4 [13,14] and 4.5 [10] and, during long time immersion, could induce demineralization around the edges of the restorations, particularly into the partially demineralized dentin, leading to a distorted or false positive result. The second point is the application of nail varnish on the surface to isolate the specimens and solely expose its margins. Dehydration is essential to achieve adhesion of the nail varnish by evaporation of the solvent and it takes approx. 15 min drying time [19]; restored dentin specimens has been shown to alter gap widths within 12 min [20], which must damage dentin and also lead to distorted results.

The silver methenamine stain was basically designed by Gromori [21] to improve the possibilities of histologic visualization of carbohydrate derivatives in animal tissues, and has been widely used in Grocott's technique [22] for staining of fungi in tissue sections. It was also used for visualization of hypo-mineralized areas in human teeth [23-25], and these studies concluded that silver methenamine staining could serve as a useful tool to visualize collagen of hypo- and unmineralized areas of dental hard tissues, and should be employed to demonstrate abnormal patterns of mineralization. Indeed, these conclusions apply to the situations where nanoleakage could be observed.

Thus, the aim of the present investigation was to critically examine the internal adaptation and nanoleakage patterns following silver methenamine staining, using the two currently accepted bonding approaches, i.e., a total-etch wet bonding technique adhesive system and a self-etching primer technique system; In addition, the total-etch adhesive system was used after collagen depletion.

2. Materials and Methods

2.1. Preparation of the specimens

The materials, manufacturers, compositions and batch numbers for this study are listed in Table 1. Freshly extracted lower bovine incisors (n=15), obtained from a local *abattoir*, stored frozen for no longer than 1 month, were used for the test. The teeth were ground on their labial surfaces with a water irrigated grinding wheel using 320-grit silicone carbide paper (SiC) to obtain flat dentin surfaces and were then finished with 600-grit SiC. Standardized cavities with margins totally located in dentin, with a C-factor of 4.3 (1.8 mm diameter/1.5 mm deep), were made using a diamond bur number 2294 (KG Sorensen Ltda. – Brazil).

The teeth were randomly divided into 3 Groups of 5 specimens each. For Group 1 (PB), the dentin surfaces were etched with 37% phosphoric acid gel (Dentsply Caulk, Milford, DE, USA) for 15 s, rinsed with running water for 20 s, and the excess water removed by blotting with tissue paper, leaving the dentin visibly moist. Adhesive system Prime & Bond 2.1 (PB) was applied using a saturated disposable brush, gently air dried with oil-free compressed air from a syringe for 5 s, and light cured for 20 s.

For Group 2 (CSEB), the adhesive system Clearfil SE Bond (CSEB) was used. The primer was applied with a saturated disposable brush for 20 s, dried with mild air flow, followed by application of the adhesive resin, air dried gently, and light cured for 10 s.

For Group 3 (PB/SH), after acid-etching as for Group 1, a 10% sodium hypochlorite solution (SH) was lightly applied for 1 min, rinsed with running water for 20 s, and the excess water removed by blotting with a tissue paper leaving the dentin visibly moist; adhesive PB was applied and light cured for 20 s.

For all Groups the cavities were restored with composite resin Z 250, placed in bulk, and light cured for 30 s. All light-curing procedures used a 3M XL3000 curing unit (3M Dental Products).

The restorations were finished with 1000-grit SiC under water in order to remove composite overhangs, and immediately immersed in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer at 7.2 pH, for 24 h at 37° C. After fixation, the specimens were rinsed with 15 ml of 0.2M sodium phosphate buffer at pH 7.2 for 1 h, in 3 baths of 20 min each, and finally rinsed for 1 min with distilled water.

The restorations were then half-sectioned parallel to the tooth long axis with a low-speed diamond saw (Model 650, South Bay Technology Inc. San Clemente, CA, USA) under water. One half of each specimen was used for silver methenamine staining.

2.2. Silver methenamine staining

The manufacturers and batch numbers of the reagents used to prepare the silver methenamine solution are listed in Table 2.

The silver methenamine solution was mixed shortly before use, and prepared from the following solutions: 3% hexamethylene tetramine solution (100 ml), 5% silver nitrate solution (5 ml) and 5% sodium borate solution (12 ml).

Immediately after mixture, the freshly prepared solution was transferred to a glass dish, where the specimens were immersed. The dish was left for 90 min at 60° C in a preheated oven. After this period, the specimens were rinsed in distilled water for 3 min. Specimens were then transferred to a 0.2% gold chloride bath for 30 s, rinsed in distilled water for 1 min, and placed in 3% a sodium thiosulphate bath for 3 min, before finally rinsing copiously in tap water. The specimens were then stained.

Silver methenamine solution (10 ml) and 50% silver nitrate solution (10 ml) were also prepared for a pH measurement at 25° C (pHmetro Digimed DM-20 µP, Digicrom, São Paulo-SP, Brazil).

The internal restoration surfaces where then lightly finished with 1000-grit SiC paper under water, lightly polished with 6, 3, 1 and 0.25 µm-grit diamond paste using a polish cloth under water and ultrasonically cleaned for 10 min between the grits. The specimens were immersed in a 50% phosphoric acid solution for 3 s, rinsed for 20 s with distilled water and were then air-dried at room temperature for 24 h.

The specimens were mounted on aluminium stubs and observed by SEM (JEOL, JSM-5600LV, Scanning Electron Microscope, Japan), at low-vacuum with back-scattered electron images.

2.3. Nanoleakage and internal adaptation measurements

The measurements of internal gaps and silver penetration were calculated with a 500X magnification directly on the SEM microscope monitor, using a multi-point measuring device, observing all the internal cavity margin lengths, in steps of approx. 100 µm. The gaps and leakage scores were calculated separately, and expressed as the percentage of the total internal length. After obtaining means and standard deviations (SD), the results for each Group, were submitted to a one-way ANOVA and to multiple comparisons using Tukey's Test, and $p < 0.01$ as the level of significance .

3. Results

Tables 3 and 4 summarize the results for internal gap opening and nanoleakage for each Group, respectively. For occurrence of internal gap opening no significant statistical difference was observed when both adhesive systems were used according to the manufacturer's instructions in Groups 1 (PB) 11.2% ± 2.19, and 2 (CSEB) 8.5% ± 1.63, although a slight better performance could be noted for Group 2 (CSEB). When the collagen was depleted by SH action, a significantly higher reduction in internal gap opening could be observed: Group 3 (PB/SH) 3.2% ± 1.46.

The nanoleakage scores were statistically different for all the Groups tested. Group 1 (PB) presented the highest leakage score 38.7% ± 7.45 of the internal cavity walls, followed by Group 2 (CSEB) 20.6% ± 7.21. For Group 3 (PB/SH) no penetration of silver was observed along the dentinal walls. Penetration of silver along the cavity walls was observed with or without internal gap opening in all specimens of Groups 1 (PB) and 2 (CSEB) and no relationship could be noted between internal gap openings and silver staining. The nanoleakage patterns of representative resin/dentin interfaces are shown in Figures 1-5. The presence of silver deposits in the adhesive layers or in the composite was rarely noted for all the Groups tested.

Group 1 (PB) presented a greater and more intense silver deposition, mainly concentrated in the pulpal wall and in the pulpal/axial angles of the cavities, decreasing towards the margins. The silver depositions could be observed mostly at the base of the hybrid layer. Most tubules took up silver within the openings, following the resin tags for approx. 10 μm (Fig. 1 and 2).

For Group 2 (CSEB), the silver deposition presented a less intense pattern (Fig. 3 and 4) when compared to Group 1 (PB). It was less continuous, and usually demonstrated a dashed line aspect. The dentinal tubules rarely took up silver. The silver deposits were mostly located at the base of the hybrid layer, but were sometimes also observed at the top, in a higher frequency than for Group 1 (PB).

For Group 3(PB/SH), there was no evidence of silver penetration within the resin/dentin interface when the collagen was depleted by SH (Fig. 5).

The pH measurements of the solutions, at 25° C, were 4.3 for the 50% silver nitrate solution and 8.1 for the silver methenamine solution.

4. Discussion

The configurations of this study comported factors that are proved to extremely challenge the bonded walls, such as the high C-factor cavity preparation (4.3), using bulk restorative composite placement [26] and a continuous and high intensity light-curing [27].

The two adhesive systems used in this study, PB and CSEB, represent the two currently accepted strategies to achieve micromechanical retention between resin and dentin. PB is a single bottle adhesive system, employing acetone as the volatile solvent and represents the total-etch wet bonding approach, i.e., its action demands a previous acid-etching procedure [28]. The major drawback of this technique is its sensitivity, due to its moisture dependence [29]. In addition, the collagen network exposed by the acid-etching procedure is not easily filled by the adhesive resin, which leads to the formation of a demineralized zone within the hybrid layer [30]. CSEB uses a self-etching primer that partially demineralizes the smear layer and the intact dentin, using them as bonding substrate, and represents a simple method to prevent the collagen from collapsing, avoiding its unprotected exposure. When these systems are applied there is no need for etching, rinsing and drying, so that the risk of over-etching, over-drying or over-moistening the dentin is eliminated [28].

No significant difference was observed in the results for internal gap opening, for the two bonding systems used in this study, Group 1 (PB) and Group 2 (CSEB), but a statistically significant decrease in internal gap formation was observed for Group 3 (PB/SH), where PB was applied after collagen depletion with SH (Table 3). A morphological SEM study of demineralized dentin surfaces which had been treated with SH revealed an altered eroded mineral surface, that appeared to be more compatible with bonding resins [31], moreover, collagen depletion has been shown to increase the bond strengths for acetone-based adhesives [32-35], such as PB, which could explain this significantly improved internal adaptation for Group 3 (PB/SH).

Since the phenomenon of the incomplete monomer infiltration through the collagen network was first described and established, by observing the penetration of silver nitrate along gap free margins for several bonding systems [2,3], the so-called nanoleakage [3] has caused some concern about the real consequences for the long-term bond. Although there is still no clear evidence of the negative effects of nanoleakage on the adhesion of composite materials to dentin [36], undoubtedly, the existence of such a pathway in gap free cavity margins brings a certain sensation of impotence and unreliableness to dentin bonding procedures. Indeed, this kind of leakage may allow for the penetration of bacterial products and dentinal or oral fluid along the interface, which may result in hydrolytic degradation of either the adhesive resin or the exposed collagen, thereby compromising the stability of the bond [37]. However, an *in vitro* study showed that although bond strengths have decreased over time, almost no change in silver penetration was observed [11]. The questions about the real influence of the nanoleakage phenomenon for the long-term bond, have prompted several studies in an attempt to establish relationships between nanoleakage patterns and several variable factors such as bonding systems [3,6,7,10-14], long-term storage [11,12], *in vivo/ in vitro* influence [5], load cycling [13], thermocycling [6,14], bond strength [4,10] and collagen depletion [9,15]. Until now, all studies have shown a nanoleakage pattern within the hybrid layer, regardless of the bonding systems used (employing either distinct volatile solvents or with different bonding approaches), and even the absence of marginal gaps [3,6,7,10-14]. For the long-term storage factor, although two studies demonstrate the decrease in bond strengths over time [11,12], just one found an increase in nanoleakage during a 12-month storage period [12]. No difference in nanoleakage formation was found between *in vivo*/ *in vitro* procedures [5]. Load cycling and thermocycling regimens were shown not to influence nanoleakage [6,13,14]. The only situation where nanoleakage was not detected occurred after collagen depletion with SH [9]. Another study [15] related no difference in nanoleakage when SH was used for collagen depletion, however for that study, the SH did not completely remove the collagen matrix, and the silver nitrate action is based upon its reaction to collagen fibers [18].

Group 1 (PB) presented a significantly higher score (Table 4) and a more intense silver deposition, mostly concentrated at the base of the hybrid layer, following the resin tags through the tubules entrances (Fig. 1 and 2). This pattern of silver deposition can be attributed to the total-etch bonding approach itself, resulting from an incomplete penetration of adhesive resin into the whole thickness of the demineralized dentin [30], and has been related to total-etch bonding systems [12,13]. For Group 2 (CSEB), silver deposition was significantly less intense (Table 4) and less continuous when compared to Group 1 (PB), appearing as a thin line at the base of the hybrid layer (Fig. 3 and 4). This pattern may be attributed to the to the self-etching bonding technique itself [12,13]. CSEB simultaneously etches and primes the dentin, the mild acidic action removes less mineral content and exposes less collagen, allowing a more complete penetration of the adhesive resin through the demineralized dentin surface, thus creating a less porous hybrid layer, which allowed significantly less silver deposition. When collagen was depleted prior to PB application for Group 3 (PB/SH), no silver deposition was observed within the adhesive resin-dentin junction (Fig. 5). These results can be explained by the silver methenamine staining technique and the acetone-based adhesive associated to a collagen depletion. As mentioned above, the lack of collagen has been shown to enhance the bond between dentin and acetone-based systems [32-35], and the silver methenamine staining technique is based upon its reaction to collagen [23-25], as well as silver nitrate technique [18]. Similar results were also found with the silver nitrate technique [9].

The results of this study for nanoleakage are in agreement with those already published in the literature, appearing to validate the silver methenamine staining technique. According to Sögaard-Pedersen [23], the silver methenamine reactive sites in dentin and in pre-dentin are most likely to be unmineralized collagen fibers, since silver methenamine stains these fibers unespecifically. A possible drawback for the 50% silver nitrate solution technique is its low pH which, according to our measurements was 4.3 at 25° C. Indeed, this low pH, associated with a long immersion time (24 h), could induce an increase in the demineralization pattern around the exposed margins of the restoration, particularly into the partially demineralized dentin zone, leading to an augment in silver uptake, which could be avoided by the use of a higher pH solution, such as silver methenamine (pH 8.1 at 25° C). Moreover, the low pH associated with the high silver concentration of the 50% silver nitrate solution, demands the application of an isolating agent such as a nail varnish, in order to avoid total silver staining of all the structures involved in the adhesion. The drying time required for the nail varnishes to adhere, approx. 15 min [19], was demonstrated to significantly increase gap widths [20] and could damage dentin, leading to distorted results. The silver methenamine technique did not require the application of a isolating agent, since it has a significantly lower silver content, higher pH, and it is specifically designed to visualize collagen of hypo- and unmineralized areas of dental hard tissues.

Further studies are necessary to clear the role played by nanoleakage for the long-term bond, as well the obscure points related to the methods used to evaluate such a phenomenon. The silver methenamine staining technique appears to represent a valuable tool for accessing nanoleakage.

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Table 1. Materials used in this study

Materials	Composition	Batch number	Manufacturer
Prime & Bond 2.1	Etchant: 37% H_3PO_4 Adhesive: UDMA, PENTA, R5-62-1 resin, Bis-EMA, Butylated hydroxitoluene, 4-Ethyl dimethyl aminobenzoate, Cetilamine hydrofluoride, Acetone	67510	Dentsply Caulk, Milford, DE, USA
Clearfil SE Bond	Primer: MDP, HEMA, Hydrophilic dimethacrylate, Camphorquinone, N,N-Diethanol p-toluidine, Water, Ethanol. Adhesive: MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, Camphorquinone, N,N-Diethanol p-toluidine, silanated colloidal silica.	51208	Kuraray Co. Ltd., Japan
Z 250	Bis-GMA, UEDMA, Bis-EMA, zirconia/silica filler	9AM	3M Dental Products, St. Paul, MN, USA

Table 2. Reagents used to prepare silver methenamine solution

Reagents	Batch number	Manufacturer
Hexamethylene tetramine	20K0941	Sigma Chemical Co., St. Louis, MO, USA
Silver nitrate	49053	Labsynth Produtos para Laboratórios Ltda. Diadema-SP, Brazil
Sodium borate	44961	Labsynth Produtos para Laboratórios Ltda. Diadema-SP, Brazil

Table 3. Occurrence of “internal gap opening”

Groups	Mean (%)	SD*
Group 1 (PB)	11.2	2.19 A
Group 2 (CSEB)	8.5	1.63 A
Group 3 (PB/SH)	3.2	1.46 B

*Same letters indicate no statistical difference ($p<0.01$).

Table 4. Occurrence of “nanoleakage”

Groups	Mean (%)	SD*
Group 1 (PB)	38.7	7.45 A
Group 2 (CSEB)	20.6	7.21 B
Group 3 (PB/SH)	0.0	0.00 C

*Same letters indicate no statistical difference ($p<0.01$).

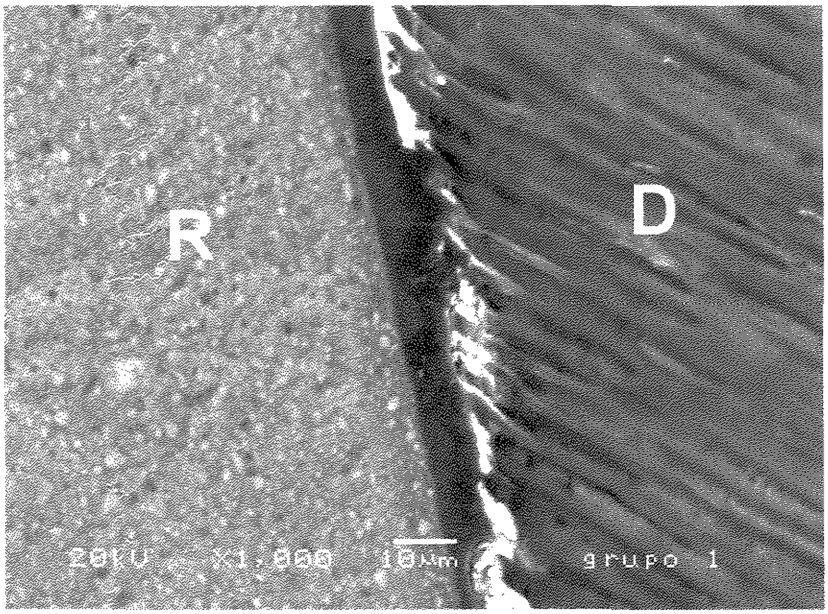

Figure 1. Back-scattered SEM photomicrograph of the interface bonded with PB. Intense silver penetration occurred at the base of the hybrid layer and along the dentin tubules, following the resin tags for approx. 10 µm (bar). D= dentin; R= composite resin.

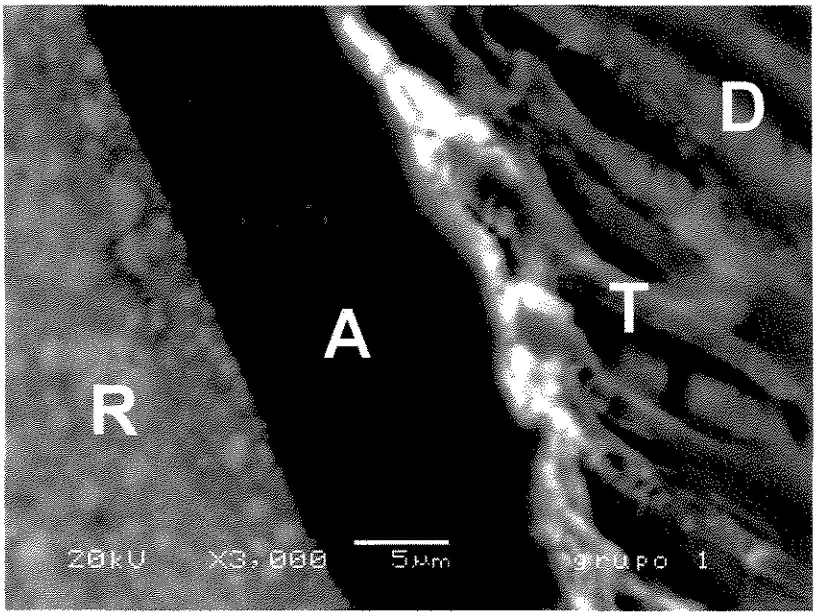

Figure 2. Back-scattered SEM photomicrograph in higher magnification (x3000) of the interface bonded with PB. An intense silver impregnation can be observed at the base of the hybrid layer and at the entrance of the dentin tubules. R= composite resin; A= adhesive resin; T= resin tag; D= dentin.

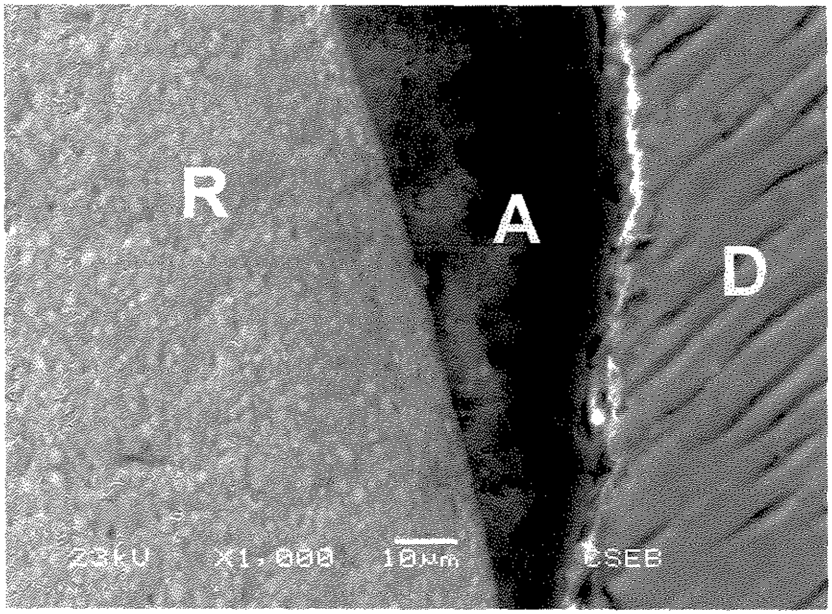

Figure 3. Back-scattered SEM photomicrograph of the interface bonded with CSEB. Note the less intense silver impregnation when compared to PB (Fig. 1) and the dashed line aspect. Note also the absence of silver deposits along the tubules and the gap observed along the wall. R= composite resin; A= adhesive resin; D= dentin.

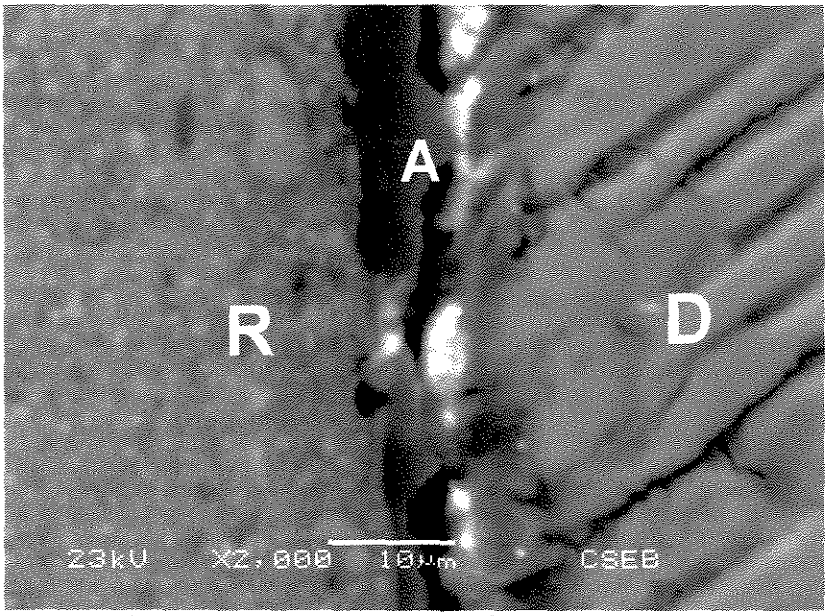

Figure 4. Back-scattered SEM photomicrograph of the interface bonded with CSEB in higher magnification (x2000). Note the minor intensity and continuity of silver deposition when compared to PB (Fig. 2) and the absence of silver impregnation within the tubules. R= composite resin; A; adhesive resin; D= dentin.

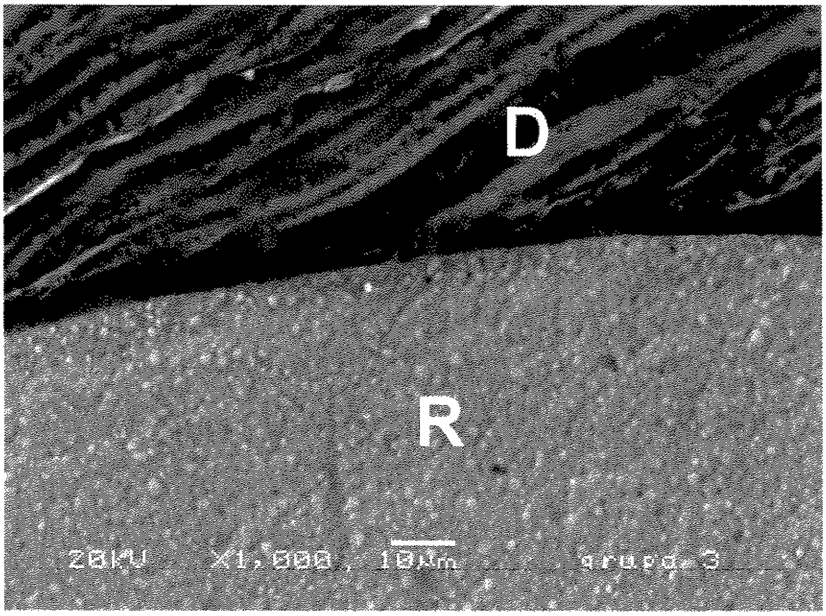

Figure 5. Back-scattered SEM photomicrograph of the interface bonded with PB, after collagen depletion with SH. No silver penetration can be detected along the interface. R= composite resin; D= dentin.

4. CONCLUSÕES GERAIS

Os resultados deste estudo *in vitro* nos permitiram concluir que:

1. A aplicação de uma camada intermediária de resina com baixo módulo de elasticidade entre a resina adesiva e o compósito restaurador estabelece um gradiente resiliente, que minimiza os efeitos de contração de polimerização na interface de união resina/dentina.
2. Os pré-tratamentos de superfície atualmente recomendados para união à dentina promoveram alterações morfológicas distintas e significativas na superfície dentinária, e devem influenciar nos resultados dos procedimentos de união.
3. A qualidade marginal de cavidades com alto fator-C e margens em dentina foi significativamente melhorada com a remoção do colágeno para o sistema adesivo que utiliza o condicionamento ácido total e acetona como solvente. O uso de uma camada intermediária de resina de baixa viscosidade melhorou significativamente a qualidade marginal para o sistema auto-condicionante.
4. Os sistemas adesivos utilizados neste estudo apresentaram nanoinfiltração quando usados de acordo com as recomendações dos fabricantes, contudo o sistema auto-condicionante apresentou um menor grau de infiltração. A remoção do colágeno para o sistema adesivo de condicionamento ácido total melhorou a adaptação interna das restaurações e evitou a ocorrência de nanoinfiltração. A metenamina de prata mostrou-se um método apropriado para o estudo da nanoinfiltração.

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