PATRÍCIA ALMADA SACRAMENTO

"ESTUDO COMPARATIVO DOS EFEITOS DE ANTIMICROBIANOS UTILIZADOS PARA A LIMPEZA DE CAVIDADES OU INCORPORADOS AO SISTEMA ADESIVO NA RESISTÊNCIA E DEGRADAÇÃO DA UNIÃO RESINA/DENTINA SUBMETIDA AO ENVELHECIMENTO"

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para a obtenção do título de Doutor em Odontologia – Área de Odontopediatria.

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DEDICATÓRIA

*Dedico esta tese ao meu Pai **Paulo**, à minha mãe **Célia**, e aos meus **irmãos Daniele, Juliana e Paulo Henrique** que sempre me apoiaram em minhas atividades e são as pessoas mais queridas e importantes na minha vida.*

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"Embora ninguém possa voltar atrás e fazer um novo começo, qualquer um pode começar agora e fazer um novo fim."

(Chico Xavier)

RESUMO

Os objetivos nesta tese, apresentada em dois capítulos, foram avaliar o efeito da clorexidina-CHX e do monômero antibacteriano 12-metacriloxidodecilpiridíneo-MDPB na resistência e degradação da união resina/dentina desmineralizada por até doze meses de armazenamento. Capítulo 1: O objetivo deste estudo *in vitro* foi avaliar o efeito da CHX e/ou de diferentes sistemas adesivos na penetração dos monômeros na dentina desmineralizada, na formação e espessura da camada híbrida utilizando a técnica de Microscopia Confocal de Varredura a Laser (MCVL). Foram utilizados 3 sistemas adesivos: Clearfil SE Bond- SE (Kuraray), Clearfil Protect Bond - PB (Kuraray) e Adper Single Bond 2- SB (3M/ESPE) e um agente antimicrobiano: solução de digluconato de clorexidina 2% (FGM) preconizado para a limpeza da cavidade. Trinta terceiros molares hígidos foram distribuídos aleatoriamente em 6 grupos de acordo com os sistemas adesivos SE, PB e SB, com ou sem aplicação prévia da CHX. A dentina média foi exposta e desmineralizada artificialmente com gel ácido de carboximetilcelulose previamente ao procedimento de união. Os dados de MCVL foram analisados pelo teste estatístico Análise de Variância e teste de Tukey ($P<0,05$). A CHX e os sistemas adesivos não interferiram com a penetração dos monômeros na dentina desmineraliza. Todas as amostras apresentaram formação de camada híbrida, sendo mais espessa e menos homogênea nos grupos do SB, independente do uso da CHX. Concluiu-se que a CHX e os diferentes sistemas adesivos não afetaram a penetração dos monômeros em dentina desmineralizada. Somente o sistema adesivo interferiu com a espessura e homogeinidade da camada híbrida. Capítulo 2: O objetivo deste estudo *in vitro* foi: avaliar a influência CHX, do MDPB e o tempo de armazenamento na resistência e degradação da união resina/dentina desmineralizada através do teste de microcisalhamento (µSBS) e análise da nanoinfiltração. Foram utilizados 2 sistemas adesivos: Clearfil SE Bond-SE (Kuraray), Clearfil Protect Bond - PB (Kuraray). Cento e vinte terceiros molares hígidos foram distribuídos aleatoriamente em 12 grupos de acordo com os sistemas adesivos SE e PB, com ou sem aplicação prévia da CHX na superfície de união e tempo de armazenamento de 24 horas, 6 e 12 meses. A dentina média foi exposta e desmineralizada artificialmente. Após 24 horas de armazenamento o SE apresentou os menores valores de µSBS, não havendo diferença após 6 e 12 meses de armazenamento. A CHX não afetou os valores de µSBS. O modo de falha e a avaliação da nanoinfiltração foram analisados de forma descritiva. A nanoinfiltração da interface resina/dentina foi verificada em todos os grupos, havendo maior penetração da prata nos grupos de CHX. Um aumento no depósito de prata e decréscimo nos valores de µSBS foi notado em todos os grupos após 6 meses de armazenamento. A CHX e o MDPB não foram capazes de inibir a nanoinfiltração e o decréscimo na resistência de união. Conclui-se que a CHX não afetou a penetração dos sistemas adesivos na dentina desmineralizada, afetou a formação da camada híbrida, porém, não afetou os valores de resistência de união imediatos, que foram alterados pelo tempo de armazenamento, bem como a nanoinfiltração da prata na interface adesiva, após 6 e 12 meses.

PALAVRAS-CHAVE: sistemas adesivos, clorexidina, resistência de união, nanoinfiltração, microscopia confocal a laser, cárie.

ABSTRACT

The objective of this Thesis, presented in two chapters, was to evaluate the effect of chlorhexidine-CHX and the antibacterial monomer 12-metacriloxydodecylpiridinium-MDPB on the bond strength and bonding degradation of the resin/demineralized dentin interface over twelve months of storage time. Chapter 1: The objective of this in vitro study was to evaluate the effect of CHX and/or different adhesive systems, in the penetration of monomers in demineralized dentin, as well as on the formation and thickness of the hybrid layer using Confocal Laser Scanning Microscopy (CLSM). Three adhesive systems were used: Clearfil SE Bond- (Kuraray), Clearfil Protect Bond - PB (Kuraray) and Adper Single Bond 2 - SB (3M/ESPE), and an antibacterial agent: 2% chlorhexidine solution (FGM) used for cavity disinfectant. Thirty sound third molars were randomly distributed into 6 groups according the adhesive systems SE, PB and SB, with or without previous CHX application. The middle dentin was exposed and the artificial caries lesion was developed with a carboxymethylcellulose acid gel previously the bonding procedure. CLSM images were analysed by the Analysis of Variance and Tukey's post hoc tests ($P<0.05$). The CHX and the MDPB did not interfere with the penetration of the adhesive systems in demineralized dentin. All the groups presented a hybrid layer formation, with significantly thicker and lower homogeneity in the SB groups, regardless of the CHX application. It was concluded that the CHX and the different adhesive system did not affect the penetration of monomers in the demineralized dentin. Only the adhesive system affected the thickness and homogeneity of hybrid layer. Chapter 2: The objective of this in vitro study was to evaluate the influence of CHX, MDPB and storage time in regard to the bond strength and the bonding degradation of resin/demineralized dentin interface by microshear (µSBS) and nanoleakage evaluation.Two adhesive systems were used: Clearfil SE Bond- SE (Kuraray), Clearfil Protect Bond- PB (Kuraray). One hundred twenty sound third molars were randomly distributed into 12 groups according to the adhesive systems, SE and PB, with or without previous CHX application on the bonding surface, and storage time of 24h, 6 and 12 months. The middle dentin was exposed and a artificial caries lesion was developed artificially. After 24h of storage, the SE groups presented the lower µSBS values, but they did not have statistical significant differences after 6 and 12 months of storage. The failure mode and the nanoleakage were evaluated descripitivelly. The nanoleakage of the resin/dentin interface was verified in all the groups, having a greater silver deposit in the CHX groups. An increase in the silver deposit and decrease in the µSBS values were noticed in all the groups after 6 months of storage. The CHX and the MDPB were not able to inhibit the nanoleakage and a decrease in bond strength. It was concluded that the CHX did not affect the penetration of the adhesive systems in the demineralized dentin, affected the hybrid layer formation, but it did not affect the imediate values of the bond strength, which were modified with the storage time, as was the nanoleakage in bonding interface, after 6 and 12 months.

KEY WORDS: adhesive systems, chlorhexidine, bond strength, nanoleakage, Confocal Laser Scanning Microscopy, caries.

SUMÁRIO

INTRODUÇÃO GERAL

A associação entre técnicas conservadoras e o desenvolvimento de materiais restauradores adesivos têm trazido mudanças na filosofia de preparos cavitários.

Preparos cavitários extensos, principalmente em cavidades profundas têm sido substituídos por técnicas mais conservadoras, com a remoção somente do tecido dentinário cariado irreversivelmente alterado e altamente infectado e a preservação da dentina afetada (Fusayama *et al.*, 1966, Tyas *et al.*, 2000). Assim, a dentina mais seca que a dentina sadia, com alteração de cor e mais resistente ao corte que a dentina infectada deverá ser deixada na parede pulpar para evitar exposição da polpa. (Kidd *et al.*, 1996).

Neste sentido, bactérias em atividade podem permanecer nas cavidades com a incompleta remoção da dentina cariada, podendo sobreviver e se desenvolver, propiciando a progressão da lesão. Portanto, materiais restauradores que contenham em sua composição antimicrobianos (Imazato *et al.,* 2007) ou o uso de agentes de limpeza no preparo cavitário previamente ao procedimento restaurador podem ser úteis para tentar reduzir a possibilidade de progressão da lesão causada por estes microrganismos remanescentes ou devido à penetração de microrganismos na interface de união material restaurador/dentina (Imazato *et al*, 2002; Imazato *et al*., 2007).

Na tentativa do controle do crescimento e desenvolvimento destes microrganismos são utilizados agentes de limpeza para a remoção de detritos que não foram retirados durante o preparo cavitário, tais como, raspas de dentina e esmalte, bactérias, pequenos fragmentos ou partículas abrasivas dos instrumentos rotatórios e óleo, provenientes dos aparelhos de alta e baixa velocidade de rotação (Francischone *et al.,* 1984).

Um dos produtos utilizados para a limpeza da cavidade é a clorexidina, potencialmente bactericida (Brännström, 1984). A clorexidina é um anti-séptico com amplo espectro de ação que atua sobre bactérias Gram positivas e negativas, aeróbicas e anaeróbicas, fungos e leveduras. Por possuir carga positiva apresenta grande afinidade pela carga negativa de alguns grupos bacterianos (Hogu, 1964). A clorexidina pode interferir no metabolismo das bactérias por vários mecanismos: inibir a produção de ácido, inibir a proteólise, interferir na membrana, incluindo a síntese de adenosina trifosfato (ATP) nos "Streptococcos" (Emilson, 1981). Em baixas concentrações (0,12%) seu efeito é bacteriostático, e em altas concentrações (2%) é bactericida (Costa *et al*., 1999). O uso da clorexidina foi generalizado durante as últimas duas décadas para o controle químico do biofilme bacteriano e desinfecção de cavidades (Atac *et al.*, 2001; Imazato *et al.*, 2002), tornando um importante coadjuvante no tratamento preventivo de lesões de cárie recorrentes (Lynch, 1996).

Além do uso de agentes de limpeza, materiais restauradores com propriedades antimicrobianas também são úteis na odontologia que preconiza preparos cavitários mais conservadores (odontologia minimamente invasiva), pois com a evolução dos sistemas adesivos, e a criação de sistemas autocondicionantes em que os monômeros são capazes de desmineralizar o substrato e infiltrá-lo ao mesmo tempo, a *smear layer* é preservada e "incorporada" na camada híbrida. Desta forma permite que parte dos microrganismos presentes na dentina afetada sejam incorporados à camada híbrida, ou permaneçam na dentina afetada. A vantagem da manutenção da lama dentinária pelos sistemas de união autocondicionantes se deve ao condicionamento da dentina e permeação desta pelos monômeros acontecer de forma simultânea, diminuindo a camada de dentina desmineralizada não permeada pelos monômeros (Michelich *et al.*, 1980 e Olgart *et al.*, 1974). Além dessas vantagens, a utilização de sistemas adesivos autocondicionantes tem permitido a união satisfatória entre o adesivo e a dentina (Kelsey *et al*., 2005; Miranda *et al*., 2006; Marquezan *et al*., 2008).

Se por um lado a manutenção da *smear layer* conduz a possibilidades positivas, por outro lado, permite a possibilidade da permanência de bactérias remanescentes no preparo cavitário e que podem multiplicar-se através dos nutrientes fornecidos pela lama dentinária ou fluido dentinário (Brännström & Nyborg, 1973), causando uma possível progressão da lesão de cárie. A viabilidade desse paradoxo trazida com o uso de sistemas adesivos autocondicionantes suscitou o aparecimento de um sistema adesivo com atividade antibacteriana, podendo ser utilizado na tentativa de assegurar o tratamento restaurador de mínima intervenção.

Em 2006, Imazato *et al*., descreveram um novo sistema adesivo autocondicionante contendo no primer 5% de um monômero antimicrobiano, o monômero brometo de 12-metacriloiloxidodecilpiridínio (MDPB), sendo este sistema adesivo comercializado inicialmente como Clearfil Protect Bond, e posteriormente como Clearfil SE Protect (Kuraray Medical, Kurashiki, Japão). O MDPB é um monômero sintetizado pela combinação de um agente antimicrobiano derivado do amônio quaternário e um grupo metacrilato, exibindo atividade antibacteriana contra bactérias orais (Imazato *et al*., 1994). A incorporação do MDPB aos sistemas adesivos é considerada um método potencial de melhoria da atividade antibacteriana do sistema adesivo (Imazato *et al.*, 1998). O *primer* contendo MDPB mostrou-se promissor para inativação de bactérias residuais da cavidade dentária em estudos *in vitro* e *in vivo* (Imazato *et al*., 1997, 2001, 2004).

Em preparos cavitários realizados *in vivo* os procedimentos adesivos são realizados em diferentes substratos dentinários, desde o substrato hígido, encontrado nas paredes laterais da cavidade, como no substrato dentinário com características alteradas (dentina afetada por cárie, dentina esclerótica ou até mesmo a cariada).

O uso de agentes antimicrobianos em preparos cavitários mais profundos seja prévia ou concomitantemente ao uso de materiais restauradores reporta a sua importância, pois a maioria dos substratos de adesão tratados clinicamente não se constitui em dentina sadia, mas sim, em dentina afetada por cárie, com remanescente bacteriano presente, principalmente nas técnicas de preparos cavitários mais conservadores.

Nesses substratos alterados (dentina desmineralizada, afetada por cárie), a resistência de união dentina/resina pode ser afetada, pois, está intimamente relacionada à qualidade do substrato dentinário (Pashley *et al*., 1995). O substrato alterado por cárie apresenta presença de maior quantidade de água, menor resistência coesiva, menor rigidez e menor módulo de elasticidade (Ito *et al*., 2005) sendo, portanto um substrato diferente da dentina sadia. Baseando-se nessas características, a literatura tem demonstrado que a dentina afetada por cárie apresenta menor resistência de união em relação à sadia, independentemente do tipo e técnica de utilização do sistema adesivo. (Say *et al*., 2005 e Pereira *et al*., 2006) Esses resultados têm sido atribuídos à dificuldade dos monômeros penetrarem na dentina afetada por cárie, pois, depósitos minerais ácido-resistentes presentes nessa condição, diminuiriam o módulo de elasticidade e a resistência coesiva da dentina afetada pela cárie (Duke & Lindemuth, 1991; Van Meerrbeek *et al*., 1994). Deve-se considerar que clinicamente, grande parte do procedimento restaurador é realizada em substrato dentinário alterado (dentina afetada por cárie) (Erhardt *et al*., 2008). Em adição, poucos são os estudos relatando a eficiência da união resina/dentina afetada por cárie em longo tempo de armazenamento (Hebling *et al*., 2005; Carrilho *et al*., 2007).

Embora a união resina/dentina seja momentaneamente estável, sabe-se que a degradação hidrolítica do polímero resinoso (Mohsen & Craig, 1995) ou do colágeno por enzimas endógenas presentes na dentina (De munk *et al*., 2009) é um fenômeno esperado, deteriorando a interface de união dentina/resina, reduzindo a eficiência dessa união. Tanto a composição do sistema adesivo, quanto às características do substrato dentário e a técnica do procedimento adesivo (tempo de condicionamento ácido e a umidade) influenciam no processo de degradação da interface resina/dentina.

A nanoinfiltração aparece como uma conseqüência da falta de penetração do sistema adesivo no substrato dentinário desmineralizado pela ação do condicionamento ácido da superfície dentinária, permitindo a penetração de líquidos da polpa e da cavidade oral nas porosidades que se encontram dentro e adjacentes à camada híbrida. A nanoinfiltração tem sido demonstrada ocorrer também em sistemas adesivos autocondicionantes (Yuan *et al*., 2007). Apesar de ser menos extensa que a microinfiltração e provavelmente em curto prazo não ter importância clínica, a nanoinfiltração pode comprometer a estabilidade da união entre o sistema de união e a dentina (Pioch *et al*., 2001, de Goes & Montes, 2004).

Constatada a necessidade de procedimentos adesivos associados aos métodos de mínima intervenção, com a remoção parcial da dentina cariada, deixando remanescentes de dentina afetada por cárie no interior da cavidade terapêutica e a premente necessidade de se controlar a viabilidade das bactérias remanescentes, é de fundamental importância a utilização de agentes antimicrobianos quer seja na limpeza da cavidade ou incorporados aos sistemas adesivos. Entretanto, não se conhece o efeito do MDPB, componente do sistema adesivo autocondicionante, com efeito antimicrobiano, e da clorexidina, utilizada na limpeza da cavidade terapêutica na resistência de união e na degradação da interface de união resina/dentina em longo prazo.

PROPOSIÇÃO GERAL

Os objetivos gerais desta Tese[1] foram:

Avaliar *in vitro* o efeito da clorexidina e/ou de diferentes sistemas adesivos na penetração dos sistemas adesivos na dentina desmineralizada, formação e espessura da camada híbrida da interface de união resina/dentina desmineralizada e avaliar a influência destes antimicrobianos e o tempo de armazenamento em água na resistência e degradação da união resina/dentina desmineralizada. As variáveis analisadas foram a resistência e degradação da união por microcisalhamento e nanoinfiltração, e a morfologia da interface por Microscopia Confocal de Varredura a Laser.

A Tese será apresentada na forma alternativa e assim dividida em dois capítulos:

Capítulo 1

A CLSM analysis of hybrid layer formation by different adhesives systems used after chlorhexidine using on demineralized dentine.

Capítulo 2

Influence of cavity disinfectant and adhesive systems on the bonding procedure in demineralized dentin – long term evaluation.

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[1] Este trabalho de Tese foi realizado no formato alternativo, com base na deliberação da CCPG 001/98, da Universidade Estadual de Campinas (Unicamp).

CAPÍTULO 1

A CLSM analysis of hybrid layer formation by different adhesives systems used after chlorhexidine using on demineralized dentine[2].

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Abstract:

Purpose: To evaluate the use of a cavity disinfectant and adhesive systems in the penetration of adhesive systems on demineralized dentine in the formation and thickness of the hybrid layer. **Materials and Methods:** Thirty third molars were selected, sectioned to expose the middle dentine. Demineralized dentine (DD) was produced *in vitro* using an acid gel model. DD-specimens were distributed into 6 groups (n=10). A 2% chlorhexidine solution (CHX) was used as a cavity disinfectant, and three adhesive systems: Clearfil SE Bond(SE), Clearfil SE Protect(PB) and Adper Single Bond 2(SB). A fluorescent dye was added to primer (SE/PB) or adhesive (SB) prior to their application to allow the observation of bonding agent penetration in the demineralized dentine. The adhesives were then applied to the DD-specimen surface according to the manufacturer's instructions, and a composite resin block was built on the surface (2 mm height). After 24h, the specimens were sectioned perpendicularly to the bonding area and analysed by Confocal Laser Scanning Microscopy(CLSM). The penetration of the adhesive as well as the formation and thickness of the hybrid layer were measured using the *software* associated with the CLSM. Data were analysed using two-way ANOVA and the Tukey post-hoc test ($p<0.05$). **Results:** The CHX and adhesive systems did not interfere with the penetration of the adhesive system in demineralized dentine. All samples presented penetration of the adhesive systems on the substrate and formation of the hybrid layer, and it was significantly thicker and not homogeneous for SB regardless of the use of CHX. There was no statistically significant difference between the two self-etch adhesive systems. **Conclusion:** Treatment with CHX or the adhesive system did not affect the penetration of the adhesive system in the demineralized dentine. Only the adhesive systems interfered with the thickness and homogeneous formation of the hybrid layer.

Keywords: chlorhexidine; adhesive systems; dentine; Confocal Laser Scanning Microscopy

Introduction

The bonding mechanism of adhesive systems to dentine is micromechanical and is essentially based on the infiltration of resin components into the etched dentine surface and the subsequent formation of a resin infiltration layer (hybrid layer), resin tags, and adhesive lateral branches[25].

Deeper cavity preparation has been replaced by more conservative techniques, with the removal only of the softer, highly contaminated and irreversibly demineralized carious dentine, preserving the affected dentine[36]. Therefore, restoring procedures have currently been executed in caries-affected dentine[12]. This altered dentine showed a lower amount of mineral, lost during the caries lesion process. At the same time, tricalcium phosphate crystals were found to occlude the dentine tubule lumen and prevent the penetration of resin monomers[22,38]. Lower bond strength and thicker hybrid layers have been found in caries-affected dentine than normal dentine[42].

Some studies have shown that some active bacteria can remain after dentine caries partial removal and would induce recurrent caries as well as damage to the pulp depending on the restoration sealing ability. Also, due to the low bond strength obtained when bonded to caries-affected dentine, a gap on the bonding interface could allow for the bacterial substrate to seed the restoration[29] and sometimes cause the progression of caries lesion which would resulting in a secondary caries lesion[41].

Many attempts to developing dentine bonding systems and filling materials with antibacterial activity have been performed[2,4]. Restorative materials with antibacterial activity would be helpful in preventing the harmfull effects caused by remainder oral bacteria[31]. For this reason, adhesive systems that exert antibacterial effects have been highlighted[15] indeed 2% chlorhexidine solution is often used as a cavity disinfectant[16,19].

Chlorhexidine has been widely used as an antimicrobial agent and it is useful in the setting of disinfecting teeth before the placement of restorations[16,19]. It is not known, however, whether such an antibacterial agent may affect the bonding strength of the restorative material to dentine. The results of in vitro studies are controversial regarding the disinfectant's effect on adhesion[6,14,23,32]. Previous studies seem to demonstrate that chlorhexidine application prior to acid-etching has no adverse effects on immediate composite-adhesive bonds in dentine[8.] However, Tulunoglu et al.,[32] found that chlorhexidine-containing cavity disinfectant increased microleakage scores when used prior to the implementation of Syntac and Prime&Bond dentine adhesive systems. They reported that there might have been some negative interaction between the cavity disinfectants and dentine bonding agents. Vieira et al.,[40] investigated the effect of chlorhexidine as a cavity disinfectant on bond strength of primary dentine and found that 2% chlorhexidine had an adverse effect on the adhesive system (Single Bond) when used prior to etching. Others studies have reported that chlorhexidine used as a cavity disinfectant can alter the dentine structure[14,24]. Chlorhexidine possesses cationic properties. Because oral surfaces are primarily negatively charged, the positive ionic charge of the chlorhexidine easily binds to the negatively charged phosphate groups of the dentine[19] compromising the bonding ability of the adhesive systems[14,24].

The use of cavity disinfectant agents or self-etch adhesive systems with antibacterial activity, such as MDPB (12-methacryloyloxydodecylpyridinium bromide), which is present in Clearfil SE Protect can contribute to the inhibition of bacterial growth[16] and does not seem to disturb bonding ability. However, bonding strength per se, it is not enough to predict the behavior of the longevity of the adhesive bonding area, as the relationship between the adhesive system and the dentine surface, as well as the hybrid layer formation and degradation are the most important factors that compromise bonding longevity.

The introduction of confocal laser scanning microscopy (CLSM), which is used in combination with fluorescent dyes, has provided a valuable new technique for the visualization of bonding structures, such as the hybrid layer and resin tags in dentine. The advantages of such a technology include a non-destructive examination and the lack of need for drying of the specimen, which minimises the risk of technical artefacts[27]. Also, by using a fluorescent dye that is mixed individually with each of the components of the bonding systems (e.g., primer or adhesive resin), the behavior of each component can be studied individually[27]. In the case of contrasting by means of fluorescent dyes, it would appear to be much easier to interpret the images. However, the possibility that the dyes might have a leaching ability must be considered[27].

The aim of this study was to evaluate the use of chlorhexidine solution and different adhesives systems in the penetration of an adhesive system on demineralized dentine and the formation and thickness of the hybrid layer. The null hypothesis tested was that the chlorhexidine and/or adhesive system would not affect the penetration of different adhesive systems and/or the formation of the hybrid layer in demineralized dentine.

Materials and methods

This study was performed under the protocol approved by the Research Ethics Committee of the Dental School of Piracicaba (#083/2005, #072/2007), University of Campinas, Brazil. Thirty extracted sound human third molars were selected, hand cleaned with periodontal scales and then with pumice slurry using a Robinson brush. Next, they were stored in chloramine T 1% at 4º C until two months after extraction. Teeth with caries or cracks were excluded. The location of the middle dentine was determined in each tooth using a bitewing X-Ray, and the middle dentine, which was considered to be half the distance from the enamel-dentine junction to the pulp, was determined. Occlusal enamels were ground flat using a water-cooled mechanical polisher (Metaserv 2000, Buehler, UK LTD, Lake Bluff, IL, USA) until it reached the middle dentine depth surfaces. The roots of the teeth were cut at the cementoenamel junction and discarded.

The specimens were then randomly divided into six groups of five specimens each based on chlorhexidine solution treatment and adhesive systems used: I) Clearfil SE Protect and chlorhexidine treatment (PB+); II) Clearfil SE Protect and no chlorhexidine treatment (PB-); III) Clearfil SE Bond and chlorhexidine treatment (SE+); IV) Clearfil SE Bond and no chlorhexidine treatment (SE-); V) Adper Single Bond 2 and chlorhexidine treatment (SB+); VI) Adper Single Bond 2 and no chlorhexidine treatment (SB-). The brand names, main components, manufacturer names, batch numbers, pH values and application methods of adhesive systems are shown in Table 1. Clearfil AP-X (Kuraray Corp., Osaka, Japan– batch # 00985B) was used to complete the bonding procedures.

Specimens were coated with a red acid-resistant nail varnish (Colorama; CEIL Com. Exp. Ind. Ltda., São Paulo, SP, Brazil), with the exception of the coronal dentine area which would later be submitted to the development of artificial caries.

Demineralized dentine was produced in vitro using an acid gel model. Specimens were immersed in 5 mL of 6% carboxymethylcellulose acid gel (Proderma Pharmacy, Piracicaba, SP, Brazil) at pH 5.0 and 37ºC. The gel contained 0.1 M lactic acid titrated to pH 5.0 with a concentrated KOH solution[37,5,18]. The specimens remained in the gel for 24 hours without renewal. This model produced a demineralized depth of approximately 40 μm, confirmed in a pilot study of polarized light microscopy. After the development of artificial caries, specimens were washed two times in an ultrasound bath with distilled water for fifteen minutes each and dried with tissue paper.

Immediately before the restorative procedure, the smear layer was standardized using a 600-grit abrasive paper for one minute. Then, for the specimens from Groups SE+, PB+ and SB+ a 2% chlorhexidine (CHX) solution (FGM Joinvile Santa Catarina, Brazil) were used as a therapeutic primer prior to acid-etching for SB groups and prior primer application for SE and PB groups; a drop of the solution was placed on the demineralized surface, maintained inert and the samples were then gently blot dried after a dwell time of 60 seconds[20].

Adper Single Bond 2 adhesive systems, as well as the primer component of Clearfil SE Protect and Clearfil SE Bond were mixed with 0.16mg/mL of the fluorescent dye Rhodamine B isothiocyanate (Sigma Chemicals, Dorset, UK). The adhesive systems were then applied according to the manufacturers' instructions, and a composite resin (AP-X, Kuraray, Japan) block (2 mm height) was built on the bonding surface, using the incremental technique (1 mm each layer). Each layer of composite resin was individually light-cured for 40 seconds with an Elipar Tri-light unit (ESPE – America Co., Seefeld 82229 - Germany). Light intensity was periodically measured (470mW/cm^2). The specimens were placed in canola vegetable oil (Liza, Mairinque, SP, Brasil) at 37º C for 24 hours[9]. Then, bonded specimens were bucco-lingually sectioned (Isomet, Buheler, Lake Bluff, IL, USA) using oil as lubricant to get slices 0.5 mm thick[9]. Two slices from each tooth were obtained (n=10) for each group.

Confocal Laser Scanning Microscopy (CLSM) analysis of the dentine/resin interface was performed with a Leica TCS SP2-SE Microscope (Leica Microsystems Gmbh, Manhein, Germany). A HeNe 543 gas laser was used as the light source. The intensity of the excitation light as well as the amplification of the photomultiplier was kept constant during the investigation period. CLSM was recorded in the fluorescent mode. The detected light was conducted through a 543-nm long-pass filter: thus, fluorescent light emitted from the specimen was discriminated from reflected and scattered light. The visualized layer was selected 10 µm below the sample surface and images were recorded with an oil immersion objective (40X, numerical aperture 1.25). The size of the images was 62.5 X 62.5 μm^2, and the resolution was 512 X 512 pixel. Images were recorded at three standardized areas for each sample (centre and two extremities). The penetration of the adhesive systems considered as positive if penetration occurred and negative if the penetration not occurred, and the formation of the hybrid layer were evaluated directly on the computer screen. The measurements of the hybrid layer thickness were obtained using a tool of the software LEICA LCS LITE FOR LEICA TCS SP2 (.LEI) which was taken by tracing a straight line perpendicular to the middle dentine, from the top until the base of the hybrid layer in each area of each sample and the results were expressed in µm.

Also the qualitative analysis of the hybrid layer was classified based on the homogeneity of the hybrid layer formation; it was considered as homogeneous, if it had no break in its structure, and it was considered as not homogeneous, if it had one or more break in its structure.

CLSM evaluation was performed double blind by two different operators. In cases of discrepancy between the two readers, a mean thickness was recorded; for the qualitative analysis the irregular structure prevailed.

Statistical analysis

Inter-examiner data from the two operators were analysed using the Spearman correlation test ($p<0.05$). Statistical descriptive analysis was performed for the penetration of the adhesive systems. Two-way ANOVA and Tukey's post-hoc test were used to compare the formation and thickness of the hybrid layer measurements between the adhesive system and previous treatment with the chlorhexidine solution. The statistical significance level was set at $\alpha = 0.05$.

Results

The Spearman test demonstrated a 98% of positive correlation between operators.

The chlorhexidine solution did not interfere with the penetration of the adhesive system to the demineralized dentine. In all of the samples occurred penetration of the adhesive systems on the demineralized dentine and the hybrid layer was clear and present throughout the sections analysed. The averages thicknesses and percentages of homogenous hybrid layer formation measured are presented in Table 2. Two-way ANOVA revealed no interaction between adhesive system and chlorhexidine ($p>0.05$). Tukey-test revealed a statistically significant difference among adhesive systems concerning the formation and the thickness of the hybrid layer ($p< 0.001$). The hybrid layer was significantly thicker for Group SB+ and SB- (4.37µm and 4.22 µm respectively). Groups SB+ and SB- showed the lowest percentage of homogenous hybrid layer formation at 33% and 40% respectively (Table 2 and Figure 1) when compared to PB+/PB- and SE+/SE-, that showed 100% of homogeneous hybrid layer formation.

Discussion

The results of this study show that the adhesive system as chlorhexidine solution did not influence the penetration of the adhesive system. However, adhesive systems significantly influence the formation and thickness of the hybrid layer in caries-affected dentine. Thus, the null hypothesis was partially accepted.

Imazato et al.,[15] suggested that the viscosity of the adhesive resin was increased by the addition of a large amount of MDPB, the concentration of MDPB incorporation was set at 5% as the maximum value. While the handling characteristics were not hampered, they speculate that an inferior infiltration of the resin into the primed dentine due to a slight increase in the viscosity could occur.

In this study, the CSLM evaluation showed that the increased in viscosity by MDPB[15] was not able to interfere the penetration of the adhesive system in caries affected dentine, as there was no difference between SE and PB. The SE adhesive system contains a similar composition to PB, except by the MDPB antibacterial monomer and fluoride that are present in PB. In all of the samples that used the adhesive system PB, which contains MDPB, a clear and homogeneous hybrid layer was verified. Also, the statistical analysis did not show statistically significant differences concerning the thickness of the hybrid layer between the PB and SE adhesive systems. These results are in accordance with those of Imazato et al.,[15] who suggested that the viscosity of the adhesive resin could be increased by the addition of a large amount of MDPB; however, the concentration of MDPB incorporation at PB was set at 5% as the maximum value, what does not interfere with the penetration of the adhesive system.

In addition, the incorporation of antibacterial components into an adhesive system (e.g. fluoride and MDPB) has been shown to exhibit an antibacterial effect against S *mutans* and also against other bacteria in human dentine carious lesion[17]. The adhesive system containing an antibacterial monomer MDPB compared with other adhesive systems or with disinfectant agents as chlorhexidine by using different microbiological methods, was able to inactivate the bacteria better than the other materials[33,34].

The results of this study indicate that the use of an MDPB-containing primer for the restoration of carious cavities could be beneficial as it does not negatively affect the formation of surrounding hybrid and resin tags and it can control residual bacteria [17].

This study showed that the treatment of the cavity with the disinfectant 2% chlorhexidine solution prior the adhesion procedure did not affect the penetration of the adhesive system to demineralized dentine and the formation of the hybrid layer. Chlorhexidine is an anti-septic with a large spectrum of action, and its use has become widespread during the last two decades for the chemical control of bacterial biofilms and the disinfection of cavities[1,16]. Earlier studies evaluating bond strength have shown that a 2% chlorhexidine solution applied on dentine surfaces prior to etching does not affect bond strength[12]. Besides Leonardo et al.,[21] demonstrated that CHX can be adsorbed to and be released from dentine for as long as 48 to 72 hours after endodontic preparation, this does not adversely affect monomer penetration into dentine and hybrid layer formation.

This result is consistent with the findings of Turkun et al.,[35] and Meiers & Kresin[23] who reported a positive effect of CHX on the bonding of self-etch adhesives and with Erdemir et al.[11] who observed the highest bond strength values of C&B Metabond (etch and rinse self cure composite) to root canal dentine with CHX irrigant.

The literature has demonstrated that the caries-affected dentine has lower bond strength values in comparison to sound dentine, independent of the type of adhesive system and technique used[26,28]. These results have been attributed to the lack of hybridization of monomers in the peritubular of caries-affected dentine; therefore, acid-resistant mineral deposits would diminish the elasticity modulus and the cohesive strength of the caries-affected dentine[38]. In this study, the analysis of CLSM in demineralized dentine substrate showed a hybridization and penetration of the adhesive system in all of the samples.

Concerning adhesive system types, SB exhibited the largest hybrid layer significantly thicker than PB and SE. In addition, no statistically significant difference was observed between the two self-etch adhesives systems. SB combines the primer and adhesive in one solution for application after etching the substrate with 35% to 37% phosphoric acid (pH=0.6 to 0.7) for 15-20 seconds[13]. Therefore, this adhesive system creates a micro-mechanical interlocking within the etched dentine by means of resin tags, adhesive lateral branches and hybrid layer formation. Compared with the total–etch adhesive system, self-etch adhesive systems as PB and the SE, have a primer with a mild potential for demineralization, pH=1.9 and pH=2.0 respectively. The weaker acidic primer in self-etch bonding systems results in less obvious change in the dentine wall structure than the stronger total etch systems. The primer demineralises dentine only up to a depth of 1 μm. Moreover, this superficial demineralization occurs only partially, which keeps residual hydroxyapatite still attached to the collagen[39]. These different patterns etching can interfere with the thickness of the smear layer, degree of demineralization and consequently with their bonding ability in the substrate[6]. The configuration of the different hybrid layers and resin tags is due to the different pH values of the primer and the etching procedure. Van Meerbeeck et al.,[39] subdivided the adhesive systems into “strong”, “intermediary strong” and “mild” according to their etching aggressiveness. SB (etching acid pH=0.6) is considered “strong”, while PB and SE (primer pH=1.9 and bond pH=2.8; primer pH=2.0 and bond pH=2.0) are considered to have “mild” aggressiveness. The more aggressive the adhesive, the greater the demineralization it causes, resulting in an increase in hybrid layer thickness.

Concerning the uniformity of the hybrid layer, the adhesive system SB accounted for the majority of not homogeneous hybrid layer and was statistically different from the other adhesive systems. Different from the self-etch systems (PB and SE), the SB is a total-etch adhesive system and has etching stage that is separate from the primer and adhesive. Also, the acid used in the etching stage is strong and causes a great deal of demineralization thus, the primer and adhesive could not penetrate into all the demineralized dentine resulting in some irregularity or the lack of a homogeneous hybrid layer, in addition to being larger than the others. A great advantage of self-etch adhesive systems is the homogeneous hybrid layer formation.

One of the primary advantages of using the CLSM for evaluating the penetration of the adhesive system as well as, the formation and the thickness of the hybrid layer of different bonding agents is that samples can be kept humid during the examination. As drying of the samples is not necessary, this leads to a decreased risk of shrinking artefacts. Furthermore, the subsurface can be analysed without destroying the specimen thus, preparation artefacts can also be excluded[3].

In this study the MDPB incorporated into dentine bonding primer/resin and the chlorhexidine did not cause an adverse effect on the penetration of the adhesive system on the demineralized dentin. The progression rate of secondary caries is an important factor that determines the longevity of restorations[30] thus, antibacterial monomers or disinfectants are appreciated to restorative dentistry and should be encouraged in the bonding procedure since progression rate of caries could be limited by these procedure increasing the durability of the restorations.

Conclusion

Treatment with a 2% chlorohexidine disinfectant or the use of different adhesive systems did not affect the penetration of the adhesive system. The formation and the thickness of the hybrid layer in demineralized dentine were influenced by the adhesive systems. A total-etch adhesive system provides a thicker hybrid layer and a lower percentage of homogenous hybrid layer formation than self-etching adhesive systems.

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Clinical Relevance

The previously application of 2% chlorhexidine or the use of antibacterial-containing adhesive systems did not affect the penetration of the adhesive system.

However the formation and the thickness of the hybrid layer are only affected by the adhesive systems in demineralized dentine. The total-etch adhesive system presented a thicker but with a lower percentage of homogeneous hybrid layer. This fact could influence the bonding degradation of resin/dentine interface.

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Table 1 – Description of adhesive system, composition, manufacturer, batch number, pH values and application technique of the materials used in this study.

Adhesive system	Composition	Manufacturer (Batch number)	pH value *	Application Technique **
Adper Single Bond 2 (SB)	Etching acid: Phosphoric acid 35% Primer/Bond:HEMA; Bis-GMA;dimethacrylates methacrylates; ethanol; water; UDMA, Bisphenol-A glycerolate, polyalkenoic acid copolymer canphorquinone	3M Dental Products, St. Paul, MN, USA (51202)	pH = 0.6 (etching acid) pH = 4.7 (primer + Bond)	a (15 s), b (30s), c, d, e, i (10 s)
Clearfil SE Protect (PB)	**Primer:** water, MDP, MDPB, HEMA, Hidrophilic dimethacrylate, **Bond:** MDP, HEMA, Bis-GMA, Hydrophobic dimethacrylate, di-Camphorquinone, N-Diethanol-p-toluidine,silanated colloidal silica, surface treated NaF	Kuraray Medical Inc, Kurashiki, Tokyo, Japan (Primer: 00047A) (Bond: 00072A)	pH = 1.9 (primer) pH = 2.8 (Bond)	f (20 s), e, g, i (10 s)
Clearfil SE Bond (SE)	**Primer:** water, MDP, HEMA, Hidrophilic dimethacrylate, N-Diethanol-p-toluidine, camphorquinone **Bond:** MDP, HEMA, Bis-GMA, Hidrophobic dimethacrylate, Camphorquinone N-Diethanol-p-toluidine, silanated colloidal silica	Kuraray Medical Inc, Kurashiki, Tokyo, Japan (Primer: 00830A) (Bond: 01212A)	pH = 2.0 (primer) pH = 2.0 (Bond	f (20 s), e, g, i (10 s)

*manufacturer's information

**Application technique: a: acid etch; b: rinse surface; c: remove excess moisture; d: apply one-bottle adhesive; e: gently air dry; f: apply self-etch primer; g: apply adhesive; h: apply mixture; i: photoactivate. MDP: 10-methacryloyloxydecyl dihydrogen phosphate; MDPB: 12-methacryloyloxydodecylpyridinium bromide; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol-A diglycidil ether dimethacrylate; UDMA: urethane dimethacrylate

Table 2 – Mean (μm) and standard deviation for the thicknesses and percentage of homogeneous formation of the hybrid layer measured by CLSM in demineralized-dentine.

Groups	Mean (μm) and Standard deviation	Homogeneous hybrid layer formation
PB+	1.61 ± 0.37 ^{A}	100 % ^{A}
PB-	1.58 ± 0.40 ^{A}	100 % ^{A}
SE+	1.89 ± 0.41 ^{A}	100 % ^{A}
SE-	2.00 ± 0.66 ^{A}	100 % ^{A}
SB+	4.37 ± 1.38 ^{B}	33 % ^{B}
SB-	4.22 ± 1.87 ^{B}	40 % ^{B}

Similar letters means no statistical significant difference.

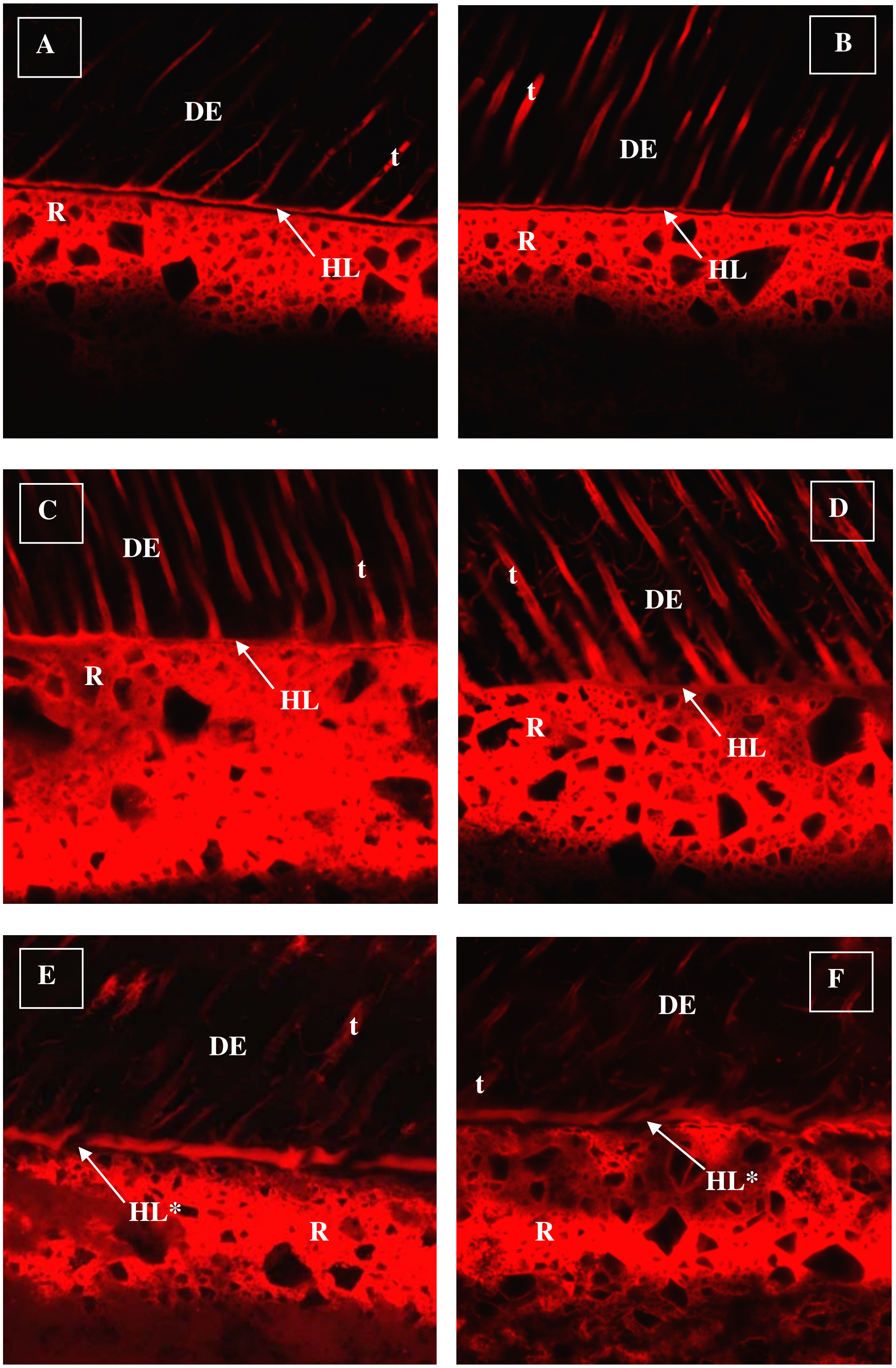

Figure 1

Figure Legends

Figure 1- CLSM images of penetration of dentine-bonding agents. (A) Clearfil SE Protect with chlorhexidine, (B) Clearfil SE Protect without chlorhexidine, (C) Clearfil SE Bond with chlorhexidine, (D) Clearfil SE Bond without chlorhexidine, (E) Adper Single Bond 2 with chlorhexidine and (F) Adper Single Bond 2 without chlorhexidine. Note the penetration of the primer into the dentinal tubules and a homogeneous and thin hybrid layer (HL) are visible in figures A, B, C and D. In figures E and F, a thick hybrid layer is visible, but it is not uniform (HL*). Resin tags – t; dentine- DE; composite resin –R.

CAPÍTULO 2

Influence of cavity disinfectant and adhesive systems on the bonding procedure in demineralized dentin – long term evaluation.

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Abstract

Objective: To evaluate the influence of a 2% chlorhexidine solution-CHX on the bond strength and nanoleakage of two self-etch adhesive systems in demineralized dentin by 12 months. **Methods***:* The middle dentin from sound third molars was exposed, and demineralized in vitro. Twelve groups were set using different adhesive systems (Clearfil SE Protect-PB and Clearfil SE Bond-SE,) dentin treatment (with or without the CHX application) and water-storage times (24h, 6 and 12 months). Composite resin completed the restorative procedure to produced specimens to microshear bond strength (μSBS) test and for nanoleakage. Data of μSBS were submitted to a three-way ANOVA and Tukey's test. The failure mode and nanoleakage was performed by descriptive analyses. **Results:** There was a statistically significant interaction only between the adhesive system and CHX, and adhesive system and water-storage times. SE showed the lowest μSBS just at 24h water-storage time regardless of CHX. A significant decrease in μSBS values after 6 months of water-storage occurred in all of the groups and was maintained until 12 months. Adhesive failure increased with storage time. All groups showed nanoleakage at the resin/dentin interfaces and an increased silver deposition was noticed after 6 and 12 months of water-storage time. The highest percentages of nanoleakage were found in CHX groups. **Significance:** CHX did not interfere with μSBS values for either self-etch adhesive systems, but water-storage interferes. Bond strength decreased for both adhesive systems after 6 and 12 months, regardless of CHX application. Nanoleakage increases with water-storage time and with CHX after water-storage time.

Keywords: micro-shear bond strength; chlorhexidine; adhesive systems; bonding degradation; nanoleakage; caries-affected dentin.

1. Introduction

The traditional approach of maximal intervention and invasiveness in oral care has been substituted by a contemporary approach of minimal intervention based on the retention of healthy tooth tissue and the use of modern adhesive systems. Thus, the bonding surface that is encountered after caries excavation consists of carious affect dentin (Erhardt et al., 2008). This zone of dentin shows a low mineral content that is lost during the caries lesion process and intratubular deposition of calcium phosphate crystals can also be found occluding the dentin tubule lumen, which prevents the resin monomer penetration (Marshal et al., 2001; van der Veen et al., 1996). These structural variations in caries-affected dentin may be a challenge in providing effective bonding (Tagami et al., 1992).

Bonding to dentin substrates, regardless of whether they consist of sound or caries affected tissues, is a complex procedure. There is a general consensus that resin-dentin bonds created by contemporary hydrophilic dentin adhesives deteriorate over time (Toledano et al., 2007). Thus, a gap on the bonding interface allows that bacterial can come down to the restoration (Shenalz et al., 2004) and sometimes caries lesion progression would be observed. Bacteria adversely left in cavity preparations can also survive for more than a year (Sharma et al., 2009).

Bacteria have been shown to proliferate from the smear layer even in the presence of a good seal from the oral cavity, allowing toxins to diffuse to the pulp and resulting in irritation and inflammation of pulpal tissues (Brannnstrom et al., 1986).

Adhesive restorations are based on the concept of micromechanical bonding (Van Meerbeek et al., 2001). In dentin, polymerized monomers interlock the exposed collagen network of the demineralized superficial dentin, forming the 'so-called' hybrid layer (Nakabayashi et al., 1992). The hybrid layer is responsible for improving the bonding between the dentin and the adhesive material (Ferrari et al., 2000). New adhesive systems have been developed in an attempt to reduce the steps, simplify clinical bonding procedures and improve the bonding quality, these are the self-etch adhesive systems. Their bonding mechanism is based on the simultaneous etching and priming of the smear-covered dentin using a acidic primer (Nakabayashi & Saimi, 1996), followed by the application of an adhesive resin, eliminating the separate acid-etching and rising steps, reducing its sensitivity and increasing the success rates of restorative procedures (Tay & Pashley, 2001). In addition, they would be able to penetrate into whole demineralized dentin, due to the simultaneous conditioning and monomer penetration, leaving less of the demineralized collagen network uncovered. Another benefit of the newer adhesive systems (Clearfill SE Protect) was the incorporation of an antibacterial monomer, 12-methacryloyloxydodecylpyridinium bromide (MDPB), which shows strong bactericidal activity before being polymerized (Imazato et al., 1999). Additionally, MDPB is immobilized in the polymer network, and the cured resin containing MDPB has been demonstrated to exhibit an inhibitory effect on the growth of bacteria on its surface (Imazato et al., 1998). It would exert a potential benefit in terms of caries prevention if imbibed into demineralized dentin (Imazato et al., 2002). This is especially important in the minimal intervention technique, which removes only the softer, highly contaminated and irreversibly demineralized carious dentin, whereas preserving the affected dentin (Tyas et al, 2000). It would present only sparse amounts of bacteria in the dentin tubules

(Meiers & Kresin, 1996) and inhibit invading bacteria at the resin-dentin interfaces after the placement of a restoration (Yildirim et al, 2008).

An alternative method for the use of restorative materials free of antibacterial characteristics at caries-affected dentin is the use of an antibacterial agent used as a cavity cleaner disinfectant. The most common agent is the 2% chlorhexidine solution (CHX) (Ersin et al., 2006). Studies in vitro are controversial regarding the use of disinfectants prior to adhesion procedures. Previous studies demonstrated that chlorhexidine application prior to acid-etching has no adverse effects on immediate composite-adhesive bonds in dentin (el-Housseiny & Jamjoum, 2000; de Castro et al., 2003). However, Tulunoglu et al (1998) found that chlorhexidine-containing cavity disinfectants increased microleakage scores when used prior to the implementation of Syntac and Prime&Bond dentin adhesive systems.

Because caries-affected dentin constitutes a significant fraction of the surface area of the majority cavity preparations (Erhardt et al., 2008), and the simultaneous use of an antibacterial monomer or cavity disinfectant in immediate and long-term bond strength evaluations are necessary to uncover the degradation mechanisms of this pathologically altered substrate, the aim of this study was to analyse the influence of an antibacterial monomer, a cavity disinfectant and water-storage times on bonding degradation and nanoleakage in human demineralized dentin using a microshear bond strength test (μSBS) and nanoleakage in an in vitro study. The failure modes and bonded interfaces were evaluated with a scanning electron microscope (SEM). The null hypothesis tested was that the water-storage time, the adhesive system and neither of the cavity disinfectant affects μSBS and microstructure, failure site types and nanoleakage of the resin/demineralized dentin interfaces.

2. Material and Methods

This study was conducted after approval from the Ethical Committee of Piracicaba Dental School, University of Campinas (Protocol 083/2005). Sound human molars were donated by patients from the clinic of Piracicaba Dental School, State University of Campinas.

2.1 Demineralized dentin preparation

One hundred and twenty sound human third molars were obtained with informed consent from patients under a protocol approved by the Ethical Committee of Piracicaba Dental School, University of Campinas. They were cleaned with periodontal scales and pumice slurry in a Robinson brush, and stored in a saline solution at 4º C in a 0.9% NaCl supplemented with 0.02% sodium azide for no more than two months after extraction. The middle dentin was determined in each individual tooth by the bitewing x-ray technique. The site located half the distance from the enamel-dentin junction to the pulp was determined as the middle dentin and marked with a pen in each tooth. Occlusal portions were sectioned at that point (Isomet, Buheler, Lake Bluff, IL, USA). The roots were cut at the cementoenamel junctions and discarded. Middle dentin blocks were coated with a red acid-resistant nail varnish (Colorama; CEIL Com. Exp. Ind. Ltda., São Paulo, SP, Brazil), except for the coronal dentin area. Middle dentin blocks were immersed in 5 mL of 6% carboxymethylcellulose acid gel (Proderma Pharmacy, Piracicaba, SP, Brazil) at pH 5.0 and 37ºC. The gel contained 0.1 M lactic acid titrated to pH 5.0 with a concentrated KOH solution (Van der Veen & ten Bosch, 1996). The specimens remained in the gel for 24 hours without renewal. By this demineralization procedure, the exposed surfaces exhibited signs of dentin-like carious lesions with wet and soft surfaces. This model produced a caries depth of 40 μm on average, confirmed in a pilot study involving of polarized light microscopy. After artificial caries development, specimens were washed two times in an ultrasound bath with distilled water for fifteen minutes each and dried with tissue paper.

2.2. Microshear bond strength test

Sixty demineralized middle dentin blocks were randomly divided into 12 groups (n=5) according to the adhesive system, the application of 2% chlorhexidine solution (CHX) in the substrate and storage time in distilled water (Table1). Brand names, main components, manufacturers, batch numbers, pH values and application methods of the adhesive systems are shown in Table 2. Charisma, light-cured composite resin, shade A3.5 (Heraeus Kulzer, Werheim, Germany – batch # 010201) was used to complete the bonding procedure of μSBS and nanoleakage. Immediately before the restorative procedure, the smear layer was standardized using a 600-grit abrasive paper for 60 seconds. Then, in the Groups of 3, 4, 7, 8, 11 and 12, a 2% chlorhexidine solution (FGM Joinvile Santa Catarina, Brazil) drop was placed on the demineralized surface, and was maintained undisturbed as a therapeutic primer and gently blot-dried after a dwell time of 60 seconds (Komori et al., 2009). The adhesive systems were applied as recommended by the manufactur instructions. The method for μSBS using silicon moulds was previously described by Moraes et al., (2008). Standardized elastomer moulds (Speedex, Vigodent SA, Brazil) with three cylinder-shape orifices (0.8 mm diameter) were used to obtain specimens for the μSBS. The composite resin Charisma shade A3.5 (Heraeus Kulzer, Werheim, Germany – batch # 010201) was light-cured for 20 seconds with Elipar Tri-light unit (3M ESPE – America Co., Seefeld 82229 -Germany). The output irradiance of the curing unit was periodically measured (470 mW/cm^2). After storing the samples in distilled at 37°C for 24h, the elastomer mould was removed by gently cutting it using a feather blade. All resin cylinders were checked with a stereomicroscope (Model XLT30, Nova Optical Systems, SP, Brazil) at 40X magnification to check for samples presenting flaws, irregularities or bonding defects. Specimens with defects were eliminated. The cross-sectional area was verified with an electronic digital calliper (Mitutoyo, Japan), to obtain resin cylinders with a cross-sectional surface area of about 1.0 mm^2. According the storage time, each group was submitted to μSBS. A thin steel wire (0.2 mm in diameter) was looped around each cylinder and aligned with the bonding interface. The test was conducted in a universal testing machine (Instron model 4411, Canton, MA, USA) at a cross-head speed of 0.5 mm/min until failure. The bond strength values were calculated in MPa. For each group, five teeth were tested and the average value of three resin cylinders was recorded as the bond strength for each specimen. All of the fractured specimens were mounted on aluminium stubs, sputter-coated with gold (Balzers-SCD 050 Sputter Coater, Liechtenstein), and examined under SEM (JEOL-JSM 5600LV, Tokyo, Japan) at 80X and 450X magnification. Modes of failure were classified as cohesive (in dentin -CD or resin -CR), adhesive (A) or mixed failure (M) (Figure 1).

2.3. Interfacial nanoleakage

Sixty teeth were used for the interfacial nanoleakage. They were randomly divided into 12 groups, according to the adhesive system, the application of 2%

chlorhexidine solution (CHX) in the substrate and storage time in distilled water (Table 1). For the nanoleakage specimens, the occlusal portions were cut (Figure 2a). Hence, the caries-affected dentin that was previously described in this study was produced in the two adjacent middle dentin exposed surfaces. The same adhesive systems were applied on these two adjacent surfaces (Figure 2a). Immediately before the restorative procedure, the smear layer was standardized and in the Groups of 3, 4, 7, 8, 11 and 12 a 2% chlorhexidine solution was applied as previously described. A resin sandwich of dentin/resin/dentin (Figure 2b) was accomplished by filling the two adjacent surfaces of middle dentin in each tooth with a Charisma, light-cured composite resin, shade A3.5 (Heraeus Kulzer, Werheim, Germany – batch # 010201) that was light–cured for 20s in each surface (mesio, distal, buccal and lingual). After 24 h, each set (the sandwich of dentin/resin/dentin) was mesio-distally (Figure 2c) sectioned (Isomet, Buheler, Lake Bluff, IL, USA) under water lubrication producing 0.8 mm thick slices (Figure 2d). The slices were immersed in buffered distilled water and stored according to the groups for different storage times at 37º C. The distilled water was weekly changed (Fraga et al., 1997). One dentin/resin/dentin specimen from each tooth from each experimental group was used for the nanoleakage evaluation. Slices were coated with two layers of a red acid-resistant nail varnish (Colorama; CEIL Com. Exp. Ind. Ltda., São Paulo, SP, Brazil) applied up to 1 mm from the bonded interfaces. They were immersed in distilled water for 20 min prior to immersion in the tracer solution in total darkness for 24 h. An ammoniacal silver nitrate solution was prepared by a dissolution of 25 g of silver nitrate crystals (Sigma Chemical Co., St. Louis, MO, USA) in 50 mL of distilled water and 50 mL of 28% ammonium hydroxide (Sigma) yielding a solution of pH = 11.0. Then, the slices were thoroughly rinsed in distilled water and immersed in a photodeveloping solution for 8h under a fluorescent light. Again, the slices were thoroughly rinsed in distilled water and one side of each slice was polished using a water-cooled mechanical grinder (Metaserv 2000, Buehler, UK LTD, Lake Bluff, IL, USA) using 600-, 1200-and 2000-grit silicon carbide abrasive papers (Carbimet Disc Set, # 305178180, Buehler, UK LTD) and with a 3-µm and a 1-µm diamond paste with cloth. Specimens were then demineralized with 37% phosphoric acid for 5 s, washed with distilled water for 30 s, and dried with a tissue paper. Following, they were deproteinized with 10% NaOCl for 5 min, washed in an ultrasound bath, dried with tissue paper and left to dry for 24h at room temperature. Finally, the prepared slices were mounted on aluminium stubs, sputtercoated with carbon (Balzers-SCD 050 Sputter Coater, Liechtenstein) and examined with a SEM (JEOL-JSM 5600LV, Tokyo, Japan) under magnifications of 300X and 800X. A single reading in the two dentin/resin interfaces of the resin sandwich was carried out for each slice (n=10). The penetration of silver into the adhesive dentin interface, hybrid layer and adhesive layer was evaluated via descriptive analysis and scored as: no leakage, score 0; leakage only in the base of the hybrid layer and around resin tags, score 1; leakage inside the hybrid layer and/or the adhesive, score 2 (Figure 3).

2.4. Statistical analysis

Bond strength data were analysed with three-way ANOVA and Tukey's post hoc tests ($p<0.05$). The data from the nanoleakage of resin-dentin interfaces and failure modes data were used for descriptive analyses.

3. Results

Concerning µSBS, a three-way ANOVA test showed no significantly statistical interaction among the three factors studied. A three-way ANOVA revealed a significant interaction between CHX application and the adhesive system (F=0.0008, p=0.04491). The means of µSBS values and standard deviations, percentage scores for the failure mode and nanoleakage scores for all the groups are shown in Table 5. Both adhesive systems showed similar µSBS values when used with or without chlorhexidine (Table 3). A significant interaction between the water-storage times and adhesive systems was found (F=4.6031, p=0.01483). After 24h of water-storage time, SE showed lower µSBS values than PB. However, SE and PB showed similar µSBS values after 6 and 12 months of water-storage time. These results were significantly lower than those founded after 24h of water-storage time (Table 4). At 24h of water-storage time, the failures were predominantly adhesive and mixed, and in 6 and 12 months of storage time the failures were predominantly adhesive. Cohesive failures in the material were not observed in any group (Table 5). Only four (3.33%) from a total of 120 interface resin/dentin samples were not evaluated because a gap was observed; these specimens were excluded from the experiment. All groups showed nanoleakage at the resin/dentin interfaces. An increase in silver deposition could be noticed after 6 months of water-storage time. The score 0 was the most frequently reported after 24 hours. The score 1 was found in all storage periods indistinctly. In addition, for groups with 6 and 12 months of water-storage time, the score 2 was the most frequently observed in the CHX groups (Table 5 and Figure 4).

4. Discussion

This study measured the bond strength and nanoleakage of two adhesives systems in demineralized-dentin associated with previous CHX application over twelve months of water-storage time.

Although ours results indicated that μSBS values of SE were significantly lower than those of PB after 24h, this difference disappeared after 6 months of water-storage time. This was seen when the two self-etch adhesive systems showed significantly decreased μSBS values that were maintained until 12 months of water-storage time. These results agree with previous studies that used self-etch adhesives systems (Ansari et al., 2008; Erhardt et al., 2008; De Munk et al., 2009). Therefore, the tested null hypothesis was partially rejected.

According to the manufacturer, SE and PB present similar compositions with the exception, mainly, of the MDPB and sodium fluoride in PB. MDPB contains C=C bonds that are capable of undergoing a free radical polymerization (Ansari et al., 2008). However, MDPB would be immobilized within the polymer network and would not induce weakness or degradation in the bonding layer through dissolution and substitution by water (Imazato et al., 2006) not interfering, in this way, with the decrease in bond strength.

No statistically significant difference concerning μSBS values between the groups with or without previous CHX application was observed, corroborating the findings of De Munk et al., (2009) that used self-etch adhesive systems. However, they used sound-dentin and a μTBS test. In addition, our results are in accordance with previous studies that used total etching adhesive systems in caries-affected dentin with previously CHX application (Komori et al., 2009). The literature lacked studies with self-etch adhesive system in caries-affected dentin with previous CHX application. Also, there was no consensus of bond strength using self-etch adhesive systems with previous CHX application in sound dentin. De Castro et al., (2003) found that CHX does not interfere with the bond strength of a self-etch adhesive, although Campos et al., (2009) and Ercan et al., (2009) found that CHX application interfere with bond strength when used with self-etch adhesive systems. However these studies were performed in sound dentin only. Hiraishi et al., (2009) verified that the previous treatment of sound-dentin with CHX showed a negative effect in the bond strength of two luting cement (Panavia F, Kuraray Medical Inc; RelyX Unicem, 3M ESPE).

The susceptibility of resin components to hydrolysis has been identified as a cause for decreased of bond strength (Ansari et al., 2008). Contemporary simplified adhesives present an increase in the concentration of ionic and polar comonomers that make them more hydrophilic and results in increased water absorption (Malacarne et al., 2006). Concerning bond strength degradation, it is expected to occur more expressively in caries-affected dentin than in sound dentin (Erhardt et al., 2008). The collagenolytic activity exhibited by unbounded, partially demineralized human dentin, in the absence of bacteria, is associated with the morphological disintegration of dentinal collagen fibrils (Pashley et al., 2004). Compared with sound dentin, the prevalence of exposed unprotected collagen fibrils within the hybrid layers formed in caries-affected dentin may be greater (Haj-Ali et al., 2006). Thus greater collagenolytic activity may be expected in caries-affected dentin. These features would explain the decrease in μSBS values and the increase in nanoleakage leves observed in this study.

In this study, the CHX did not prevent nanoleakage and higher levels of nanoleakage were observed in the groups with previous CHX application, corroborating a study by Tulunoglu et al., (1998). For the nanoleakage analysis, two main methods exist for silver impregnation in collagen fibrils displayed in the hybrid layer: one using silver metenamina and another using a silver nitrate solution. The silver metenamina technique was initially developed to visualize the derived from carbohydrates in animal tissues. The mechanism of action of this technique is specific to the pigment collagen fibrils displayed in hypo- or un-mineralized areas of the dental substrate without the necessity of the dehydration of the samples (De Goes & Montes, 2004). In the technique with silver nitrate solution, the concentration of this element is 50% of the volume with a pH of 3.4 in the conventional version, which provides enough acidic characteristics able to demineralize the dentin. In this way, crystal dissolution of apatite and remaining of calcium phosphate amorphous in the interface of the self-etch adhesive systems after the immersion for 24 hours in the solution (Pereira et al., 2001) could compromise the veracity of the results (De Goes & Montes, 2004).

The current study presented a modification in the methodology preparation of the silver nitrate solution described by Tay et al., (2002). We used a simplified methodology, excluding the titration stage and pH adjustment. This provided a new and easier method of ammoniacal silver nitrate solution preparation used for the nanoleakage analysis, and was verified in a pilot study to have no differences between the two solutions with different pH.

The distrust regarding the use of silver nitrate solutions was dismissed by Tay et al., (2002). They tested the hypothesis of the use of a basic version (pH = 9.5) and verified that for self-etch adhesive systems, there was no difference in the use of the conventional version (acid, pH=3.4) versus the ammoniac one (basic, pH=9.5). They also verified that nanoleakage in resin/dentin interface was independent of the acidity of the solution.

The presence of silver uptake along the hybrid layer, or in the final length of the resin tags, and the increase in silver deposition after 6 and 12 months of evaluation, are in accordance with Erhardt et al., (2008). Concerning the greater nanoleakage observed in the groups with previous CHX application, Tulunoglu et. al., (1998) verified in a in vivo study using adhesives systems in non-carious primary teeth that the groups with previous CHX application presented higher microleakage than those without CHX. They stated that cavity disinfectants alter the ability of the hydrophilic resin to seal the dentin in a negative interaction. In addition, self destruction via the release of endogenous matrix metalloproteinases (MMPs) was identified as a cause of the reduction in bond strength and increase in nanoleakage (Osorio et al., 2002). CHX is an effective synthetic MMP-inhibitor that can directly inhibit the collagenolytic activity of MMP-2, -8 and -9 (Gendron et al., 1999), founded in human dentin (Martin-De Las Heras et al., 2000). The inhibitory effect of CHX on MMPs is thought to be related to a cation-chelating mechanism, wherein the sequestration of metal ions such as calcium and zinc would hamper the activation of the catalytic domains within MMPs (Hannas et al., 2007). However, De Munk et al., (2009) conclude in their study that MMP inhibitors appeared to be effective in reducing bond degradation only for the etch & rinse adhesive, not for the self-etch adhesive. They stated that perhaps the acid treatment with 37% phosphoric acid for 15-20 seconds used in total-etch adhesives systems facilitates the release of the enzymes because of their aggressive potential of demineralization (pH=0.3) (Van Meerbeeck et al., 2001) whereas the mild potential demineralization (primer pH=1.9 and bond pH=2.8; primer pH=2.0 and bond pH=2.0 PB and SE, respectively) (Van Meerbeeck et al., 2001) self-etch adhesives were not able to release the enzymes. Also, they stated that when CHX application was not rinsed off, the resultant concentrations in the hybrid layer are more significantly affected the stability of the hybrid layer. The amphipathic properties of CHX may interfere with resin infiltration, or its cation-chelating properties might interact with calcium salts remaining from acid etching, because these are known to prevent the chelation-mediated inhibition of a low-concentration CHX solution (Gendron et al., 1999). Water absorption of adhesive interfaces most likely remains the principal mechanism of bond degradation, whereas endogenous enzymes appear to contribute to bond degradation in only etch & rinse adhesives (De Munk et al., 2009).

Concerning failure mode analysis, it was showed, in general for all groups, more adhesive failures and a low percentage of cohesive failures. Such results are possibly explained by the high viscosity of the self-etch adhesive systems (Moraes et al., 2008) that might have interfered with the wettability to the bonding system surface and also because carious surfaces present a modified substrate that are difficult for resin monomers penetration (Yoshyama et al., 2003; Nakajima et al., 2005). Ours results indicated that previous treatment of a cavity with CHX did not interfere with μSBS values, but did have a negative effect in nanoleakage after water-storage time.

The antibacterial monomer incorporated to the self-etch adhesive system did not hinder nanoleakage and μSBS even after 6 months of water-storage time. Water-storage time hindered nanoleakage and μSBS, and increases the adhesive failures.

Because the reduction of microorganisms on cavities is appreciative, other studies with cavity disinfectants that can exert their function without inferring with adhesive procedures are necessary.

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Table 1 –Distribution of groups regarding the adhesive system, CHX application and water-storage time.

CHX application for 60 seconds	Water-storage time	Adhesive system	
		Clearfil SE Bond	Clearfil Protect Bond
No	24 h	G1	G2
Yes	24 h	G3	G4
No	6 months	G5	G6
Yes	6 months	G7	G8
No	12 months	G9	G10
Yes	12 months	G11	G12

Table 2 – Description of Adhesive System, Composition, Manufacturer and batch number, pH values and Application Technique of the materials used in this study.

Adhesive System/ composite resin	Composition	Manufacturer (Batch number)	pH value *	Application Technique **
Clearfil SE Protect (PB)	**Primer:** water, MDP, MDPB, HEMA, Hidrophilic dimethacrylate, **Bond:** MDP, HEMA, Bis-GMA, Hydrophobic dimethacrylate, di-Camphorquinone, N-Diethanol-p-toluidine,silanated colloidal silica, surface treated NaF	Kuraray Medical Inc, Kurashiki, Tokyo, Japan (Primer: 00047A) (Bond: 00072A)	pH = 1.9 (primer) pH = 2.8 (Bond)	a(20 s), b, c d (10 s)
Clearfil SE Bond (SE)	**Primer:** water, MDP, HEMA, Hidrophilic dimethacrylate, N-Diethanol-p-toluidine, camphorquinone **Bond:** MDP, HEMA, Bis-GMA, Hidrophobic dimethacrylate, Camphorquinone N-Diethanol-p-toluidine, silanated colloidal silica	Kuraray Medical Inc, Kurashiki, Tokyo, Japan (Primer: 00830A) (Bond: 01212A)	pH = 2.0 (primer) pH = 2.0 (Bond	a(20 s), b, c d (10 s)
Charisma light-cured composite resin	—	Heraeus Kulzer, Werheim, Germany – batch # 010201	—	d (20 s)

*manufacturer's information

**Application technique: a: apply self-etch primer; b: gently air dry; c: apply adhesive; d: photoactivate.

MDP: 10-methacryloyloxydecyl dihydrogen phosphate; MDPB: 12-methacryloyloxydodecylpyridinium bromide; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol-A diglycidil ether dimethacrylate; UDMA: urethane dimethacrylate

Table 3 - Microshear bond strength values (MPa) in caries-affected dentin with regard to CHX application and adhesive systems.

Adhesive systems	CHX application	
	No	Yes
Clearfil Protect Bond	6.55 ^{Aa}	6.67 ^{Aa}
Clearfil SE Bond	5.53 ^{Aa}	5.67 ^{Aa}

Similar capital letters means no statistical significant differences between averages of numbers in the same row.

Similar small letters means no statistical significant differences among averages of numbers in the same column.

Table 4 - Microshear bond strength values (MPa) in caries-affected dentin with regard to adhesive systems and different storage times.

Adhesive systems	Storage time		
	24h	6 months	12 months
Clearfil Protect Bond	15.42 ^{Aa}	2.70 ^{Ba}	1.72 ^{Ba}
Clearfil SE Bond	12.34 ^{Ab}	2.91 ^{Ba}	1.56 ^{Ba}

Similar capital letters means no statistical significant differences between averages of numbers in the same row.

Similar small letters means no statistical significant differences among averages of numbers in the same column.

Table 5 – Means (standart deviation) for bonding strength to dentin, and percentage of scores for the failure modes and nanoleakage scores with regard to the adhesive system, CHX application and water-storage time.

Group	Bond Strength n= 5 (MPa)	Failure modes (%) n= 15			Nanoleakage scores* (%)		
		Adhesive	Mixed	Cohesive in dentin	Score 0	Score 1	Score 2
G1	12.28 (2.91)	27	37	36	11	78	11 n=10
G2	16.24 (2.71)	55	45	0	11	78	11 n=8
G3	12.39 (2.37)	30	60	10	10	90	0 n=10
G4	14.60 (3.65)	38	54	8	0	90	10 n=8
G5	2.95 (0.77)	75	17	8	20	60	20 n=10
G6	2.32 (0.60)	70	30	0	0	70	30 n=10
G7	2.88 (1.30)	100	0	0	0	44	56 n=8
G8	3.09 (0.92)	90	10	0	0	50	50 n=10
G9	1.36 (0.22)	63	0	37	0	70	30 n=10
G10	1.11 (0.59)	80	0	20	0	50	50 n=10
G11	1.76 (0.35)	67	22	11	0	44	56 n=10
G12	2.34 (0.76)	91	0	9	0	44	56 n=8

* no leakage, score 0; leakage just in the base of hybrid layer and around resin tags, score 1; leakage inside hybrid layer and/or adhesive, score 2

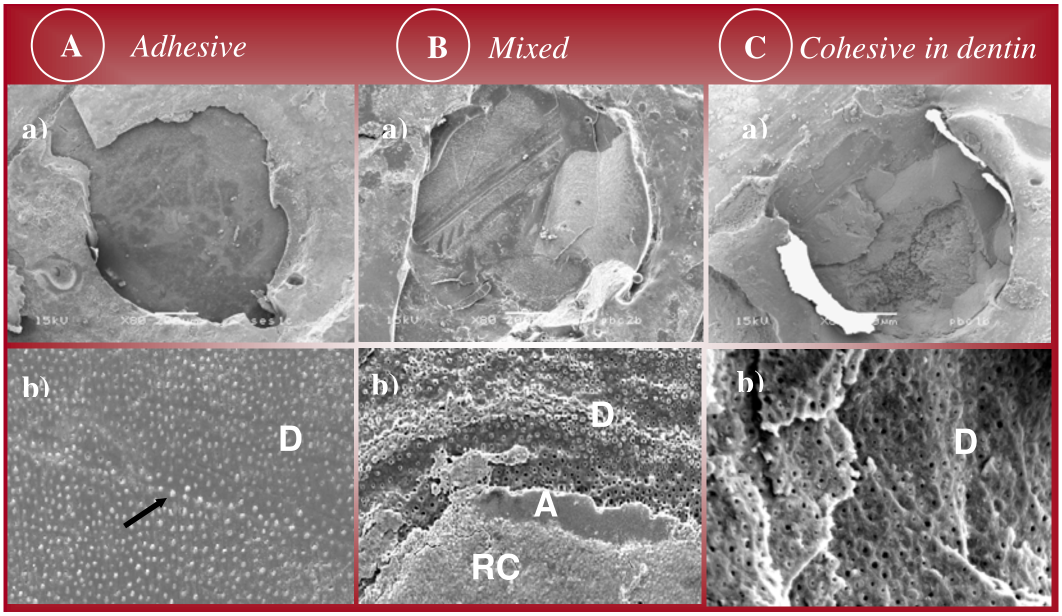


Figure 1.

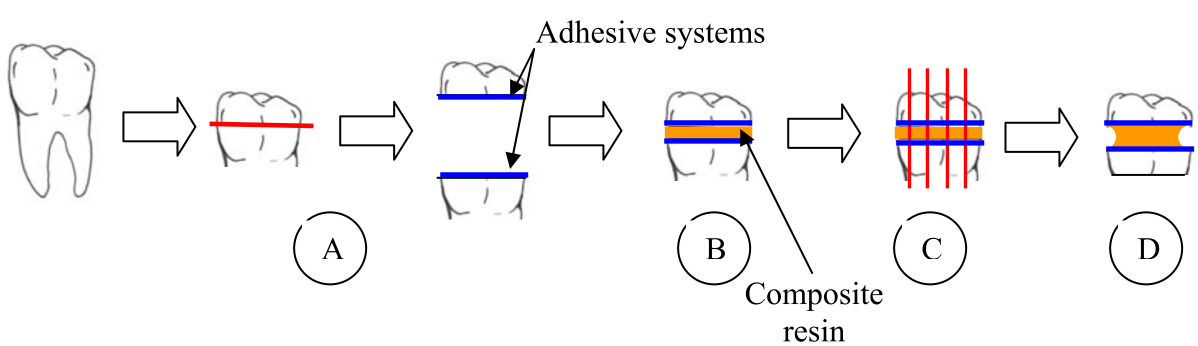


Figure 2.

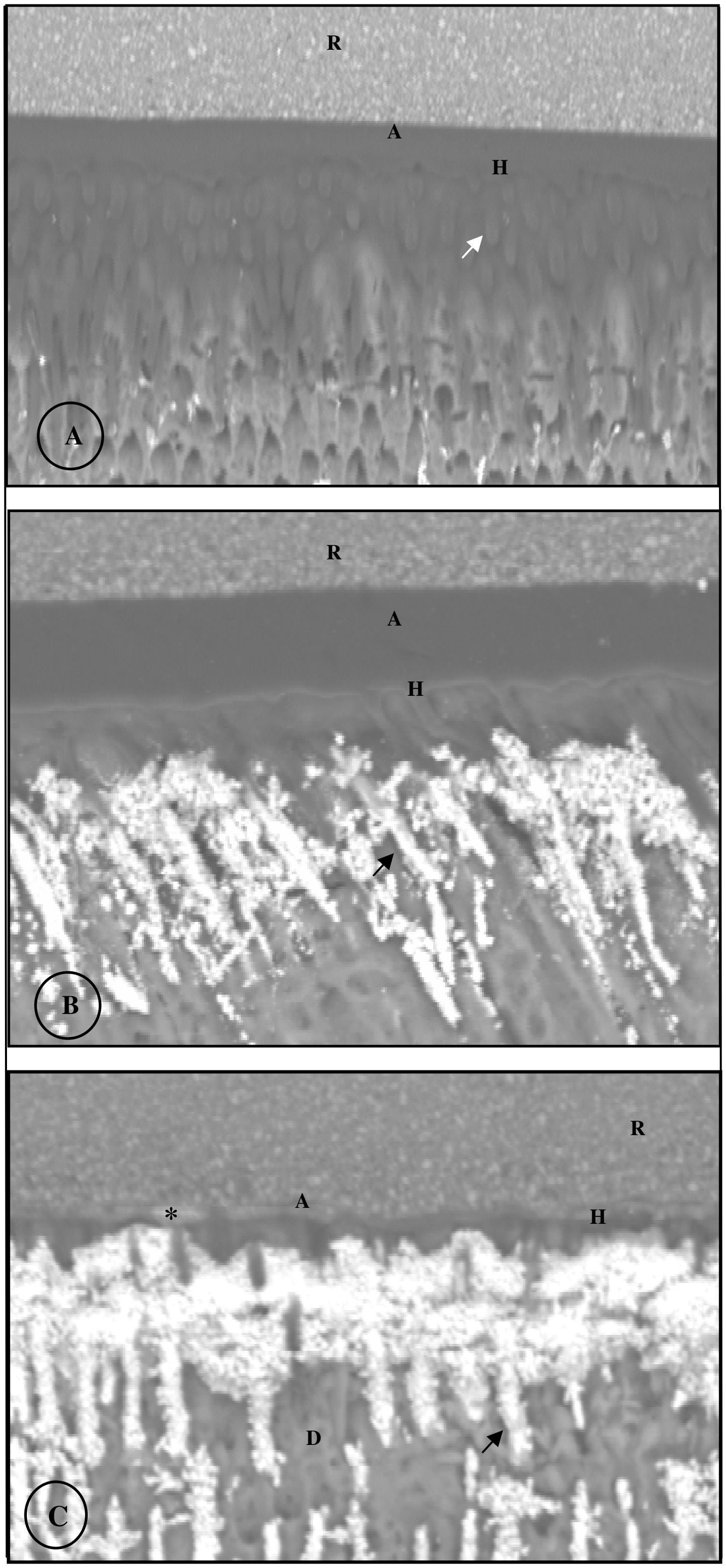
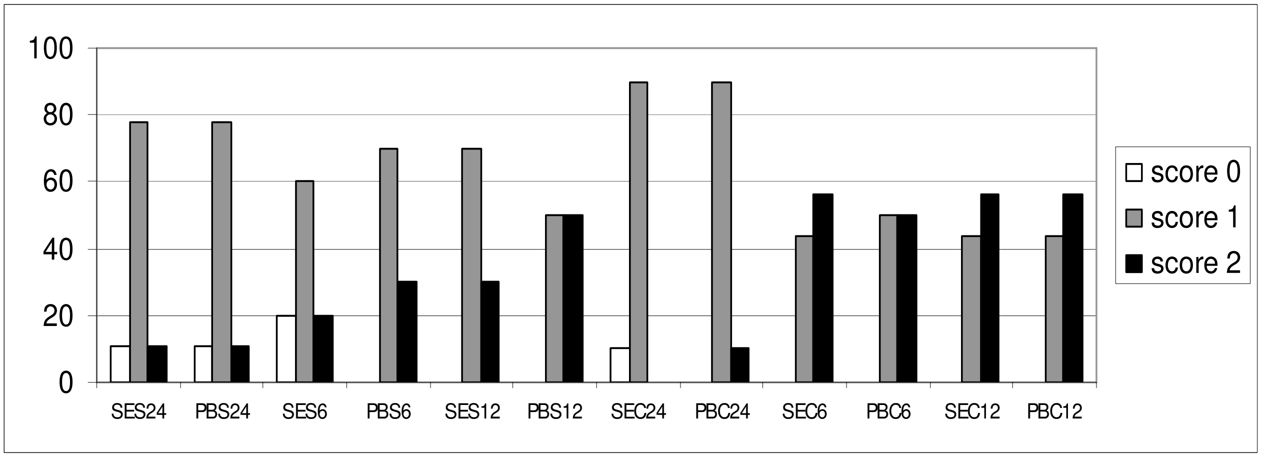


Figure 3.



100
80
60
40
20
0
SES24
PBS24
SES6
PBS6
SES12
PBS12
SEC24
PBC24
SEC6
PBC6
SEC12
PBC12
score 0
score 1
score 2

Figure 4.

Figure 1 – a) SEM electromicrography 80x magnification; **b)** higher magnification (450x). R- composite resin (material); D – dentin. **A** – Adhesive failure under hybrid layer. Note the plane surface with opened tubules; some of them with remain full of adhesive (arrow). **B** – Mixed failure. Note that three different layers could be seen: dentin, adhesive system and composite resin. **C -** Cohesive failure in dentin. Note the dentin layer in different levels.

Figure 2 – Resin sandwich block of dentin/resin/dentin and mesio-distal section of slices for nanoleakage analysis. **a)** middle dentin exposed; **b)** resin sandwich block of dentin/resin/dentin; **c)** mesio-distal sections; **d)** slice of dentin/resin/dentin.

Figure 3 – Stereomicroscopy of 800X magnification. Nanoleakage scores: **a)** no leakage, score 0; **b)** leakage just in the base of hybrid layer and around resin tags, score 1; **c)** leakage inside the hybrid layer and/or the adhesive, score 2. R – composite resin; A – adhesive; D – dentine; H – hybrid layer; resin tag free of silver (white arrow) and covered with silver (black arrow). Silver in hybrid layer (asterisk).

Figure 4 – Percentage of nanoleakage in demineralized-dentin with or without previous CHX application during 12 months of water-storage time. Clearfil SE Bond with no CHX application after 24 hours water-storage time-SES24; Clearfil Protect Bond with no CHX application 24 hours water-storage time-PBS24; Clearfil SE Bond with no CHX application 6 months water-storage time-SES6; Clearfil Protect Bond with no CHX application 6 months water-storage time-PBS6; Clearfil SE Bond with no CHX application 12 months water-storage time-SES12; Clearfil Protect Bond with no CHX application 12 months water-storage time-PBS12; Clearfil SE Bond with CHX application 24 hours water-storage time-SEC24; Clearfil Protect Bond with CHX application 24 hours water-storage time-PBC24; Clearfil SE Bond with CHX application 6 month water-storage time-SEC6; Clearfil Protect Bond with CHX application 6 months water-storage time-PBC6; Clearfil SE Bond with CHX application 12 months water-storag time-SEC12; Clearfil Protect Bond with CHX application 12 months water-storage time-PBC12.

CONCLUSÃO GERAL

Baseado nos resultados obtidos e considerando as limitações destes estudos, concluiu-se que:

1. A clorexidina e o MDPB não afetaram a penetração do sistema adesivo na dentina desmineralizada.
2. O tipo de sistema adesivo influencia a espessura e homogeneidade da camada híbrida: Adper Single Bond 2 apresentou a camada híbrida mais espessa, entretanto, a menos homogênea.
3. O sistema adesivo é um importante fator a ser considerado quando é testada a adesão em dentina desmineralizada, após 24 h de armazenamento: o sistema adesivo Clearfil SE Protect produziu os maiores valores de resistência de união, não havendo diferença entre os sistemas adesivos após 6 e 12 meses de armazenamento.
4. Os valores de resistência de união na dentina desmineralizada não foram afetados pela aplicação prévia da clorexidina, independente do sistema adesivo.
5. A clorexidina apresentou efeito negativo na infiltração dos grânulos de prata e foi incapaz de inibir a nanoinfiltração e a sua progressão, assim como o decréscimo na resistência de união.
6. O MDPB não apresentou efeito negativo na infiltração dos grânulos de prata, mas foi incapaz de inibir a nanoinfiltração e a sua progressão, assim como o decréscimo na resistência de união.

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[2] De acordo com a norma da FOP/ UNICAMP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o MEDLINE.

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COMITÊ DE ÉTICA EM PESQUISA
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CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Interação molecular da dentina decídua e permanente frente a diferentes tratamentos. Análise química, morfológica, micro-mecânica e estudo da interface sistemas de união/dentina"**, protocolo nº 083/2005, dos pesquisadores Ana Flávia Sanches Borges, Patricia Almada Sacramento e Regina Maria Puppin Rontani, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 22/10/2008.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Molecular Interaction of deciduous and permanent dentin submitted to different treatments. Chemical, morphological, micro-mechanical analysis and adhesive systems/dentin interface study"**, register number 083/2005, of Ana Flávia Sanches Borges, Patricia Almada Sacramento and Regina Maria Puppin Rontani, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 10/22/2008.

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

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

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

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Uso de agentes antibacterianos na dentina afetada por cárie. Análises biológica e da morfologia da interface de união"**, protocolo nº 072/2007, dos pesquisadores éfani Caroline de Freitas Banzi e Regina Maria Puppin Rontani, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 10/10/2007.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Use of antibacterial agents in the affected dentine for caries. The bonding interface morphology and biological analysis"**, register number 072/2007, of éfani Caroline de Freitas Banzi and Regina Maria Puppin Rontani, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 10/10/2007.

Cínthia Machado Tabchoury

Profa. Dra. Cínthia Pereira Machado Tabchoury
Secretária
CEP/FOP/UNICAMP

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

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
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- <u>Em formato alternativo</u>:

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Folha de rosto (primeira folha interna)

Ficha catalográfica (verso da folha de rosto)

Folha de aprovação

Dedicatória (Opcional)

Agradecimentos (Opcional)

Epígrafe (Opcional)

Resumo

Abstract

Lista de Abreviaturas e Siglas (Opcional)

Sumário

Introdução (trata-se, como no formato tradicional, da parte inicial do texto, da formulação clara e simples do tema investigado, constando a delimitação do assunto tratado, sua justificativa e objetivos da pesquisa)

Capítulos (devem ser inseridas as cópias de artigos de autoria ou co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem. Cada capítulo deve conter sua indicação, seguido do número (em arábico) correspondente. Ex.: Capítulo1, Capítulo 2 e assim sucessivamente)

Discussão ou Considerações Gerais (de caráter <u>opcional</u>, esta parte poderá conter argumentos para estabelecer relações entre os artigos apresentados nos capítulos)

Conclusão

Referências (não devem ser inseridas as referências já relacionadas nos trabalhos apresentados nos capítulos)

Bibliografia (Opcional)

Glossário (Opcional)

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Anexo (Opcional)

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