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"AVALIAÇÃO DA RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS À DENTINA DESPROTEINIZADA ATRAVÉS DO USO DE HIPOCLORITO DE SÓDIO"

Tese apresentada à Faculdade de Odontologia de Piracicaba – UNICAMP, para a obtenção do título de DOUTOR em CLÍNICA ODONTOLÓGICA – Área de Concentração DENTÍSTICA

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PIRACICABA

- 2008 -

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA
Bibliotecária: Marilene Girello – CRB-8ª. / 6159

Soeiro, Caio Raphael Martins.

So21a Avaliação da resistência de união de sistemas adesivos à dentina desproteinizada através do uso de hipoclorito de sódio. / Caio Raphael Martins Soeiro. -- Piracicaba, SP: [s.n.], 2008.

Orientadores: Luiz André Freire Pimenta, Mario Alexandre Coelho Sinhoreti.

Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Adesivos dentários. 2. Colágeno. I. Pimenta, Luiz André Freire. II. Sinhoreti, Mario Alexandre Coelho. III. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. IV. Título.

(mg/fop)

Título em Inglês: Evaluation of adhesive systems bond strength to deproteinized dentin by the use of sodium hypochlorite

Palavras-chave em Inglês (Keywords): 1. Dental adhesives. 2. Collagen

Área de Concentração: Dentística

Titulação: Doutor em Clínica Odontológica

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Data da Defesa: 05-12-2008

Programa de Pós-Graduação em Clínica Odontológica

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 05 de Dezembro de 2008, considerou o candidato CAIO RAPHAEL MARTINS SOEIRO aprovado.

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AGRADECIMENTOS

À Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas em nome do seu diretor Prof. Dr. Francisco Haiter Neto.

Ao Prof. Dr. Luiz André Freire Pimenta pela oportunidade de estar envolvido com a pesquisa e o ensino. A minha gratidão pela orientação durante toda a minha pós-graduação.

Ao Prof. Dr. Jacks Jorge Júnior pela coordenação dos cursos de pós-graduação.

À Profa. Dra. Renata Cunha Matheus Rodrigues Garcia pela coordenadoria do programa de pós-graduação em Clínica Odontológica.

Aos professores da Área de Dentística, pela dedicação em transmitir os conhecimentos necessários à nossa formação enquanto mestres e doutores.

À Prof. Dra. Gláucia Maria Bovi Ambrosano, que além da oportunidade do estágio a mim oferecido na Área de Bioestatística do Departamento de Odontologia Social da FOP, o qual foi extremamente proveitoso, válido e enriquecedor, esteve sempre ao meu lado como uma grande amiga.

Aos professores que prontamente aceitaram em participar da banca examinadora do meu exame de qualificação, Prof. Dr. Lourenço Correr Sobrinho, Profa. Dra Regina Maria Puppin Rontani e Prof. José Augusto Rodrigues, obrigado pela dedicação em ler nosso estudo e trazer informações e sugestões de grande valia ao enriquecimento deste trabalho.

Ao Prof. Dr. Mário Alexandre Coelho Sinhoreti, um agradecimento muito honroso pelo aceite na co-orientação desta tese. Extendo aqui os votos de gratidão por este em nome do meu orientador, Prof. Dr. Luiz André Freire Pimenta.

Aos colegas de doutorado, um abraço muito especial por termos divididos juntos as alegrias e dificuldades durante esse período de convivência e aprendizado.

Um agradecimento especial se faz necessário a duas personalidades que sempre me serviram de referência enquanto pesquisadoras e seres humanos. Muito obrigado a Vanessa Gallego Arias e Ana Karina Barbieri Bedran de Castro.

Aos funcionários da Área, Sr. Pedro, Mônica e Fernandinha, dedico a gratidão pela ajuda durante esse período.

Aos grandes e verdadeiros amigos que estão sempre comigo compartilhando as vitórias e as derrotas, ... obrigado.

Indubitavelmente o grande agradecimento e minha eterna gratidão são dedicados aos meus pais, Neri e Antonio Carlos, à minha irmã Thais, a meus avós Carmem e Albertino (*in memorian)*. A estes, todo o carinho e amor que eu possa retribuir será pouco por tudo o que fazem por mim. Nada disso teria valor sem vocês. Muito obrigado.

Graças a Deus sempre, por nunca me abandonar nos momentos difíceis e estar sempre trazendo luz meus caminhos.

Friso por fim a menção ao órgão de fomento CAPES, pela cessão de bolsa de doutorado no período de 2006.

Agradeço ainda a todos que direta ou indiretamente contribuiriam para o desenvolvimento deste trabalho.

RESUMO

A utilização de procedimentos adesivos está consolidada na odontologia atual, sendo protocolo indispensável à realização de uma ampla gama de procedimentos preventivos e restauradores. Os substratos dentais, esmalte e dentina, diferem quanto a sua composição, morfologia e, conseqüentemente, em relação à sua interação com os sistemas adesivos. A remoção do colágeno exposto pelo condicionamento ácido, procedimento também conhecido por desproteinização da dentina condicionada, é sugerido para remover alguns dos problemas enfrentados na união à dentina, como a degradação do colágeno e a incompleta infiltração do sistema adesivo. Além disso, a remoção da matéria orgânica poderia aumentar a energia livre de superfície da dentina condicionada, facilitando seu molhamento pelo sistema adesivo. O uso de hipoclorito de sódio, em concentração de 10%, apresentado em forma de solução ou gel, tem sido estudado na remoção das fibrilas colágenas dentinárias. O objetivo desta tese foi avaliar o comportamento de sistemas adesivos com diferentes solventes frente à desproteinização. Três estudos focam diferentes aspectos dessa união, tais como tempo de aplicação do sistema adesivo, tipo de solvente do agente de união, armazenagem a longo prazo em água a 37^{o}C e ainda a avaliação da aplicação do hipoclorito sob a apresentação líquida ou em forma de gel. Os resultados mostram que a remoção do colágeno dentinário ainda se mostra muito heterogênea e dificilmente aplicável em procedimentos clínicos futuros visando o aumento dos valores de resistência de união dos sistemas adesivos ao substrato dentina. Estudos futuros necessitam ser realizados visando definir com clareza o real papel da remoção do colágeno na união adesiva dentinária.

Palavras-chave: Adesão dentinária, sistema adesivo, remoção de colágeno, hipoclorito de sódio.

ABSTRACT

The use of adhesives systems is well-known at current dentistry. Most of prevent and restorative procedures requires an adhesive technique. The dental tissues, enamel and dentin, differ from its composition, morphology and, thus, their relation to adhesive systems available. The collagen removal after acid conditioning (dentin deproteinization), could remove some of troubles founded by adhesion between adhesives and dentin tissue, as collagen degradation and incomplete infiltration of adhesives systems thought demineralized dentin, as well an increase in wettability of those adhesives. The use of 10% sodium hypochlorite, as solution or gel, had been studied in its capacity to remove dentin collagen fibrils. The aim of this thesis was to evaluate the behavior of some adhesive systems applied in a demineralized and deproteinized dentin substrate. Three studies present different characteristics of this union: influence of adhesive application time, adhesive solvent type, storage of restored specimens in water at 37^{o}C and a comparison of NaOCl efficacy used in a solution or gel form. According to the results, the dentin collagen removal is not completely effective in an increase of bond strength values, regardless adhesive system or technique applied. Further studies need to be done to disclose the hole of sodium hypochlorite in dentin bonding.

Key-words: Dentin adhesion, adhesive systems, collagen removal, sodium hypochlorite.

SUMÁRIO

INTRODUÇÃO

A utilização de procedimentos adesivos está consolidada na odontologia atual, sendo protocolo indispensável à realização de uma ampla gama de procedimentos preventivos e restauradores.

Os substratos dentais, esmalte e dentina, diferem quanto a composição, morfologia e, conseqüentemente, em relação à interação com os sistemas adesivos.

A estrutura altamente mineralizada do esmalte, após tratamento com substâncias ácidas adequadas, transforma-se em uma superfície microporosa devido à dissolução de cristais de hidroxiapatita. Essa superfície tem, então, alta energia de superfície e permite uma boa interação com o sistema adesivo que penetra nas microporosidades, sendo lá polimerizado, formando prolongamentos de resina. A união esmalte-resina é considerada clinicamente confiável. Por outro lado, a dentina é um substrato dinâmico e complexo. Ao contrário do esmalte, a dentina apresenta alto conteúdo orgânico e é essencialmente úmida. A dentina apresenta uma estrutura tubular que varia de acordo com a profundidade. Os túbulos dentinários são preenchidos pelo fluido intratubular que está em direta relação com os prolongamentos odontoblásticos e com a periferia do tecido pulpar. Na técnica adesiva que se utiliza do condicionamento ácido total, a dentina é tratada com um ácido que remove a lama dentinária resultante do preparo cavitário, desmineraliza a dentina superficial, expondo uma rede de fibrilas colágenas e desoblitera a embocadura dos túbulos dentinários. O sistema adesivo é, então, aplicado sobre a dentina ácido-condicionada e úmida, com o intuito de preencher os espaços resultantes do ataque ácido e obliterar os túbulos dentinários abertos, promovendo assim uma camada intimamente unida à dentina e uma superfície adequada para receber o material restaurador.

Entretanto existem dificuldades na obtenção de uma boa adesão ao substrato dentinário. Entre alguns dos possíveis problemas podem ser citados: a incompleta infiltração do sistema adesivo na zona de dentina desmineralizada; a permeabilidade de alguns sistemas adesivos; uma inadequada polimerização do adesivo; a difusão de monômeros resinosos, supostamente citotóxicos, em direção à polpa, via túbulos dentinários; a degradação do adesivo;.a degradação do colágeno exposto e o selamento não-efetivo da totalidade dos túbulos dentinários.

A remoção do colágeno exposto pelo condicionamento ácido, procedimento também conhecido por desproteinização da dentina condicionada, reduziria a degradação do colágeno e a incompleta infiltração do sistema adesivo na dentina desmineralizada. Além disso, a remoção da matéria orgânica poderia aumentar a energia de superfície da dentina condicionada, facilitando o molhamento pelo sistema adesivo.

O uso de hipoclorito de sódio, em concentrações que variam de 1 a 10%, apresentado em forma de solução ou gel, tem sido estudado na remoção das fibrilas colágenas dentinárias, promovendo assim um substrato relativamente semelhante ao substrato do esmalte, o qual já é comprovadamente efetivo na união aos materiais adesivos.

Dessa forma, a resolução teórica de alguns problemas do procedimento adesivo ao substrato dentinário na técnica do condicionamento ácido total, pela utilização de desproteinizantes, justificam o desenvolvimento destes estudos apresentados nesta tese; **"Avaliação da resistência de união de sistemas adesivos à dentina desproteinizada através do uso de hipoclorito de sódio"**.

Esta tese está dividida em três capítulos; de acordo com a resolução CCPG/002/06;

Capítulo 1:

O tratamento com hipoclorito de sódio e o tempo de aplicação de um adesivo afetam a resistência à união por cisalhamento à dentina bovina ? *(Sodium*

hypochlorite treatment and adhesive application time do affect shear bond strength to bovine dentin ?)

Capítulo 2:

Influência da camada híbrida na resistência à união de dois sistemas adesivos – um estudo a longo prazo. *(Hybrid layer influence on bond strength of two adhesive systems – A long therm study).*

Capítulo 3:

Efeito do hipoclorito de sódio 10% gel e solução na resistência à união por cisalhamento à dentina para três sistemas adesivos a base de acetona. *(Effect of 10% sodium hypochlorite gel or solution on shear bond strenght of three acetone-based adhesive systems in dentin).*

OBJETIVO

O objetivo destes estudos é avaliar se a desproteinização com hipoclorito de sódio é eficaz na união em dentina para diferentes sistemas adesivos.

1) Avaliar a resistência de união à dentina de diferentes agentes de união, através da remoção das fibrilas colágenas dentinárias após o condicionamento ácido total.
2) Avaliar a utilização do Hipoclorito de Sódio 10% na efetividade em remover as fibrilas colágenas dentinárias.
3) Três diferentes estudos na abordagem da união de sistemas adesivos sem formação de camada híbrida.

CAPÍTULO 1

SODIUM HYPOCHLORITE TREATMENT AND ADHESIVE APPLICATION TIME DO AFFECT SHEAR BOND STRENGTH TO BOVINE DENTIN ?

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ABSTRACT

Purpose: The aim of this study was to evaluate the influence of different application times on the shear bond strength (SBS) of adhesive system to demineralized and deproteinized dentin. **Materials and Methods:** Eighty bovine incisors were sectioned and the buccal surfaces were ground flat to expose dentin and polished on a water-cooled mechanical grinder with Al_2O_3 abrasive paper. The specimens were divided in 4 groups (n=20). The dentin was etched with 35% phosphoric acid for 15s, washed for15s and blot dried. Except for the control group, a solution of 10% NaOCl was applied with a dwell time of 60s, washed for 30s and dried. The adhesive system applied was Single Bond (SB)/3M/ESPE. G1-Control Group: SB light-cured after 10s. of waiting time (WT); G2-NaOCl-10% + SB light-cured 10s. (WT); G3-NaOCl-10% + SB/light-cured 20s. (WT); G4-NaOCl-10% + SB/light-cured 30s. (WT). A composite resin Filtek-Z250 was inserted in a bipartite teflon matrix and cured for 40s. The specimens were stored in distilled water for 24 hours at 37^{o}C. The SBS tests were performed in an EMIC universal testing machine with a crosshead speed of 0.5mm/min. The means (MPa$\pm$SD) were analyzed with one-way ANOVA and Tukey´s post hoc test ($p \leq 0.05$). **Results:**

The results, expressed in MPa, were: G1:18.6±3.5[a]; G2:14.1±3.9[b]; G3:13.9±3.6[b] and G4:11.9±3.2[b]. Statistical differences were observed between G1 and the other groups, while no statistical significant differences were pointed between G2, G3 and G4. **Conclusion:** The dentin pre-treatment with sodium hypochlorite may interfere negatively on SBS of Single Bond regardless of the adhesive application time.

INTRODUCTION

One of the challenges in restorative dentistry is to develop adhesive systems to restorative materials that provide an effective bond to dental tissues, and consequently, offer successful restorative treatment regarding to mainly bond strength, controlling leakage resulting in no post-operative sensitivity and reduced occurrence of secondary caries.[7,35]

While the bond strength of composite resin to etched enamel has achieved a predictable high level,[14,15,24,33] the bond strength of composite resin to etched dentin had been inconsistent.[2,7] These results are due to the inherent properties of dentin, such as high organic content, variations in its intrinsic composition, the presence of fluid and odontoblastic processes in the tubules, and the presence of the smear layer.[21,22,27]

The adhesive principle of most dentin bonding systems is a micromechanical interlocking of unfilled bonding resin with the collagen network of decalcified dentin.[12,17,19,28] It has been repeatedly shown that the presence of a rich organic collagen zone at the surface of conditioned dentin is important for interaction with adhesive resin, resulting in the formation of a hybrid layer.[16,17,20,35]

A durable bonded restoration originates from the diffusion of bonding resin through and completely occupying space within the demineralized dentin matrix. The physical and/or chemical reaction of the resin with the dentin at the leading

edge of tissue demineralization is the site at which the bonding mechanism is established.[9]

It has been suggested that dentin bonding agents do not fully diffuse through the collagenous zone that remains after acid conditioning of the dentin, thus leaving the region of dentin tubules partially filled.[26] It has also been observed that poor infiltration of adhesive resin into the rich collagen area of demineralized dentin might leave gaps in the hybrid layer that, after long-term exposure to water, are vulnerable to degradation, as described by nanoleakage.[26,30,34]

The treatment of the dental tissue with sodium hypochlorite to remove collagen fibers and the influence of the collagen-rich demineralized zone on bond strength have been considered in some studies as an alternative to improve the adhesion.[9,12,23,24,36,38] Gwinnett observed that the collagen zone offered no direct, quantitative contribution to the interfacial bond strength, which was probably derived from complete resin diffusion into the porous, partially demineralized dentin below.[8]

Others studies suggest that collagen removal significantly improves bond strength when some multiple-bottle and one-bottle adhesive systems were applied,[4,5,12,23,24,25,32,36,38] while no improvements, or a decrease on SBS were demonstrated for other adhesive systems.[9,10,12,23,24,25,36] Acetone based adhesive systems generally shows higher shear bond strength (SBS) values when applied in a collagen unproved dentin.[3,25] One possible explanation may be related to the ability of acetone based primers to penetrate into the deeper layer of the demineralized dentin.[8] However, water based adhesive systems does not offer constant results in shear bond strength associate to NaOCl pre-treatment in dentin.[3,25]

The results observed for water-ethanol based adhesive system could also be speculated with the application time of the adhesive after collagen removal. Thus, an increase of this time could interfere in a higher penetration of adhesive into tubules and demineralized dentin, in a greater evaporation of solvent, improving bond strengths. Based on that, the purpose of this study was to evaluate

the influence of application time of a water-based adhesive system in SBS values when applied in a bovine deproteinized dentin.

The null hypothesis to be tested was that the different application time of adhesive system would not interfere on SBS and the collagen removal technique would not reduce or increase the SBS when compared with the Control Group.

MATERIALS AND METHODS

Specimen Preparation

Eighty extracted bovine incisors were collected and stored in distilled water. From each tooth, a square piece of the coronal facial surface was sectioned with a double-face diamond disk (KG Sorensen, São Paulo, SP, Brazil) and mounted on a ¾-inch-diameter PVC ring, parallel to the base of the ring. The tooth fragments were embedded with self-curing polyester resin (Piraglass, Piracicaba, SP, Brazil) to hold the specimens. Afterward, the specimens were ground on a water-cooled mechanical grinder (Maxigrind, Solotest, São Paulo, SP, Brazil) using 320-grit Al_2O_3 abrasive paper (Carborundum Abrasivos, Recife, PE, Brazil) to expose the dentin, and polished with 400- and 600- grit Al_2O_3 abrasive paper to obtain 5- to 6-mm^2 areas of flat, standardized dentin surfaces.

Bonding Procedure

The specimens were stored in distilled water at 37°C and assigned in four groups (n=20). Prior to surface treatment, a 3-mm-diameter area was left uncovered as a bonding site by placing a piece of vinyl tape with a 3-mm-diameter hole over the dentin.

In all groups, the dentin was etched with 35% phosphoric acid (3M/ESPE, St. Paul, MN, USA) for 15s, washed for 15s and blot dried. For the Control group (G1), the adhesive system Single Bond (SB) (3M ESPE) was applied according the

manufacturer's instructions. Adhesive was applied in two consecutive layers. The surface was dried for 5s with air using a triple syringe with a standardized air pressure with 20cm of distance from the syringe tip to dentin. Then the adhesive was light cured (Optilux 500/Demetron-Kerr, Danbury, CT, USA) for 10s. The light intensity was measured periodically with the curing-unit radiometer, and ranged from 540 to 570mW/cm^2. For group 2, 3 and 4, a 10% sodium hypochlorite solution (NaOCl), pH 12.0 was applied for 60s[3,4,35] washed for 30s and blot dried. For group 2, the adhesive system was applied according the manufacturer's instructions as the group 1/Control: SB was applied in two consecutive layers; the surface was dried for 5s with air and then, the adhesive was light cured. For group 3, it was used 10s of waiting time before curing the adhesive system. SB was applied in two consecutive layers; the surface was dried for 5s with air and the adhesive was light cured 10s. For group 4, it was used 20s of waiting time and SB was applied as previously described. Filtek-Z250 (3M/ESPE) was inserted in a bipartite circle teflon mold with 3-mm-diameter central hole and 5mm of high. It was clamped to dentin surfaces such that the mold was positioned exactly over the treated dentin. The matrix central role was completely filled with composite in two increments and cured for 40s each increment. The teflon mold was removed and the composite resin was light cured for an additional 40s in opposite directions, at the left and right side of specimen. The restoration of the specimens was done following an arbitrary sequence. The specimens were stored in distilled water for 24h at 37^{o}C before the bond strength test.

The materials used in this study and its components are described on table 2.

Bond Strength Test

Each specimen was mounted in a custom-made jig attached to the universal testing machine EMIC DL500 (EMIC, São José dos Pinhais, SP, Brazil) with the dentin surface parallel to the tip's trajectory. A compressive load was applied using

a steel knife-edge placed over the specimen so that the shear force was applied directly to the bond interface. The specimens were loaded to fail at crosshead speed of 0.5 mm/min using a calibrated 10N load cell. Means and standard deviations were calculated in MPa.

Statistical Analysis

The data were subjected to one-way ANOVA to detect possible interactions (application time of adhesive system vs. treatment). Multiple comparison Tukey´s test was chosen to check differences between pairs of means.

RESULTS

One-way ANOVA followed by Tukey´s test detected statistically significant differences ($p<0.05$) among the Control Group and all the different dentin treatments with 10% NaOCl. No significant differences were found between different application times of adhesive system when 10% NaOCl solution was applied. The statistically significant differences are expressed in table 3 by subscripted different letters.

DISCUSSION

The integrity of the bond between dentin and resin adhesive system has important implications in improving the success of composite resin restorations.[36,37] Considerable improvement in dentin adhesion has with the introduction of hydrophilic primers and total-etch technique showed by classical studies.[6,16,19] In the one-bottle adhesive systems, the microretention necessary for adhesion to dentin is still provided basically by Bis-GMA and hydrophilic monomers that flow

into the etched dentin surface,[10] encapsulating the collagen fibers present in the demineralized zone, and forming the hybrid layer.[9,16,19,30]

Some authors suggest that the hybrid layer tags might not be important for the mechanism of adhesion between materials and dentin.[11,35]

Concerns have been raised that dentin bonding agents do not fully diffuse through the collagen network that remains after acid conditioning of dentin.[16,26,36] This unprotected collagen might potentially be a weak physical link in the long-term adhesion of dentin to resin.[25] Hydrolysis of these bands of exposed collagen not protected by resin ("nonhybridized collagen")[36] could occur with long-term exposure to oral environment. This could lead to deterioration of the adhesion between resin and dentin, resulting in decreased bond strength[18] or increased microleakage.[25]

Wakabayashi et al. indicated that bond strengths after long-term water immersion were significantly higher for those specimens in which the collagen was removed after acid conditioning, suggesting that degradation of the bond was due to hydrolysis of unprotected collagen fibers.[38]

The removal of collagen can improve the resin diffusion through increasing dentin permeability and modification in its composition, showing a tissue with mineral exposed on surface.[36] A durable adhesion could be get directly with dentin hidroxiapatite partial demineralized exposed by collagen removal.[25]

Speculations about the water chaser of adhesive systems and its ability to infiltrate etched and deproteinized dentin has been studied.[12,25] Practically all current dental adhesive systems contain resin monomers (HEMA or others) dissolved in acetone, water, ethanol, or some combination of these solvents.[29] It is considered that acetone and ethanol effectively displace water, and therefore better facilitators of resin primer infiltration into the collagen network as compared to water based adhesive systems.[13]

Our study, showed that collagen removal decreased the shear bond strength values of water-ethanol based adhesive system Single Bond. These

results are in agreement with some studies that evaluated the same adhesive system in collagen unproved dentin.[12,25]

Other aspect to be considered to explain the results, especially regarding Single Bond, is that not only the water chaser but also the type of monomer could influence the results. Arias et al. demonstrated that the low bond strength observed for Single Bond when 10% NaOCl solution was used to remove the collagen fibrils could be related with chemical interactions that may occur between mineralized dentinal collagen and Single Bond and also with the application form of the NaOCl.[1]

The water-ethanol based adhesive Single Bond also shows increased values of SBS after collagen removal in other study.[3] Ciuchi et al. observed an increase in SBS using sodium hypochlorite and a water-based adhesive system.[4]

Gwinnett has not found statistical differences between specimens with and without hybrid layer, using water or organic solvents based adhesives. Consequently, the author concluded that collagen zone is not crucial factor on SBS.[8,9]

Toledano et al. described an increase in wettability for etched and deproteinized dentin. It could be expected that this dentin would be a suitable substrate for wetting by hydrophilic resins because of the modification of its surface tension and morphology.[31] Since all hydrophilic adhesives could, potentially, improve their SBS values when applied on the dentin surface, speculations can be made concerning the differences in the methods employed in applying the adhesive system, ie, drying time and distance,[13] technique of etching and deproteinizing, dwell time of agents used on dentin surfaces,[9,12,24,38] and depth (superficial vs. deep dentin) and type of dental substrate (human vs. bovine teeth),[25,31] which may explain the varying results found among studies.

It was not observed any improvement on bond strength when the application time of adhesive system was longer, suggesting also that the diffusion of the adhesive into the tubules and into the demineralized dentin was not increased. One of the reasons that acetone based adhesive systems shows better and

constants results on SBS when applied in a depleted collagen dentin could be the higher capacity of acetone to evaporate and displace water than the water-ethanol present in Single Bond composition.

It is clinically important to enhance the adhesion between adhesive and dentin, because such improved adhesive strength leads to better retention of restorations, reducing the chance of failures. The null hypothesis of this study was partially accepted since that the different application time of the adhesive system before curing did not interfere on SBS, but the collagen removal technique negatively interfere on SBS values.

The use of NaOCl seems not to be useful as dentin pre-treatment when a water-ethanol based adhesive system was used, although the role of sodium hypochlorite pre-treatment in dentin bonding is not completely disclosed yet. Thus, further research should be conducted for the effective improvement in dentin adhesion before the use of sodium hypochlorite as a clinical procedure can be indicated.

CONCLUSION

Under the conditions of this study and materials employed, the following conclusions can be made:

- Collagen removal decreases the bond strength using the water-ethanol based adhesive system Single Bond.
- The different application times of adhesive system Single Bond did not interfere on shear bond strength values on deproteinized dentin.

Table 1

Schematic sequence of the procedure for each group:

Group	Dentin acid-etching	Collagen removal	Adhesive system	Application time of adhesive system	Composite resin
1	H_3PO_4 35% - 15s.	None	Single Bond	10s.	Filtek Z250
2	H_3PO_4 35% - 15s.	NaOCl 10% (60s.)	Single Bond	10s.	Filtek Z250
3	H_3PO_4 35% - 15s.	NaOCl 10% (60s.)	Single Bond	20s.	Filtek Z250
4	H_3PO_4 35% - 15s.	NaOCl 10% (60s.)	Single Bond	30s.	Filtek Z250

Table 2

Materials Composition:

Material	Composition	Batch Number
Single Bond (Adhesive System) (3M ESPE)	Bis-GMA, HEMA, Water, Ethanol, Polyalkenoic acid, Acid Copolymer	1FX
Filtek Z250 (Composite Resin) (3M/ESPE)	Bis-GMA, Bis-EMA, Triethylene glycol dimethacrylate, Barium alumino boro silicate glass filler, Colloidal silica	55337/2

Table 3

Shear bond strength mean values (standard deviations - SD) in MPa:

GROUP	MEAN/SD
G1	18.6(3.5)[a]
G2	14.1(3.9)[b]
G3	13.9(3.6)[b]
G4	11.9(3.2)[b]

Statistical differences are expressed by subscripted different letters, (α= 0.05)

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CAPÍTULO 2

HYBRID LAYER INFLUENCE ON BOND STRENGTH OF TWO ADHESIVE SYSTEMS – A LONG THERM STUDY

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ABSTRACT

The aim of this study was to evaluate the bond strength of two adhesive systems applied on demineralized and deproteinized dentin, after 24 hours, 6 months and one year of water storage. One hundred and twenty bovine incisors were sectioned, and had the buccal surface ground with Al_2O_3 abrasive paper to expose a flat dentin surface. The specimens were divided in 12 groups (n=10). The dentin was etched with 35% phosphoric acid for 15s, washed during 15s and blot dried. Control groups/(CG): The adhesive systems were applied following the manufacture's instructions: Single Bond/(SB)(G1/G5/G9) and Bond One/(BO)(G2/G6/G10). Other groups: 10% NaOCl solution was applied for 60s, washed for 30s and blot dried. Afterwards, SB was applied on G3/G7/G11 and BO on G4/G8/G12, respectively. A resin composite Filtek Z-250 cylinder build-up was made on the surface of specimens. Shear bond strength/(SBS) test was performed 24h after restorative procedures in the universal testing machine for G1 to G4. After 6 months of storage, G5 to G8. After 1 year of storage, G9 to G12. The means (MPa±SD) were analyzed by two-way ANOVA and Tukey´s post hoc test ($p \leq 0.05$). The results were expressed in MPa: G1:13.0±4.9(a); G2:13.3±4,3(a); G3:7.3±7.1(b); G4:13.0±4.5(a); G5:12.9±4.1(a); G6:11.0±4.4(a); G7:2.1±3.6(b);

G8:11.3±4.9(ab); G9:10.8±5.5(a); G10:11.9±2.5(a); G11:3.6±3.6(b); G12:8.6±4.0(b). Statistical differences were observed between G3/G7/G11/G12 (lowest values) and other groups, while no statistical differences were observed among G1/G2/G4/G5/G6/G8/G9/G10. The dentin pre-treatment with 10%NaOCl solution negatively interfere on SBS for SB regardless the storage time. For BO, the collagen removal did not interfere on SBS for 24h and 6 months of storage, while after 1 year, the absence of hybrid layer negatively influenced on SBS.

INTRODUCTION

The clinical success of the composite restorations depend on adhesive systems that provide durable bonding of resin composites to the tooth structure, especially dentin, and effectively seal the dentin tubules[1] to prevent microleakage, recurrent caries, and pupal irritations[2]. Dentin is a less favorable bonding substrate than enamel tissue due to the high organic content, the variation in the degree of mineralization and the presence of outward fluid movement[3,4].

The mechanism of adhesion for dentin bonding agents is generally believed to be micro-mechanical in nature as a result of resin penetration into exposed collagen on the acid-demineralized dentin surface[5]. This zone is commonly referred as hybrid layer[6] or interfusion zone[7]. This hybridization of demineralized dentin and resin monomers is composed of two interphases. The larger outermost region is a network of resin-impregnated collagen largely devoid of mineral content. Below this is a narrow partially demineralized band of dentin mainly composed of resin-encapsulated hydroxyapatite crystals[8]. Due to its elastic modulus, this resin-dentin interdifusion zone may act as a stress-absorber between dentin and resin composite[9].

The collagen fibril network of demineralized dentin represents a soft delicate bonding substrate that may contribute to the technique-sensitivity of bonding

procedures. Therefore, the longevity and durability of the collagen layer can be questionable[10].

Sano & others suggested a leakage pathway through a porous zone at the hybrid layer-adhesive interface without gap formation[11]. Therefore, it has been suggested that the dentin bonding agents do not fully diffuse through the collagen network that remains after acid conditioning of dentin. Failure to adequately penetrate the collagen network into the partially demineralized dentin may produce a weak porous layer of collagen not protected by hydroxyapatite or encapsulated by resin. Consequently, hydrolysis of the exposed collagen peptides might lead to degradation of the dentin to resin bond, resulting in decreased bond strength and increased microleakage over time[11].

One potentially positive strategy would be to remove the collagen prior to bonding by the use of sodium hypochlorite[12,13,14,15,16,17,18].

Sodium hypochlorite (NaOCl) has been used on dentin as a deproteinizing agent due to its nonspecific proteolytic property, capable of removing organic material[17,19]. The rationale is that the collagen layer offers no quantitative contribution to the interfacial bond strength[20]. Some investigations have found no significant difference after removing this collagen rich zone[21] but some studies have shown improvement on values of bond strength of some adhesive systems[1,5,17,22,23] and reducing microleakage[24]. So far, there is no conclusive data, depending on each testing methodology and/or specific composition of each dentin adhesive, the application of NaOCl upon etching may increase or decrease bond strengths[1,5,13,25].

Most published data have used different methodologies, such as, different NaOCl application time and technique, and types of adhesive systems[15,17,22,25]. Thus, attempting to analyze the influence of sodium hypochlorite on dentin bonding, the aim of this study was to evaluate the effect of 10% solution of sodium hypochlorite as a dentin pre-treatment on shear bond strength for two different adhesive systems, an acetone based and an ethanol-water based system throughout 12 months of storage in water.

MATERIALS AND METHODS

Specimen Preparation

One-hundred and twenty bovine incisor teeth were collected and stored in distilled water for no longer than 15 days. The incisors were cleaned and a square piece of the coronal buccal surface was sectioned with a double-face diamond disk (KG Sorensen, São Paulo, SP, Brazil) and mounted on a ¾-inch-diameter PVC ring, parallel to the base of the ring. The ring was filled with self-curing polyester resin to hold the specimens. The embedded specimens were ground on a water-cooled mechanical grinder (Maxigrind, Solotest, São Paulo, SP, Brazil) using 320-grit Al_2O_3 abrasive paper (Carborundum Abrasivos, Recife, PE, Brazil) to expose the dentin, and polished with 400- and 600- grit Al_2O_3 abrasive paper to obtain 5- to 6-mm areas of flat, standardized dentin surfaces. Afterwards, the specimens were stored in distilled water at 37°C before the bonding procedures.

Bonding Procedure

The specimens were randomly assigned into twelve groups (n=10). Prior to any dentin surface treatment, a 3-mm-diameter area was left uncovered (bonding site) by placing a piece of vinyl tape with a 3-mm-diameter hole over the dentin.

In all groups, the dentin was etched with 35% phosphoric acid (3M ESPE, St. Paul, MN, USA) for 15s, washed for 15s and blot dried. The groups were divided depending on the adhesive system used and the NaOCl treatment applied. The adhesive system Single Bond (SB) (3M ESPE, St. Paul, MN, USA) was applied on G1, G5 and G9 as a control groups, according the manufacturer's instructions. The adhesive was applied in two consecutive layers. The surface was dried for 5s with a standardized air pressure at 15cm of distance from the syringe to dentin. Then, the adhesive was light cured (Optilux 500/Demetron-Kerr,

Danbury, CT, USA) for 10s. The adhesive system Bond One (Pentron, Wallingford, CT, USA) was applied on G2, G6 and G10 as a control groups, following the manufacture's instructions. The adhesive was applied in two consecutive coats, for 10s reaching a shiny surface. The surface was dried for 10s with a standardized air pressure at 15cm of distance from the syringe to dentin to remove the adhesive solvent. Then, the adhesive was light cured for 10s. For groups G3, G6 and G11 (SB+NaOCl); and G4, G7 and G12 (BO+NaOCl) after etching, a 10% NaOCl solution, pH 12 was applied for 60s[25] washed for 30s and blot dried. Afterwards, the same adhesive procedures were followed as control groups. The light intensity was measured periodically with the curing-unit radiometer (Optilux 500/Demetron-Kerr, Danbury, CT, USA), ranging from 540 to 570 mW/cm^2. The treatment groups are described on table 1.

The resin composite Filtek-Z-250 (3M ESPE, St. Paul, MN, USA) was inserted in a bipartite Teflon matrix with 3-mm-diameter central hole and 4mm of high was clamped to dentin surfaces such that the mold was positioned over the treated dentin. The matrix central role was completely filled with composite in two increments (2mm) and cured for 40s each. The Teflon matrix was removed and the resin composite was light-cured for an additional 40s in opposite directions. The specimens were stored in distilled water at 37^{o}C for 24 hours in groups G1 to G4. In groups G5 to G9 they were stored at same conditions for 6 months, and for 12 months in groups G9 to G12. The distilled water was changed every week of storage for G5 to G12. The materials used in this study and its components are described on table 2.

Bond Strength Test

Each specimen was mounted in a custom-made jig attached to the universal testing machine (EMIC, São José dos Pinhais, SP, Brazil) with the dentin surface parallel to the tip's trajectory. A compressive load was applied using a steel knife-edge placed over the specimen so that the shear force was applied directly to the

bond interface. The specimens were loaded to fail at crosshead speed of 0.5 mm/min using a calibrated 10N load cell. Means and standard deviations were calculated in MPa (Table 3).

Statistical Analysis

The data were subjected to three-way ANOVA. Multiple comparison Tukey´s test (α= 0.05) was chosen to analyze differences between pairs of means and possible interactions (adhesive system vs. treatment vs. time). The statistical program used was SAS software.

RESULTS

The results of shear bond strength test performed in this study, expressed in MPa, are described on table 3. The statistical analysis showed no interaction among the factors evaluated. For Single Bond, an water-ethanol based adhesive, the collagen removal affected the shear bond strength negatively for all storage times studied. For Bond One, an acetone based adhesive system, the NaOCl treatment did not interfere on bond strength, except after one year of water storage, in which the bond strength values were decreased.

DISCUSSION

Most of the conventional bonding systems attempted to reinforce the adhesion through a reaction between the collagen-rich surface and a high-affinity functional monomer[22]. All of these systems seem to achieve resistant adhesion between the dentin and resin, mediated by the resin-reinforced layer[6]. The microretention necessary for adhesion to dentin in one-bottle adhesive systems is

still provided by bis-GMA and hydrophilic monomers that flow into the etched dentin surface[26], encapsulating the collagen fibers present in the demineralized zone, and forming the hybrid layer[6,8,27]. The dentin-resin adhesion mediated by the hybrid layer has a high initial adhesive strength, but it is difficult to maintain the initial adhesive force for long periods[28], and because of that, this study evaluated bond strength of two adhesives systems after twelve months of water storage.

In the present study, two different adhesive systems were used on dentin: an acetone based system (Bond One) and a water-ethanol based system (Single Bond) as solvents. For Bond One, the use of 10% NaOCl as a dentin pre-treatment did not interfere on shear bond strength after 24h and 6months of water storage time; however, the SBS values decreased after one year of water storage. Nevertheless, for Single Bond, the collagen removal negatively affect the SBS values even after 24 hours of water storage. Different results have been published when 10% NaOCl has been used as dentin pretreatment. Depending on the adhesive system used, SBS values can increase or decrease[10,12,16,17,28]. In general, for those adhesive systems with acetone as solvent, the use of 10% NaOCl solution increases the SBS values[12,13,15]. However, for the adhesive systems presenting ethanol as solvent, the use of 10% NaOCl solution decreases the mean SBS[15]. Due to their volatility, solvents such as acetone and, to a lesser degree, ethanol, may displace surface moisture and serve better to carry the primer monomers into the micro or nanoporosities of the etched dentin surface[29]. The acetone-based adhesive system appear to have beneficial effects from the use of sodium hypochlorite in dentin, while restorations placed by ethanol- and water-based adhesive have been negatively affected by the collagen removal technique in a *in vivo* study[17], as also demonstrated in "*in vitro"* studies[15,16,25]. It is important to highlight that the collagen removal will depend upon concentration, time and agitation NaOCl and if NaOCl is presented in solution or gel form[16].

In the present study, after 1 year of water storage, both adhesives Single Bond and Bond One were negatively influenced by collagen removal. The NaOCl application alters the dentin surface, and may change the hydrophilic properties

that might influence the intimate attachment at the interface, improving the compatibility to hydrophobic resins[30]. Also it has been shown that the diameter of tubules orifices increased after NaOCl treatment of acid-etched and demineralized dentin due to the loss of demineralized peritubular dentin. The diameter of the lateral branches of the tubules also increased and they were more numerous when compared with the conventional etching procedures[13,20]. Nevertheless, according to our results, the use of a 10% NaOCl solution as an alternative to improve adhesion between dentin and adhesive resin is not effective for both adhesive systems tested, when compared to the conventional etching procedures. The acetone based adhesive system used in this study, Bond One was negatively influenced by 10% NaOCl solution dentin treatment after 1 year of water storage, and for Single bond, water-ethanol based adhesive system, as expected according to previous studies, the collagen removal decreased the bond strength, even right after 24 hours of water storage. The aim of this study was to evaluate, firstly, if collagen removal could achieve better bond strength values for both adhesives, and it was not confirmed by the results even after 24 hours of storage. Secondly, if collagen removal, after 1 year of storage in water at 37^{o}C, could better maintain the adhesion obtained after bonding procedures; however, again, this fact was not confirmed. Therefore, based on the variability of the results observed in different studies, further research is needed attempting to clarify the real role of sodium hypochlorite in dentin bonding procedures.

CONCLUSION

Under the conditions of this study and materials employed, the following conclusions can be made:

- Both, Single Bond and Bond One, when applied following the manufacture's instructions, presented similar SBS values, regardless the storage time (after 6 and 12 months).

- The collagen removal technique, using 10% sodium hypochlorite solution negatively affects the SBS values when a water-ethanol based adhesive system (Single Bond) was applied, regardless the storage time in water (after 24 hours, 6 and 12 months). For Bond One, the collagen removal only affect the SBS values after 1 year of storage time in water.

Table 1

Shear bond strength results. Means and standard deviations expressed in MPa:

Group	Adhesive System/NaOCl Treatment	Storage Time
G1 SB Control	Single Bond	**24 hours**
G2 BO Control	Bond One	**24 hours**
G3 SB NaOCl	Single Bond + 10% NaOCl	**24 hours**
G4 BO NaOCl	Bond One + 10% NaOCl	**24 hours**
G5 SB Control	Single Bond	**6 months**
G6 BO Control	Bond One	**6 months**
G7 SB NaOCl	Single Bond + 10% NaOCl	**6 months**
G8 BO NaOCl	Bond One + 10% NaOCl	**6 months**
G9 SB Control	Single Bond	**12 months**
G10 BO Control	Bond One	**12 months**
G11 SB NaOCl	Single Bond + 10% NaOCl	**12 months**
G12 BO NaOCl	Bond One + 10% NaOCl	**12 months**

Table 2

Materials composition:

MATERIAL	COMPOSITION
Single Bond	Bis-GMA
	HEMA
	Water
	Alcohol
	Polyalquenoic Acid
	Acid Copolymer
Bond One	PMGDM
	HEMA
	Light cure initiator
	Acetone
Filtek Z-250	Bis-GMA
	TEGDMA
	UDMA
	bis-EMA(6)
	filler

Table 3

Shear bond strength results. Means and standard deviations expressed in MPa:

24 hours of water storage	**G1 SB Control**	**12.98±4.92(a)**
	G2 BO Control	**13.33±4.29(a)**
	G3 SB NaOCl	**7.3±7.14(b)**
	G4 BO NaOCl	**12.98±4.5(a)**
6 months of water storage	**G5** SB Control	**12.89±4.15(a)**
	G6 BO Control	**10.96±4.44(a)**
	G7 SB NaOCl	**2.14±3.64(b)**
	G8 BO NaOCl	**11.3±4.88(ab)**
1year of water storage	**G9** SB Control	**10.81±5.54(a)**
	G10 BO Control	**11.88±2.45(a)**
	G11 SB NaOCl	**3.64±3.56(b)**
	G12 BO NaOCl	**8.63±3.96(b)**

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CAPÍTULO 3

EFFECT OF 10% SODIUM HYPOCHLORITE GEL OR SOLUTION ON SHEAR BOND STRENGHT OF THREE ACETONE-BASED ADHESIVE SYSTEMS IN DENTIN.

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ABSTRACT

Purpose: The aim of this study was to evaluate the influence of 10% NaOCl gel and solution in dentin collagen removal on shear bond strength (SBS) of three acetone-based adhesive systems. **Materials and methods:** 135 bovine incisors were sectioned and buccal surface were flat ground to expose dentin and polished on water cooled mechanical grinder with Al_2O_3 abrasive paper. The specimens were randomly assigned into 9 groups (n= 15). The dentin was etched with phosphoric acid 35% during 15 sec., washed during 15 sec. and blot dried. The adhesive system Bond One® (Jeneric Pentron) was applied on G1/BO, G4/BOS and G7/BOG, according to manufacture´s instructions. The adhesive system Prime & Bond 2.1® (Dentsply) was applied on G2/PB, G5/PBS and G8/PBG, according to manufacture´s instructions; and the adhesive system One Step Plus® (Bisco) was used in G3/OS, G6/OSS and G9/OSG, according to manufacture´s instructions. The G1, G2 and G3 were used as control groups. The 10% NaOCl solution was applied on groups G4, G5 and G6, after etching, during 60 seconds, washed for 30 seconds and blot dried. The 10% NaOCl gel was applied on groups G7, G8 and G9, after etching, during 60 seconds, washed for 30 seconds and blot dried. The

specimens were restored with the composite resin Filtek Z-250® (3M/ESPE) that was inserted in a circular Teflon matrix and cured during 40seconds. The specimens were stored in distilled water for 24h. at 37°C. The shear bond strength was performed in a Universal testing machine and the data was recorded in MPa. **Results:** The data were analyzed by two-way ANOVA and Tukey's test. The means for the adhesive system Bond One were: G1=13.25$^{(b)}$, G4=16.34$^{(a)}$ G7=13.07$^{(b)}$. The means for Prime & Bond 2.1 were: G2=9.63$^{(b)}$; G5=16.09$^{(a)}$ G8=10.18$^{(b)}$ and the means for One Step Plus were: G3=13.17$^{(b)}$ G6=14.45$^{(a)}$ G9=11.01$^{(b)}$. The results showed statistical differences among G4, G5 and G6 and others groups. It could be concluded that the dentin pre treatment with NaOCl 10% solution increase the values of SBS for acetone-based adhesive systems in comparison to NaOCl 10% gel and control group for the adhesive systems tested. **Clinical significance:** It is clinically important to enhance the adhesion between adhesive and dentin, reducing the chance of failures of the restorations. The use of NaOCl seems to be one possible strategy to improve the adhesion.

INTRODUCTION

The clinical success of the composite restorations depends on adhesive systems that provide durable bonding of composite to tooth structure and effectively seal the dentin tubules (Vargas 1997) to prevent microleakage, recurrent caries, and pupal damage (Swift, Perdigão & Heymann 1995).

Etched enamel shows an irregular surface representing a perfect substrate for bonding of unfilled resins. However, the dentin is a less favorable bonding substrate due to the high organic content, the variation in the degree of mineralization and the presence of outward fluid movement (Walshaw &McComb 1995, Eliades 1994).

The mechanism of adhesion for dentin bonding agents is generally believed to be micro-mechanical in nature as a result of the penetration of resin into

exposed collagen on an acid-demineralized dentin surface (Armstrong *et. al.* 1998). This zone is commonly referred to as the hybrid layer (Nakabayashi 1982) or interfusion zone (Van Meerbeek 1992). This hybridization of demineralized dentin and resin monomers is composed of two interphases. The larger outermost region is a network of resin-impregnated collagen largely devoid of mineral content. Below this is a narrow partially demineralized band of dentin mainly composed of resin-encapsulated hydroxylapatite crystals (Nakabayashi *et. al.* 1992). Due to its elastic modulus, this resin-dentin interdifusion zone may act as a stress-absober between dentin and composite (Uno & Finger 1996).

One-bottle dentin adhesive systems are providing effective bonding to dentin with only one chemical formula for priming and bonding, combining hydrophilic and hydrophobic resins (Finger, Inoue & Asmussen 1994, Mason & others 1997, Kanca 1997, Wilder & others 1998). However the collagen fibril network of demineralized dentin represents a soft delicate bonding substrate that may contribute to the technique-sensivity of bonding procedures. Thus the longevity and durability of collagen is questionable (Prati 1999, Gwinnett 1994; 1996, Sabóia 2000; 2006).

Sano & others (1994) suggested the existence of a leakage pathway through a porous zone at the hybrid layer-adhesive interface without gap formation. It has been suggested that the dentin bonding agents do not fully diffuse through the collagen network that remains after acid conditioning of dentin. Failure to adequately penetrate the collagen network into the partially demineralized dentin may produce a weak porous layer of collagen not protected by hydroxylapatite or encapsulated by resin. Subsequent hydrolysis of the exposed collagen peptides could lead to degradation of the dentin to resin bond, resulting in decreased bond strength and increased microleakage over time (Sano & others 1994).

One strategy would be remove the collagen prior to bonding by the use of sodium hypochlorite (Gwinnett 1996, Inai *et al.* 1998, Hashimoto 2000).

Sodium hypochlorite (NaOCl) has been used on dentin as a deproteinizing agent. NaOCl is a nonspecific proteolytic agent capable of removing organic material (Inaba *et al*. 1996).

Gwinnett (1994a) theorized that the collagen layer offers no quantitative contribution to the interfacial bond strength (Gwinnett 1994a, Perdigão *et al*. 2000) demonstrated that the use of 10% NaOCl gel decreased the values of SBS for two adhesive systems. Others investigations have found no significant difference after removing this collagen rich zone (Uno & Finger 1995) but some studies showed improvement on values of bond strengths of some multi-bottle and one-bottle adhesive systems (Wakabayashi 1994, Vargas 1997, Armstrong 1998, Sabóia 2000, Bedran de Castro 2000) and reducing of microleakage (Sabóia *et al*. 2002). Depending on each testing methodology and/or specific composition of each dentin adhesive, the application of NaOCl upon etching may increase or decrease bond strengths (Vargas *et al*. 1997; Armstrong *et al.* 1998; Inai *et al.* 1998) and microleakage (Shinohara *et al.* 2004).

Different concentrations of NaOCl are used to remove collagen fibrils network. It has been reported that the higher the concentration of NaOCl, the greater the dentin bond strength until a plateau is reached at a concentration of 10%, for an application time of 60 seconds (Tanaka 1993).

However, the eventual clinical application of sodium hypochlorite only could be achieved by a gel presentation of NaOCl. For apply the 10% sodium hypochlorite in a clinical situation safely it could be more interesting to be as gel and not as a liquid solution. Few studies have perfomed the collagen removal by gel of NaOCl. Perdigão *et al.* in 2000 have studied a commercial gel of NaOCl and did not find better bond strength values in comparison to total etch-technique.

The null hypothesis to be tested was that the collagen removal by 10% sodium hypochlorite can improve shear bond strength to acetone-based adhesive systems used in this study.

MATERIALS AND METHODS

Specimen Preparation

One hundred and thirty five extracted bovine incisors were used in this study. From each tooth, a square piece of the coronal facial surface was sectioned off with a double-face diamond disk (KG Soresen, São Paulo, SP Brazil) and mounted on a 3/4 inch-diameter PVC ring. The rings were then filled with self-curing polyester resin to set the specimens. The embedded specimens were ground on a water-cooled mechanical grinder (Maxigrind, Slotest, São Paulo, SP, Brazil), using 180 and 320-grit Al_2O_3 abrasive paper to expose the dentin and polished with 400 and 600-grit Al_2O_3 abrasive paper to create a smear layer and obtain 5mm areas of flat. The specimens were stored in a humid environment at 37°C.

Bonding procedures

The specimens were randomly assigned to nine groups (n=15). Before the surface treatment, a 3mm-diameter area was left uncovered as a bonding site by placing a piece of adhesive paper with a 3mm-diameter hole over the dentin.

The adhesive systems used were Bond One (Jeneric Pentron, Wallingford, CT, USA), Prime & Bond 2.1 (Dentsply Caulk, York, Pennsylvania, USA) and One Step Plus (Bisco Inc., Schaumburg, IL, USA) (table 1). The specimens were restored with composite resin Filtek Z-250 (3M/ESPE, St. Paul, MN, USA) (Table1).

The groups received the following treatments:

Group 1: Bond One (BO) – control group

The dentin surface was etched with phosphoric acid 35% (3M/ESPE, St. Paul, MN, USA), during 15s, rinsed with water for 15s, and dried with absorbent paper. The adhesive system was applied according to manufacture's instructions:

Two consecutive coats of Bond One were applied, dried with a gentle stream air for 10 seconds. Then the adhesive system was light cured (Optilux 500/ Demetron-Kerr, Danburry, CT, USA) for 10 seconds. Re-apply Bond One, air dried for 10 seconds and no light cure was necessary.

Group 2: Prime & Bond 2.1 (PB) – control group

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s, and dried with absorbent paper. The adhesive system was applied according to manufacture's instructions: A coat of Prime & Bond 2.1 was applied, after waiting 30s., an air dried for 5s. Then the adhesive system was light cured for 10s. A second coat was applied and the excess of adhesive system was removed by an air dried, during 5s., and light cured for 10s.

Group 3: One Step Plus (OS) – control group

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s, and dried with absorbent paper. The adhesive system was applied according to manufacture's instructions: One Step Plus bottle was shaked for 3-5s. once the mixing element inside the bottle was audible. A coat of One Step Plus was applied, agitating slightly on moist dentin for 10s. and air dried for 10s. Then the adhesive system was light cured for 10s.

Group 4: Bond One + 10% NaOCl solution (BOS)

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s and dried with absorbent paper. A 10% NaOCl solution was applied during 60s, rinsed with water for 15s, and dried with absorbent paper. The same adhesive procedure was followed as group 1.

Group 5: Prime & Bond 2.1 + 10% NaOCl solution (PBS)

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s and dried with absorbent paper. A 10% NaOCl solution was

applied during 60s, rinsed with water for 15s, and dried with absorbent paper. The same adhesive procedure was followed as group 2.

Group 6: One Step Plus + 10% NaOCl solution (OSS)

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s and dried with absorbent paper. A 10% NaOCl solution was applied during 60s, rinsed with water for 15s, and dried with absorbent paper. The same adhesive procedure was followed as group 3.

Group 7: Bond One + 10% NaOCl gel (BOG)

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s and dried with absorbent paper. A 10% NaOCl gel was applied during 60s, rinsed with water for 15s, and dried with absorbent paper. The same adhesive procedure was followed as group 1. The NaOCl gel used in this study was achieved adding silica to the same 10% solution used in other test groups.

Group 8: Prime & Bond 2.1 + 10% NaOCl gel (PBG)

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s and dried with absorbent paper. A 10% NaOCl gel was applied during 60s, rinsed with water for 15s, and dried with absorbent paper. The same adhesive procedure was followed as group 2.

Group 9: Prime & Bond 2.1 + 10% NaOCl gel (PBG)

The dentin surface was etched with phosphoric acid 35%, during 15s, rinsed with water for 15s and dried with absorbent paper. A 10% NaOCl gel was applied during 60s, rinsed with water for 15s, and dried with absorbent paper. The same adhesive procedure was followed as group 3.

Restorative procedures

A bipartite teflon ring mold 3mm in diameter and 5mm high was clamped to the dentin surfaces such that the mold was positioned over the treated surface. The mold was filled with composite resin Filtek Z-250 (table 1) and light cured for 40s, thus light cured again for additional 40s in opposite directions after removing the mold. The specimens were stored in a humid environment at 37°C for 24h.

Bond strength test

Each specimen was mounted in a custom-made jig attached to a universal testing machine (EMIC, São José dos Pinhais, SP, Brazil) with the dentin surface parallel to the machine's trajectory. A compressive load was applied using a steel knife-edge placed over the specimens. The specimens were loaded to fail at a crosshead speed of 0.5 mm/min. Means and standard deviations were calculated with units expressed in MPa.

RESULTS

Means shear bond strength in MPa +/- Standard deviations (SD) for the groups are demonstrated in table 2, 3 and 4. A two-way ANOVA and Tukey´s tests were used for statistical analysis of the data. The two-way ANOVA reveled statistical significant differences in the variable (s). The Tukey´s test showed statistical differences between groups in which a 10% NaOCl solution was applied. When a 10% NaOCl solution pretreatment was used, the values of shear bond strength increased, G4/BOS = 16.34 MPa, G5/PBS = 16.09 MPa, G6/OSS = 14.45 MPa, comparing with the values of control groups, G1/BO = 13.25 MPa, G2/PB = 9.63 MPa, G3/OS = 13.17 MPa, and 10% NaOCl gel pretreatment groups, G7/BOG = 13.07 MPa, G8/PBG = 10.18 MPa, G9/OSG = 11.01 MPa.

DISCUSSION

Most of the conventional bonding systems attempted to reinforce the adhesion through a reaction between the collagen-rich surface and a high-affinity functional monomer (Wakabayashi 1994). All of these systems seem to achieve reinforced adhesion between the dentin and resin, mediated by the resin-reinforced layer (Nakabayashi 1982).

In one-bottle adhesive systems, microretention necessary for adhesion to dentin is still provided by bis-GMA and hydrophilic monomers that flow into the etched dentin surface (Hara 1999), encapsulating the collagen fibrils present in the demineralized zone, and forming the hybrid layer (Gwinnett 1996, Nakabayashi 1992, Nakabayashi 1982, Titley 1994). In the present study it was used three one-bottle acetone-based adhesive systems, Bond One, Prime & Bond 2.1 and One Step plus, which are composed by resin monomers as dimethacrylate, PENTA for Prime & Bond 2.1 and Hydroxyethyl methacrylate (HEMA) for One Step Plus and for Bond 1. These monomers are responsible to create the resin-reinforced layer.

The hybrid layer, resin tags, and adhesive filling of lateral branches of dentinal tubules have been suggested as the essential mechanisms of adhesion (Pioch 1999). It is assumed that the resin-impregnated layer has a lower young's modulus of elasticity than does the restorative resin and thus acts as an inherent elastic buffering layer that is able to absorb the resin composite's curing contraction stress (Uno e Finger 1995).

However, Kiyomura examinated the durability of the adhesion between 4-META/MMA-TBB resin and bovine dentin during long term immersion in water, most samples showed detachment of the resin from the resin reinforced layer and also from the dentin. This suggests the dentin-resin adhesion mediated by the hybrid layer has a high initial adhesive strength, but it is difficult to maintain the initial adhesive force for long periods (Kiyomura 1987, Sano 2006).

Suzuki *et. al.* reported that there will be aggregates of collagen fibrils on the intertubular dentin and around the tubules after the dentin surface is acid

demineralized, and it is difficult to diffuse adhesive resin onto the collagen layer on an adhesive surface (Suzuki *et al.* 1990).

The demineralized dentin is low in monomer permeability, and the diffusion potential of the monomers are not so high enough to penetrate towards the intact dentin before they polymerize (Pioch 2001). More over, previous studies have reported that many dentin bonding agents can produce high bond strength without the presence of exposed collagen fibrils (Saboia *et al.* 2000, Bedran de Castro *et al.* 1999). The collagen removal will depend upon concentration, time and agitation NaOCl (Gwinnett 1994b, Arias et al. 2004).

In this study the use of 10% NaOCl solution increased the values of shear bond strength for Bond One, Prime & Bond 2.1 and One Step Plus, comparing with Controls when the collagen network was not removed or partially removed mediated by 10% NaOCl gel. The adhesion values were statistically significant higher with the absence of collagen fibrils for all adhesive systems when a 10% NaOCl solution was used. However 10% NaOCl gel was not statistical different from the control group. It might be due to the worst penetration of NaOCl gel in comparison of a NaOCl solution, thus not removing all the collagen fibrils. The incomplete collagen removal might difficult the monomer penetration, decreasing the values of shear bond strength, as observed with the use of 10% NaOCl gel for all adhesive systems tested.

Wakabayashi suggested that the greater adhesive durability following the combined phosphoric acid and sodium hypochlorite treatment system resulted from the absence of the collagen layer between the dentin and resin inhibiting the penetration of water, thus maintaining the adhesive strength at the interface between the resin and dentin (Wakabayashi 1993). The NaOCl application alters the dentin surface, and may change the hydrophilic properties that might influence the intimate attachment at the interface, improving the compatibility to hydrophobic resins (Pioch 2001).

The use of 10% NaOCl showed different results, depending of the adhesive system used. For those adhesive systems which have an acetone as solvent, the

use of 10% NaOCl solution increased the values of shear bond strength. However for the adhesive systems contenting an ethanol as solvent, or HEMA as monomer (Salim *et al.* 2004, Arias *et al.* 2004) the use of 10% NaOCl decreased the means (Sabóia *et al.* 2000; Spencer *et al*. 1992). Inai *et.al* (1998), Gwinnett (1996), Pioch *et.al*. (1999) and Saboia *et al.* (2000) found an increase of shear bond strength, when using acetone containing primers, which do not have acetone as solvent. Due to their volatility, solvents such as acetone and, to a lesser degree, ethanol may displace surface moisture and serve better to carry the primer monomers into the micro or nanoporosities of the etched dentin surface (Li 2000, Kanca 1992). Also, it could be speculated that not only the chaser but also the type of monomer could influence the results.

With 10% NaOCl gel, some collagen fibrils remained allowing the formation of a "poor" hybrid layer in that areas, generating intermediate values of bond strength (Arias *et al.* 2005) (Vide em anexos desta tese Figura 1 e 2). This study are in accordance to Perdigão *et al.* (2000) when the application of a 10% NaOCl commercial gel (AD Gel) resulted in a significant decrease in bond strengths for two one-bottle adhesive systems containing different solvents (Perdigão *et al.* 2000). Nevertheless, Arias *et al.* (2001) showed that the use of 10% NaOCl gel or solution can improve shear bond strength depending on the adhesive system used. So, we could assert that the use of 10% sodium hypochlorite as a chemical factor presents great results when an acetone-based adhesive system is used. However, the clinical use of this substance is not viable yet, because we could not have a safe application *in vivo* using a solution of NaOCl. For apply the 10% sodium hypochlorite in a clinical situation safely it could be more interesting to be as gel and not as a liquid solution. In the present study the 10% NaOCl gel was prepared at the time of its use, using the same 10% NaOCl solution used in the tests, adding to it silica gel and getting a 10% sodium hypochlorite under the same conditions as the 10% NaOCl solution. Thus, we could be sure that we were testing the same substance in different presentation forms.

From this pattern, the use of a 10% NaOCl might be another way to improve adhesion between teeth and composite resin restorations using acetone-based adhesive systems, but it is still unclear the manner to transform NaOCl gel as efficient as NaOCl solution.

Table 1

Adhesive and restorative materials:

Adhesive Systems	Manufactures	Composition	Batch n°
Bond One	Jeneric Pentron	PMGDM HEMA Light cure initiator Acetone	53767
Prime & Bond 2.1	Dentsply	Dimethacrylate elastomeric resin, PENTA, photoinitiatiors, cetilamydehydrfluorite, acetone	63692
One Step Plus	Bisco	Biphenyl dimethacrylate, Hydroxyethyl methacrylate, acetone, glass frit	0200000712
Composite resin Filtek Z-250	3M ESPE	Bis-GMA, TEGDMA, UDMA, bis-EMA(6), filler	1-76-1MK A2 2004-09

Table 2

Shear bond strength mean values, standard deviations in Mpa for adhesive system Bond One:

Group	Means	Standard deviations	N
G1/BO	13.25[b]	3.55	15
G4/BOS	16.34[a]	7.63	15
G7/BOG	13.07[b]	6.10	15

Statistical differences are expressed by different letters (α= 0.05)

Table 3

Shear bond strength mean values, standard deviations in Mpa for adhesive system Prime & Bond 2.1:

Group	Means	Standard deviations	N
G2/PB	9.63[b]	4.03	15
G5/PBS	16.09[a]	6.42	15
G8/PBG	10.18[b]	7.49	15

Statistical differences are expressed by different letters (α= 0.05)

Table 4

Shear bond strength mean values, standard deviations in Mpa for adhesive system One Step Plus:

Group	Means	Standard deviations	N
G3/OS	13.17[b]	4.87	15
G6/OSS	14.45[a]	5.30	15
G9/OSG	11.01[b]	5.63	15

Statistical differences are expressed by different letters (α= 0.05)

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CONSIDERAÇÕES GERAIS

Os substratos dentais, esmalte e dentina, diferem quanto a sua composição, morfologia e, conseqüentemente, em relação à sua interação com os sistemas adesivos. Os resultados da remoção do colágeno exposto pelo condicionamento ácido através do uso de hipoclorito de sódio, se mostraram bastante heterogêneos nos estudos desta tese no que tange a união eficaz de sistemas adesivos à dentina.

No primeiro capítulo, foi avaliado o comportamento de um sistema de união a base de água e álcool como solvente após a remoção da camada híbrida dentinária através do uso de uma solução de NaOCl a 10%. Foi estudado diferentes tempos de aplicação do sistema de união visando uma maior penetração do adesivo através dos túbulos expostos. Os resultados mostraram que para o agente de união estudado (Single Bond), a técnica da remoção das fibrilas colágenas se mostrou ineficiente, exibindo um menor valor de resistência à união do que o grupo controle. O aumento no tempo de aplicação do sistema de união não modificou tais resultados. A própria literatura citada relata que, para este agente de união, a remoção das fibrilas colágenas não melhora a união do Single Bond em dentina, quando comparado a técnica convencional (*técnica do condicionamento ácido total*).

Visando conhecer melhor a influencia da da camada híbrida na união de sistemas adesivos ao substrato dentinário, foi proposto no segundo capítulo avaliar o comportamento de dois sistemas de união, um a base de água e álcool (Single Bond) e outro tendo a acetona como solvente (Bond One) após a remoção das fibrilas colágenas dentinárias através da aplicação de uma solução de NaOCl a 10%. Foi ainda objetivo deste trabalho, acompanhar o desempenho das restaurações confeccionadas sobre os corpos de prova após 6 e 12 meses de armazenagem em água, a 37^{o}C. O estudo mostrou que, de acordo com os achados no primeiro estudo, para o adesivo Single Bond, a remoção das fibrilas

colágenas interfere negativamente nos valores de resistência à união, o que não se verificou ao adesivo Bond One, onde o uso do NaOCl não influenciou nos resultados, exceto após 12 meses de armazenagem em água, onde os valores foram estatisticamente inferiores ao grupo controle. Nesse estudo, com suas limitações, pode-se comprovar que a remoção do colágeno independente do agente de união avaliado, não se mostra eficaz a longo prazo.

O terceiro capítulo traz a comparação de três diferentes sistemas adesivos, todos contendo acetona como solvente (Bond One; Prime & Bond 2.1 e One StepPlus). Também foi estudado a efetividade na remoção do colágeno dentinário com o uso de NaOCl a 10%, apresentado como solução e gel. O objetivo deste estudo foi comparar se, em sendo acetona o solvente dos sistemas adesivos, o comportamento frente a desproteinização é beneficiado e, ainda, se a apresentação do hipoclorito de sódio em forma de gel seria eficiente, visto que a aplicabilidade clinica do NaOCl líquido não seria segura aos tecidos pulpares. Os resultados mostraram uma eficiência da remoção das fibrilas colágenas dentinárias para os três adesivos testados no aumento dos valores de resistência à união quando comparados aos grupos controle, embora não se comprovou a mesma eficácia quando o NaOCl foi utilizado na forma de gel.

Dessa forma, a resolução teórica de alguns problemas do procedimento adesivo ao substrato dentinário na técnica do condicionamento ácido total, pela utilização de desproteinizantes, justificam o desenvolvimento destes estudos que foram apresentados nesta tese junto à linha de pesquisa intitulada "Avaliação da efetividade da união de sistemas adesivos à dentina através da desproteinização pelo uso de hipoclorito de sódio". Entretanto, pelos resultados obtidos, a desproteinização não pode ser considerada benéfica a aplicabilidade de alguns dos sistemas de união disponíveis no mercado estudados em dentina, haja visto a heterogeneidade nos valores de resistência a união encontrados, a perda de eficácia a longo prazo quando comparado aos grupos controle, e ainda, o resultado ineficiente do agente hipoclorito de sódio na apresentação em forma de gel, o que eventualmente viabilizaria o uso clínico desta substância.

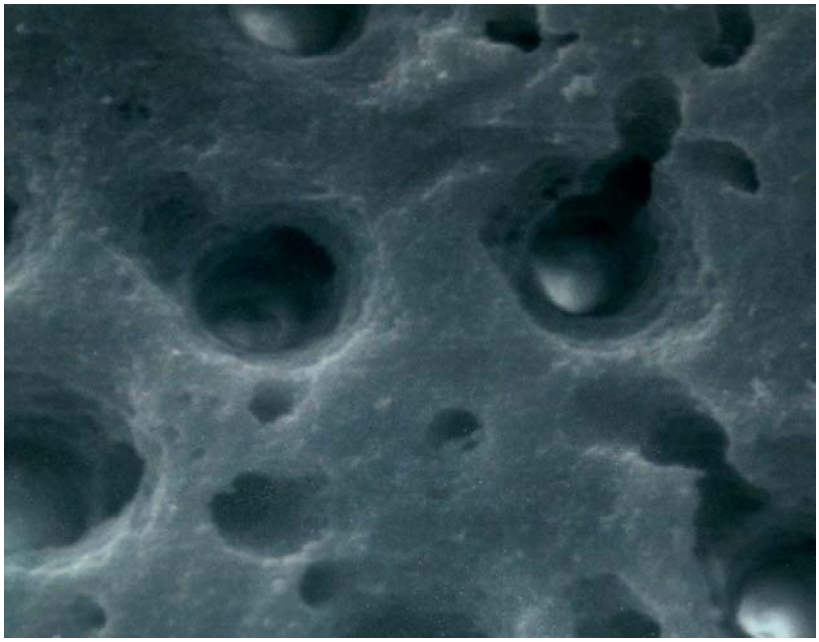
Evidente que os estudos apresentados possuem suas limitações e em virtude deste, não deve-se descartar por completo a possível união ao substrato dentinário na ausência de fibrilas colágenas. Talvez, estudos mais aprofundados da questão química envolvida na interação do NaOCl a dentina e ainda dos sistemas de união que pudessem ser mais favoráveis a aplicação sob a técnica da desproteinização dentinária. Entretanto, considerando os resultados obtidos com os estudos apresentados nesta tese, não se afirma a hipótese de que a desproteinização dentinária através do uso de hipoclorito de sódio a 10% seja eficiente para o aumento dos valores de resistência de união dos agentes adesivos testados.

De acordo com a variabilidade encontrada nos resultados destes estudos, e a heterogeneidade descrita na literatura, sugere-se a realização de novos estudos com enfoque na interação química entre os materiais adesivos e a dentina desmineralizada e desproteinizada.

CONCLUSÕES

O uso de hipoclorito de sódio enquanto agente desproteinizante dentinário não se mostrou eficaz no aumento dos valores de resistência à união pelo cisalhamento, segundo as técnicas e materiais utilizados nos estudos apresentados.

ANEXOS



Soeiro, CRM

Figura 1 – Dentina condicionada com ácido fosfórico 35% e tratada com hipoclorito de sódio 10% solução. Magnificação de 5000x. Note a ausência de porções orgânicas no substrato e um aspecto bastante "mineral" da estrutura remanescente.

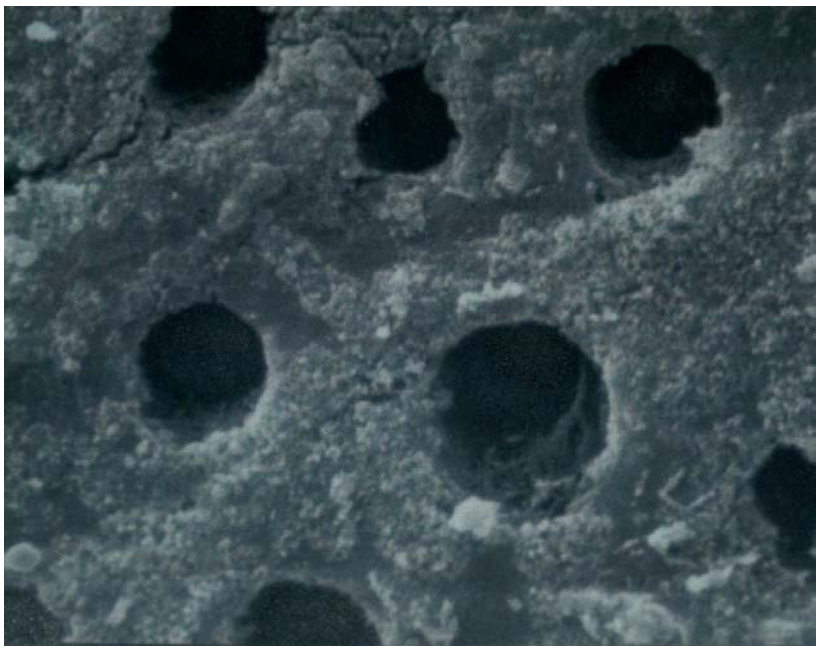


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Figura 2 – Dentina condicionada com ácido fosfórico 35% e tratada com hipoclorito de sódio 10% gel. Magnificação de 5000x. Note a presença de porções orgânicas no substrato e um aspecto misto de porção orgânica e mineral da estrutura remanescente.

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Data: Fri, 14 Jan 2005 20:25:59 -0200

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Parte(s): 2 Manuscript Soeiro et al.doc application/msword 143.69 KB

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