PATRÍCIA ALMADA SACRAMENTO

"Degradação da interface de união utilizando diferentes sistemas adesivos em dentes decíduos e permanentes tratados endodonticamente – estudo in vitro."

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

Orientadora: Profa. Dra. Regina Maria Puppin-Rontani

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DEDICATÓRIA

*Dedico esta dissertação ao meu Pai **Paulo** e à minha mãe **Célia**, que com tanto carinho me apoiaram e incentivaram as minhas decisões. Muito obrigada!! Amo muito vocês!!!*

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EPÍGRAFE

"O medo nos afasta das grandes derrotas, mas também das grandes vitórias e das boas oportunidades"

RESUMO

Durante o tratamento endodôntico são utilizadas substâncias irrigadoras com a finalidade de auxiliar a desinfecção dos condutos, sendo o hipoclorito de sódio a mais utilizada. Estas substâncias modificam a superfície do substrato dentinário, o qual é utilizado na formação da camada híbrida e união dos sistemas adesivos à dentina. Os estudos são controversos quanto à alteração da resistência de união em cavidades previamente tratadas com substâncias irrigadoras utilizadas na endodontia. Desta forma os objetivos destes estudos *in vitro* foram avaliar a resistência de união de sistemas adesivos na dentina de profundidade média de dentes decíduos e permanentes previamente tratadas com uma substância irrigadora utilizadas no tratamento endodôntico e a possível degradação da união resina/substrato por até 90 dias de armazenamento em água destilada. Neste estudo foram utilizados noventa terceiros molares e noventa molares decíduos livres de cárie, fraturas ou trincas. Dezoito grupos de dentes permanentes e dezoito grupos de decíduos foram delineados de acordo com os sistemas adesivos Adper Single Bond 2 (SB), Clearfil Protect Bond (CP), Adper Prompt L-Pop (APL); com ou sem irrigação da superfície de união com NaOCl (0.5%) e tempo de armazenamento de 24h, 45 dias, 90 dias. A dentina média foi exposta para realização do procedimento de união. Os dentes foram restaurados com resina foto-ativada Charisma cor A3.5 e submetidos ao teste de microtração (µTBS). O modo de falha, presença de *tags* e a interface resina/dentina foram avaliados por microscopia eletrônica de varredura (MEV). Os dados (µTBS) foram analisados pelo teste estatístico análise de variância e teste de Tukey ($p<0,05$). O modo de fratura, a presença de *tags* e a interface de união resina/dentina foram avaliados por MEV e os dados obtidos foram submetidos ao teste Qui-quadrado ($p<0.05$). Os resultados demonstraram que para dentes decíduos, APL mostrou o menor valor de resistência por µTBS. Ocorreu um significativo decréscimo nos valores de µTBS após 90 dias de armazenamento somente quando os dentes decíduos e permanentes não foram submetidos à irrigação com NaOCl. Para os dentes permanentes, somente APL mostrou um decréscimo nos valores de µTBS quando NaOCl foi usado. Após 90 dias de armazenamento, a presença de *tags* na maioria das amostras diminuiu, exceto para o SB na dentina decídua, o qual exibiu 100% de presença. Dentro das condições deste estudo, conclui-se que considerando-se dentes decíduos e permanentes, a irrigação com NaOCl não afetou a resistência de união após 90 dias de armazenamento, exceto quando APL foi utilizado na dentina de dentes permanentes; em adição, para dentes decíduos o fator mais importante na adesão foi o sistema adesivo utilizado, entretanto para dentes permanentes a interação sistema adesivo e NaOCl foi fator significativo.

PALAVRAS-CHAVE: sistemas adesivos, hipoclorito de sódio, dente decíduo, dente permanente, microtração, MEV, interface de união.

ABSTRACT

During the endodontic treatment irrigant substances with the purpose of assisting the disinfection of the ducts are used, being the most used the sodium hypochlorite. These substances modify the teeth substrata, which is used in the formation of the hybrid layer and bonding of the adhesive systems to the dentine. The studies are controversial of the shear bond strength in cavities previously treated with irrigants substances used in endodontic treatment ***Objective:*** To evaluate, by microtensile bond strength (μTBS) and the resin/dentin interface morphology, the 90-day-bonding degradation of primary and permanent dentin after simulating the irrigation procedure during endodontics, using different adhesive systems. ***Methods:*** Ninety sound third molars and ninety sound primary molars were divided into eighteen permanent and eighteen primary dentin groups, according to: adhesive systems (Adper Single Bond 2-**SB**, Clearfil Protect Bond-**CP**, Adper Prompt L-Pop-**APL**); dentin treatment (with or no 0.5% NaOCl irrigation) and storage time (24h, 45 days, 90 days). The middle dentin was exposed; NaOCl irrigation was accomplished according to the group, and restored with composite (Charisma-Shade-A3.5). Sticks with $1mm^2$ section area were obtained and submitted to μTBS test and obtained data were submitted to ANOVA and Tukey tests ($p<0.05$). The failure mode, resin tags presence and the resin/dentin interface were evaluated by Scanning Electron Microscopy (SEM) and obtained data were submitted to Chi-square test ($p<0.05$). ***Results:*** For primary dentin, APL showed the lowest μTBS values, regardless irrigation and storage time. For permanent dentin, μTBS values of APL decreased when NaOCl was used, and CP and SB showed similar values. There was significant decrease in μTBS at 90-day storage when primary and permanent dentin was not irrigated with NaOCl, however until 45-day storage did not affect μTBS values. The often failure mode was the mixed for both permanent and primary dentin. At 90-day storage, the resin tags presence generally decreased, except for SB in primary dentin that it remains almost 100%. **Conclusions:** concerning permanent and primary teeth, NaOCl irrigation did not affect the bonding strength after 90-day storage, except when APL was used in permanent dentin; in addition, for primary teeth the most important factor in adhesion is the adhesive system used, while for permanent teeth the interaction between adhesive system and NaOCl irrigation is significant.

KEY WORDS: adhesive systems, sodium hypochlorite, primary tooth, permanent tooth, microtensile bond strength, SEM.

SUMÁRIO

INTRODUÇÃO GERAL

O tratamento endodôntico tem como principal objetivo a descontaminação do sistema de canais radiculares, o qual é mantido através do selamento apical e coronário, contribuindo para o sucesso da terapêutica (Sritharan, 2002).

Durante o tratamento endodôntico, o saneamento do sistema de condutos é produzido pela limpeza mecânica e remoção de restos necróticos, desinfecção e modelagem das paredes dos condutos para posterior obturação. Microrganismos estão presentes em toda a extensão do sistema de canais radiculares, incluindo anastomoses e encontrados em uma variação de profundidade de 300 µm no túbulo dentinário (Horiba *et al*., 1990). Como as variações da anatomia interna do sistema de condutos dificultam a ação dos instrumentos em todas as paredes, faz-se necessário a utilização de uma solução química auxiliar para a irrigação (Spanó, 1999). Este é um procedimento importante na eliminação de microrganismos e controle da infecção endodôntica (Berber *et al*., 2006).

Entre as soluções mais usadas na irrigação endodôntica encontra-se o hipoclorito de sódio (NaOCl), produzindo clarificação, dissolução de tecido orgânico, saponificando e transformando aminas em cloraminas, desodorizando e ainda contando com a ação antimicrobiana (Spanó, 1999). Entretanto as soluções irrigadoras utilizadas no tratamento endodôntico podem interferir na qualidade da adesão, resistência (Edermier *et al*., 2004) e durabilidade de restaurações em resina composta.

Supõem se que o NaOCl atua dissolvendo a camada de colágeno e portanto, podendo interferir na formação da camada híbrida e posterior restauração do dente com materiais adesivos (Gürgan *et al*., 1999), os quais dependem da camada de fibrilas colágenas para a formação da camada híbrida (Nakabayashi *et al*., 1992).

Alguns autores verificaram que o insucesso do tratamento endodôntico está mais relacionado à falha nas restaurações do que propriamente à falha na terapêutica endodôntica, devido à recontaminação do sistema de condutos (Ray & Trope, 1995; Hommez *et al*, 2002).

Muitos materiais restauradores vêm sendo avaliados na tentativa de se evitar a infiltração coronária e a recontaminação radicular. Os sistemas adesivos e os materiais restauradores resinosos, quando corretamente utilizados e indicados, reduzem significativamente a formação de fendas entre o material e a estrutura dentária, evitando a microinfiltração (Nakabayashi *et al*., 1982).

A união ao esmalte já é considerada como um procedimento consolidado (Nakabayashi & Pashley, 1998; Kugel & Ferrari, 2000), mas a dentina por ser um substrato de maior complexidade e menor heterogeneidade apresenta dificuldades para a eficiente adesão e durabilidade da união. A complexidade do substrato dentinário pode ser expressa pela composição mineral, orgânica e fluido dentinário, nos diferentes locais, de um mesmo dente ou em diferentes dentes, como decíduos e permanentes. Sabe-se que os túbulos dentinários variam em número, podendo representar desde 5% até 22% da área total da superfície da dentina, dependendo da profundidade em relação à polpa. Além disso, a dentina possui áreas hipermineralizadas (dentina peritubular), ricas em minerais (hidroxiapatita), com poucas fibras colágenas, ao contrário da dentina intertubular, rica em fibras colágenas, (Gruverman *et al*., 2007). Em acréscimo, fatores como a modificação do substrato dentinário pode afetar o procedimento de união. A adesão à dentina não depende somente dos sistemas adesivos e materiais restauradores, mas também da qualidade do substrato dentinário (Nakajima *et al*., 1995; Pashley *et al*., 1999; Cederlund *et al*., 2002).

Segundo Yamauti *et al.*, (2003), a hidrólise do colágeno poderia ser uma das razões para a degradação da união, uma vez que o efeito do NaOCl sobre a dentina pode produzir a diminuição da resistência de união sistemas adesivos/dentina, de acordo com o aumento do tempo de tratamento da dentina. Dessa forma, os dentes tratados endodonticamente apresentam a dentina com propriedades modificadas comparada aos dentes não submetidos ao tratamento pela ação de agentes desinfetantes.

A literatura é controversa, há relatos de aumento (Wachlarowicg *et al.,* 2007) e diminuição (Nikaido *et al*., 1999; Ozturk & Ozer, 2004) da resistência da união dentina/material, dependendo do agente utilizado como irrigador. Essa controvérsia pode residir na variação da metodologia empregada nos diferentes estudos, na variabilidade do tratamento da superfície (Sano *et al*., 1994), nas características intrínsecas da dentina, na quantidade de umidade, na densidade de túbulos (Giannini *et al*.,2001) e conteúdo inorgânico.

Para a adesão ao substrato dentinário ocorrer é necessário a formação da camada híbrida, que representa um tecido reticular híbrido, formado quando a dentina é desmineralizada pelo ataque ácido e é penetrada por monômeros resinosos que polimerizam *in situ* (Nakabayashi *et al*., 1982; Nakabayashi *et al*., 1992; Pashley, 1992). Essa camada representa a união micro-mecânica do substrato dentinário aos materiais restauradores adesivos (Wang & Nakabayashi, 1991; Walshaw & McComb, 1994). Nakabayashi *et al.,* (1982), descreveram o primeiro sistema adesivo com sucesso na dentina através da técnica de condicionamento ácido total, e foram os primeiros pesquisadores a utilizar o termo "camada híbrida", para designar o embricamento de monômeros resinosos que penetraram em profundidade na dentina desmineralizada e polimerizaram ao redor das fibrilas de colágeno. Os sistemas adesivos atualmente apresentam um, dois ou três passos de aplicação, sendo do tipo *etch & rinse* ou auto-condicionantes. Nos sistemas adesivos auto-condicionantes o condicionamento ácido é realizado de maneira simultânea à aplicação do *primer*; os monômeros são capazes de desmineralizar o substrato e infiltrá-lo ao mesmo tempo, diferindo de outros sistemas em que o condicionamento ácido exige uma etapa separada para a desmineralização. Pesquisas sobre sistemas adesivos permitiram o desenvolvimento de um adesivo auto-condicionante de passo único, Adper Prompt L-Pop (ESPE Dental AG, Seefeld, Germany) cuja comercialização iniciou-se em 1999. Os fabricantes uniram o *primer* ácido ao adesivo. Neste sistema adesivo, os fotoiniciadores são mantidos em compartimentos separados dos demais componentes, havendo a incorporação destes nos demais componentes da solução apenas no momento da aplicação.

A efetividade de união de materiais adesivos pode ser avaliada através de diferentes testes de resistência de união, sendo um deles o teste de resistência à tração (microtração). Este teste utiliza áreas superficiais pequenas, em relação aos ensaios convencionais, as quais são capazes de conter poucos defeitos na interface dentina/resina (Sano *et al*., 1994). Em adição, essa técnica é capaz de prover várias amostras de um mesmo dente, portanto, permitindo a melhor comparação das amostras (Burrow *et al*., 2002.). Sano *et al*. (1994) relataram que o teste de microtração, por utilizar espécimes com áreas menores que 1 mm^2, produz uma distribuição mais uniforme do estresse, com menos defeitos. Pashley *et al*. (1999) listaram como vantagens do teste de microtração a alta resistência de união interfacial, habilidade de medir a resistência de união em pequenas regiões pré-determinadas, diminuindo as variabilidades encontradas em único dente. Este teste também permite avaliar a adesão em superfícies regulares e facilita a análise das falhas observadas por microscopia eletrônica de varredura (Armstrong *et al*., 1998).

Dentes decíduos são freqüentemente submetidos ao tratamento endodôntico, cuja substância irrigadora mais utilizada é o NaOCl, e devido às particularidades desse substrato em relação aos permanentes, faz-se necessário estudos que demonstrem o desempenho da união material/dentina tratada em dentes decíduos. Todos os parâmetros estabelecidos para a preparação de um adequado substrato dentinário para adesão têm sido estudados em dentes permanentes e os resultados extrapolados para dentes decíduos, sem levar em consideração a diferença na composição (mineral e orgânica) e morfologia de ambos os substratos (Nor *et al*., 1997 Borges *et al*., 2006).

Ainda, a incerteza da longevidade da união dente/restauração tem sido a preocupação de muitos pesquisadores. Se levado em consideração a necessidade de se utilizar substâncias que alteram a superfície e a estrutura físico-química do substrato dentário, como o NaOCl, talvez a degradação da interface, nesses casos ocorra de modo mais intenso e visível.

PROPOSIÇÃO GERAL

Os objetivos gerais desta Dissertação[1] foram:

Avaliar a resistência de união pelo teste de microtração do adesivo/substrato dentinário decíduo e permanente, tratado com uma substância irrigadora utilizada no preparo químico-mecânico durante o tratamento endodôntico, bem como avaliar os sítios de fratura, e interfaces de união quando submetidos a armazenamento por até 90 dias. Foram utilizados nestes estudos três sistemas adesivos. As variáveis analisadas foram a resistência de união por microtração e a morfologia da interface de união por MEV, para dentes decíduos e permanentes.

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

Capítulo 1

Bonding degradation and morphology of resin/dentin interface in teeth irrigated with NaOCL using different adhesive systems – An in vitro study[2].

Capítulo 2

Short period of bonding degradation and morphology of resin/dentin interface of primary teeth irrigated with NaOCL using different adhesive systems – An in vitro study[3]**.**

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

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CAPÍTULO 1

Bonding degradation and morphology of resin/dentin interface in teeth irrigated with NaOCL using different adhesive systems – An in vitro study[4].

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Bonding degradation and morphology of resin/dentin interface in teeth irrigated with NaOCL using different adhesive systems – An in vitro study.

ABSTRACT

Purpose: To evaluate NaOCl treated dentin/resin-bonding degradation during 90-day storage time, using three adhesive systems by microtensile bond strength (μTBS) and the resin/dentin interface morphology. ***Methods:*** Ninety sound third molars were used. Eighteen groups were assigned according to adhesive system (Adper Single Bond 2 -SB, Clearfil Protect Bond -CP, Adper Prompt L-Pop -APL); dentin treatment (NaOCl 0.5%) and storage time (24h, 45d, 90d). The middle dentin was exposed; NaOCl irrigation was accomplished according to the group, and restored with composite (Charisma-Shade-A3.5). Sticks with $1mm^2$ section area were obtained and submitted to μTBS test. The obtained data were submitted to ANOVA and Tukey tests ($p<0.05$). The failure mode, resin tags presence and the resin/dentin interface were evaluated by Scanning Electron Microscopy (SEM) and obtained data were submitted to Chi-square test ($p<0.05$). ***Results:*** There was significant decrease in μTBS values at 90 days storage only when dentin was not irrigated with NaOCl. APL showed decrease in μTBS values when NaOCl was used. After 90 days of storage, the resin tags presence generally decreased.

CLINICAL SIGNIFICANCE: NaOCl irrigation did not affect the bonding strength at 90 days of storage; therefore, the interaction between adhesive system and NaOCl irrigation is a significant factor to be considered on bond strength of permanent teeth.

Introduction

The bonding concept in dentin is based on micromechanical retention: monomers interlocking the exposed collagen network of the demineralized superficial dentin, and upon polymerization, results in the formation of a hybrid layer.[1,2] Hybrid layer has been described as responsible for improving the bond strength between dentin and adhesive material.[3,4]

Although the quality of the substrate is an important factor for the appropriate formation of hybrid layer.[5,6] Some clinical procedures can modify the substrate, like the irrigation with sodium hypochlorite (NaOCl) used for endontontic treatment[7] due to its antibacterial effect and organic tissue dissolution.[8,9] Sodium hypochlorite solution is an oxidizing agent that can alters the configuration or removes the organic components of dentin, producing deproteinization of collagen fibrilis.

In addition, whether NaOCl is effective to remove retained residual pulpal components, on the other hand it may play a role in decreasing the physical and mechanical properties of dentin[10]. Theoretically, the sodium hypochlorite breaks down to sodium chloride and oxygen, which could provide oxidation of some components in the dentin matrix[11] and consequently decrease the elastic modulus and flexural strength of dentin.[12] It could also affect the resin penetration into the dentin structure and/or the polymerization of monomers in the demineralized dentin, interfering in the restoration performance.[12]

Some studies have been carried out to evaluate the bond strength of resin materials upon the permanent dentin previously subjected to pulp therapy with NaOCl irrigation. Regardless of the adhesive systems[7,13] or resin luting agents[14] tested, there was a trend to decrease the bond strength. Collagen hydrolysis by NaOCl can be one of the reasons for the bond strength degradation.[15] However, Wachlarowicz *et al*.[16] reported that NaOCl treatment increased the bond strength of specific adhesive system. Interestingly, the long or even short-term effect of NaOCl irrigation on bonding adhesive performance has not been evaluated. The bonding degradation can lead to microleakage and determine the failure of the restoration and consequently of the endodontic treatment.[17] Therefore, considering the success of endodontic treatment, a successful final restoration is also important for long-term clinical success.[18]

The aim of this study was to analyze the bonding degradation after simulating of 0.5% NaOCl irrigation among three different adhesive systems in human permanent sound dentin, using microtensile bond strength test (μTBS), in three different period of time. The failure sites and bonded interfaces were also evaluated using Scanning Electron Microscopy (SEM). The hypothesis tested for permanent dentin is that there is influence of storage time, adhesive systems and NaOCl irrigation on μTBS and at resin/dentin interfaces.

Materials and Methods

Specimen preparation and bonding procedures for Microtensile Bond Strength Test and SEM Evaluation of the Dentin/Resin Interface

This study was conducted after approval of the Ethical Committee of Piracicaba Dental School, University of Campinas (Protocol 083/2005). Ninety sound human third molars teeth were selected, cleaned and stored in saline solution at 4° C for no longer than 2 months after extraction. The teeth were donated by the patients at the clinical of Piracicaba Dental School, State University of Campinas and private clinics. The localization of middle dentin was determined by X-Ray, using parallelism technique and an orthodontic wire was used as a scale during the X-Ray taking, to find the X-Ray image/real size ratio. On the X-Ray image, a parallel line to the long axis of teeth was delimited in the center of the crown in order to reach the middle dentin, which was considered as a half of the distance between the top of the dentin and the roof of the pulp chamber. Each tooth was ground flat in the occlusal surface, until middle dentin be reached on a water-cooled mechanical polisher (Metaserv 2000, Buehler, UK LTD, Lake Bluff, IL, USA) using 600-grit abrasive papers.

The specimens were randomly assigned into 18 groups according the adhesive system, dentin treatment and period of storage in distilled water (Table1). The adhesive systems, the way they were used in this study and components are described on Table 2. Charisma light-cured composite resin, shade A3.5 (Heraus Kulzer, Wehrheim, Germany – batch # 010208) was used to complete the bonding procedure.

Before the restorative treatment, the roots of the teeth were cut off 1mm beyond the cement-enamel junction. The pulp horns and enamel areas were identified with a pen marker. In all groups, the dentin was treated with the adhesive systems according to the manufacturers' directions. Additionally, according to each group, 0.5% sodium hypochlorite solution (NaOCl) was applied for thirty minutes in an ultrasound bath before the adhesive treatment to simulate the irrigation procedure during an endodontic treatment. After that, the adhesive systems were applied and a composite resin block (5 mm height) was built on the bonding surface, using the incremental technique. Each layer of composite resin was individually light-cured for 20 seconds, with an Elipar Tri-light unit (ESPE – America Co., Seefeld 82229 - Germany). Light intensity was periodically measured

($470mW/cm^2$). The pulp chamber was equally filled up. The specimens were placed in distilled water at 37º C for 24 hours.

Microtensile Bond Strength Test

For the microtensile bond strength test, three teeth for each group were used (n=3). Each teeth/restoration set was buccal-lingual and mesio-distal sectioned (Isomet, Buheler, Lake Bluff, IL, USA) in order to obtain sticks with a cross-section surface area around 1.0 mm^2. The cross-section area was verified with electronic digital caliper (Mitutoyo, Japan) and the sticks were observed with a stereomicroscope (Model XLT30, Nova Optical Systems, SP, Brazil) at 25X magnification in order to verify if the stick didn't have any color mark that indicated enamel area or pulp horns. It was obtained 20 sticks an average per tooth, and a total of 12-14 adequate sticks per tooth were used. The sticks were immersed in distilled water and stored according to the groups at 37º C. Distilled water was changed weekly.[19]

Each stick was attached to a microtensile jig with a cyanocrilate adhesive (Super Bond, Loctite, Itapevi, SP, Brasil) and then submitted to a tensile loading in a universal testing machine (Instron model 4411, Canton, MA, USA) at a cross-head speed of 0.5 mm/min. The microtensile bond strength (MPa) values were calculated from the peak load at failure divided by a cross-section area of adhesion. Means and standard deviation were calculated.

Failure Mode Evaluation

A calibrated examiner evaluated all the sticks tested at 40X magnification to on stereomicroscope (Model XLT30, Nova Optical Systems, SP, Brazil) initially classify the failure sites as cohesive (in dentin or in the material), adhesive, or mixed failure. To facilitate this analyzes 2% basic fucsin was dropped and left at the bonding area for 10 seconds, then, washed and tissue paper dried. Five representative sticks of each group were mounted on aluminum stubs,[20] sputter-coated with gold (Balzers-SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL- JSM 5600LV, Tokyo, Japan) and with a magnification of 70X, and 1100X.

SEM Evaluation of Dentin/Resin Interface

For the SEM evaluation of dentin/resin interface, two third permanent molars were used for each group described above (n=4). The bonding procedure was done according the described groups (Table 1 e 2). Each teeth/restoration set was buccal-lingual sectioned (Isomet, Buheler, Lake Bluff, IL, USA) in order to obtain slices. Four slices of each teeth, in the buccal-lingual direction were obtained. Two slices of each tooth were randomly chosen for the SEM evaluation of dentin/resin interface. The slices were immersed in distilled water and stored according to the groups at 37º C. Distilled water was changed weekly.[19] Only one side of each slice was prepared for SEM and it was polished on a water-cooled mechanical grinder (Metaserv 2000, Buehler, UK LTD, Lake Bluff, IL, USA) using 320-, 400-, 600- and 1200-grit silicon carbide abrasive paper (Carbimet Disc Set, # 305178180, Buehler, UK LTD) and with a 3-µm and 1-µm diamond paste. The specimens were ultrasonically cleaned in distilled water for 3 min in each exchange of carbide abrasive paper and between each diamond paste with detergent solution. Then, they were demineralized with 35% phosphoric acid for 15 s, washed with distilled water for 30s and dried with a tissue paper. Next, they were deproteinized with 1% NaOCl for 10 min, washed in ultrasound bath and dried with a tissue paper. The specimens were left dried in a dissecator, for 24h at room temperature. After this period, the specimens were mounted on aluminum stubs, sputter-coated with gold (Balzers-SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL- JSM 5600LV, Tokyo, Japan) with a magnification of 600X, 1200X, 2500X, and 3300X. A calibrated examiner (86% coincidence intra-examiner) evaluated the interface resin/dentin. The electromicrographs were classified according presence or absence of remaining resin tags, since all they presented hybrid layer.

Statistical analysis

Bond strength data were submitted to ANOVA. When the F ratio of bond strength test was significant, Tukey test was used to compare specific mean values at $p<0.05$. SEM resin/dentin interface data and failure mode data were submitted to Chi-Square test $p< 0.05$. Spearman Correlation test was used for correlation failure mode and μTBS values ($p<0.05$).

Results

There was significant interaction between the NaOCl irrigation and storage time ($p=0.0332$). μTBS values were not influenced by storage time until 45 days for groups without NaOCl irrigation, but, in 90 days storage it decreased significantly (Table 3).

In addition, ANOVA test showed significant interactions between NaOCl irrigation and adhesive systems ($p=0.0234$). In the groups that NaOCl was used, APL showed the lowest means values of μTBS, and CP was similar to SB. In the groups that NaOCl was not used there was no difference between the adhesive systems (Table 4).

Concerning failure sites evaluation, in general, the mixed failure was the most frequent (Figure 1 and 2). For the failure mode there was significant statistically difference when NaOCl irrigation and adhesive systems was analyzed. The highest percentage of cohesive failure on material were verified when CP was used (Figure 1 and 3). When APL and SB was used, mixed failure was the most frequent (Figure 1 and 2). The adhesive failure mode was often when NaOCl was not used (Figure 1 and 4) and in relation to cohesive failure in dentin, it was more frequent when NaOCl was used (Figure 1 and 5).

SEM images of all adhesive systems showed adequate adaptation and well established and uniform hybrid layer formation in all groups. CP group showed thinner hybrid layer than APL and SB (Figure 6). The SEM showed that the thickness resin tags in the opened tubules of the SB and APL groups were similar, but SB presented a higher number of resin tags and CP showed thinner and lower number than SB and APL (Figure 6).

The percentage of presence of remaining resin tag at the interface resin/dentin is shown in Figure 7. Comparing the results between 24 hours and 90 days, after 90 days storage SB with NaOCl treatment was noted a little reduction in the remaining tags presence; CP showed decrease of the tags presence regardless of the treatment. For APL, the NaOCL decreased 50% the presence of tags, which were maintained in 90 days of storage.

There was a weak, but significant correlation between failure mode and μTBS values ($r=0.40896; p=0.0021$). The highest μTBS values were obtained in specimens with dentin cohesive failure mode.

Discussion

Sodium hypochlorite in widely range concentrations has been used as a potential irrigant solution in endondontic treatment due to its disinfectant potential. In that case, the dentin is submitted to NaOCl for a length time. In order to simulate the irrigation procedure, this study used a 0.5% NaOCl solution to immerse the dentin samples for 30 minutes, simulating the clinical time.[21] Therefore, important features are accomplished when the irrigating solution is used in endodontic treatment: all canal length is bathing on the solution and also all dentin surfaces, from the apices throw the coronal dentin.

In this study, there was no statistically significant difference on µTBS values for all storage time with NaOCl irrigation, and until 45 days of storage without NaOCl irrigation. Also, the µTBS values among treatments in the same periods were similar (Table 3), these data corroborate Uno & Finger[22] and Arias *et al.,*[23] although the storage time of these studies were lower. There was significant decrease in µTBS values after 90 days storage only when dentin was not irrigated with NaOCl, these data corroborate others studies.[24-25] It seems that the NaOCl irrigation has protected the bonding area from degradation. It is believed that inorganic content changes can be resulted of some hydroxyapatite molecules reaction with sodium hypochlorite resulting in calcium hypochlorite and sodium phosphate, as follows: $Ca_{10}(PO)_4(OH)_2$ + $Na(ClO)_2$+ Na_3PO_4. This reaction can solubilize some hydroxyapatite molecules, causing a slow dissolution of encapsulated collagen of dentin, the so called "organic 2" component that covers the hydroxyapatite nanocrystals and 2 – 5 nm thick,[26] leaving unbound hydroxyapatite crystals[27-28] and reveling a mineral surface rich in hydroxyl, carbonate and phosphate groups.[27] In this way, after dentin NaOCl irrigating, the etching acid would increase the area of adhesion by exposition of unbound hydroxyapatite crystals and resin monomers are capable of penetrating within this area. In addition, the hydroxyl, phosphate and carbonate groups can be available for chemical bonding when used adhesive systems containing acidic monomers.[29] All this facts would protect the bonding area from degradation.

There was a significant interaction between the adhesive systems and NaOCl irrigation (Table 4). In NaOCl irrigation groups there was no statistically significant difference between one-bottle etch & rinse adhesive system (SB) and self-etching adhesive system (CP), providing the highest values of microtensile bond strength. The SB combines the primer and adhesive in one solution to be applied after etching the substrate with 37% phosphoric acid (pH=0.3) for 15-20 seconds.[30] So, this adhesive system create a mechanical interlocking with etched dentin by means of resin tags, adhesive lateral branches and hybrid layer formation, and show higher strength values to bond.[31] The higher values for CP can be explained by the mild potential of demineralization of the primer (pH=1.9) and adhesive (pH=2.8) of this adhesive system. The primer demineralizes dentine only to a depth of 1 µm. Moreover this superficial demineralization occurs only partially, keeping residual hydroxyapatite still attached to the collagen. The preservation of hydroxyapatite within the submicron hybrid layer may serve as receptor for additional chemical bonding. Furthermore, CP contains MDP (methacryloxydecyl dihydrogen phosphate), which has a chemical bond potential to the calcium in the residual hydroxyapatite.[32] The reduced thickness should not decrease the adhesive properties; a demineralization of approximately 2 µm is sufficient for a good bonding.[33] However, when compared both self-etching adhesive systems, all-in-one (APL)- and two-bottles (CP) adhesive systems, in the groups with NaOCl irrigation there was a significant difference between both ones, corroborating the results obtained by Kaaden *et al*.[34] The difference in µTBS values for both self-etching adhesive systems can be due to some differences between their components. APL has a high amount of water in the primer, raging from 70% to 80% wt as solvent and it is responsible for the lower µTBS values. Because of the high water amount and its low vapor pressure, the water does not evaporate easily and completely during the bonding procedure. The residual water can remain within the adhesive interface, and also in the hybrid layer, it compromises the monomer cure. This high water content may result in competition between the monomer (HEMA) and the remaining water inside the demineralized dentine. In this way, the bond strength performance may be affected by the residual water.[35] In addition, phase separation of the hydrophobic and hydrophilic monomer components causing blister-like spaces and globule formation of the resin within the hybrid layer has been observed in overly wet conditions.[36] The water excess may also dilute the primer and reduce its effectiveness, resulting in lower bond strength.[34]

Also, concerning the interaction between adhesive system and NaOCl irrigation the results demonstrated that APL was sensible to use NaOCl irrigation, since the µTBS values of APL were lower for dentin irrigated with NaOCl when compared with the others adhesive systems. Giovannone *et al.*[10] carrying out a push-out test observed similar results when compared self-etch and etch & rinse adhesive systems, although they found significant difference between the groups treated and not treated with NaOCl irrigation.

The resin tags are infiltration of resin monomers polymerized into the tubule that forms an intratubular digitation and provides mechanical anchoring, which helps bonding. Mechanical anchoring is greater when the resin is in contact with the tubule walls over a large area. This mechanical property can be improved when the lateral branches are filled up with monomers.[37-38]

The configuration of the different hybrid layer and resin tags is due to the different values of pH of the adhesive systems. Van Meerbeeck *et al.*, (2001)[32] subdivided the adhesive systems into “strong”, “intermediary strong” and “mild” according to their etching aggressiveness. SB (pH=0.3) and APL (pH=0.8) are considering "strong" and CP (primer pH=1.9 and bond pH=2.8) is considering "mild" aggressiveness. The more aggressive the adhesive are, greater demineralization they cause, and higher hybrid layer and large thickness resin tags in the opened tubules are formed.

The morphology and the remaining resin tags results from their interaction with the organic elements of peritubular dentin after demineralization and is influenced by the resin composition. As showed in Figure 6, the etch & rinse adhesive system (SB) presented greater contact with the opening and wall tubules and greater thickness of hybrid layer. It can be owned to the acid conditioner that causing more demineralization, roughness and a higher wettability can interfere on the bond strength in dentin.[39-40] In addition, the NaOCl treatment not influenced the resin tag morphology and formation of hybrid layer.

Further studies concerning long-term degradation and *in vivo* studies are needed to clarify the interactions of self-etching primers and all-in-one- and two-bottles adhesive systems with dentin treated with NaOCl considering the esthetic restorations performance.

Conclusion

Based on the results obtained and considering the limitations of this study it can be concluded that:

The NaOCl cannot be deleterious for adhesive/dentin bonding, since at 90 days of storage the µTBS values did not decreased in the irrigated groups. Regarding the adhesive systems, solely APL was affected by NaOCl irrigation; it had a decrease in µTBS values when NaOCl irrigation was used. The failure mode is affected by the adhesive system and the treatment of the substrate, the morphology of the resin tags and hybrid layer were affected only by the adhesive systems, but the percentage of the remaining resin tags were affected by the storage time and treatment.

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References

1- Nakabayashi N, Ashizawa M, Nakamura M.Identification of a resin-dentin hybrid layer in vital human dentin created in vivo: durable bonding to vital dentin. *Quintessence Int*. 1992; 23(2): 135-41.

2- Perdigao J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F. Effect of a sodium hypochlorite gel on dentin bonding. *Dent Mater*. 2000; 16(5): 311-23.

3- Gwinnett AJ. Quantitative contribution of resin infiltration/hybridization to dentin bonding. *Am J Dent*. 1993; 6(1): 7-9.

4- Ferrari M, Mason PN, Vichi A, Davidson CL. Role of hybridization on marginal leakage and bond strength. *Am J Dent*. 2000; 13(6): 329-36.

5- Pashley DH, Sano H, Ciucci B, Yoshiyama M, Carvalho RM. The microtensile bond test: a review. *J Adhes Dent.* 1999; 1(4): 299-309.

6- Cederlund A, Jonsson B, Blomlof J. Do intact collagen fibers increase dentin bond strength. *Swed Dent J.* 2002; 26(4): 159-166.

7- Nikaido T, Takano Y, Sasafuchi Y, Burrow MF, Tagami J. Bond strengths to endodontically-treated teeth. *Am J Dent*. 1999.; 12(4): 177-80.

8- Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. *Dent Clin North Am*. 1990; 34(1): 13-25.

9- Zehnder M, Grawehr M, Hasselgren G, Waltimo T. Tissue-dissolution capacity and dentin-disinfecting potential of calcium hydroxide mixed with irrigating solutions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2003; 96(5): 608-13.

10- Giovannone T, Migliau G, Bedini R, Ferrari M, Gallottini L. Bond strength to deep coronal dentin: effect of bonding strategies. *Minerva Stomatol.* 2007; 56(3): 105-14.

11- Yui CK, Garcia-Godoy F, Tay FR, Pashley DH, Imazato S, King NM, Lai SC. A nanoleakage perspective on bonding to oxidized dentin. *J Dent Res* 2002; 81: 628-632.

12- Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J*. 2001; 34(2): 120-32.

13- Ozturk B, Ozer F. Effect of NaOCl on bond strengths of bonding agents to pulp chamber lateral walls. *J Endod*. 2004; 30(5): 362-5.

14- *Ari H, Yaşar E, Belli S. Effects of NaOCl on bond strengths of resin cements to* root canal dentin. *J Endod*. 2003; 29(4): 248-51.

15- Yamauti M, Hashimoto M, Sano H, Ohno H, Carvalho RM, Kaga M, Tagami J, Oguchi H, Kubota M. Degradation of resin-dentin bonds using NaOCl storage. *Dent Mater.* 2003; 19(5): 399-405.

16- Wachlarowicz AJ, Joyce AP, Roberts S, Pashley DH. Effect of endodontic irrigants on the shear bond strength of epiphany sealer to dentin. *J Endod*. 2007; 33(2): 152-5.

17- Hommez GM, Coppens CR, De Moor RJ. Periapical health related to the quality of coronal restorations and root fillings. *Int Endod J*. 2002; 35(8): 680-9.

18- Erdemir A, Ari H, Gungunes H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. *J Endod.* 2004; 30(2): 113-6.

19- Fraga RC, Andrade SJ, Martins LRM, Lovadino JR. Hidrólise e resistência ao cisalhamento de uma resina composta polimerizada em condições úmidas (simulação do isolamento relativo). *Rev Bras Odontol.* 1997; 54(1): 22-4.

20- el Kalla IH, Garcia-Godoy F. Bond strength and interfacial micromorphology of four adhesive systems in primary and permanent molars. *ASDC J Dent Child*. 1998; 65(3): 169-76.

21- Borges AF, Correr GM, Sinhoreti MA, Consani S, Sobrinho LC, Rontani RM. Compressive strength recovery by composite onlays in primary teeth. Substrate treatment and luting agent effects. *J Dent.* 2006; 34(7): 478-84. Epub 2006 Jan 19.

22- Uno S, Finger WJ. Function of the hybrid zone as a stress-absorbing layer in resin-dentin bonding. *Quintessence Int.* 1995; 26(10): 733-8.

23- Arias VG, Bedran-de-Castro AK, Pimenta LA. Effects of sodium hypochlorite gel and sodium hypochlorite solution on dentin bond strength. *J Biomed Mater Res B Appl Biomater*. 2005; 72(2): 339-44.

24- Sato H, Miyazaki M, Moore BK. Influence of NaOCl treatment of etched and dried dentin surface on bond strength and resin infiltration. *Oper Dent.* 2005; 30(3): 353-8.

25- Abo T, Asmussen E, Uno S, Tagami J. Short- and long-term in vitro study of the bonding of eight commercial adhesives to normal and deproteinized dentin. *Acta Odontol Scand.* 2006; 64(4): 237-43.

26- Brik A, Haskell E, Brik V, Scherbina O, Atamanenko O. Anisotropy effects of EPR signals and mechanisms of mass transfer in tooth enamel and bones. *Appl Radiat Isot.* 2000; 52(5): 1077-83.

27- Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. *Deproteination.Biomaterials*. 2001; 22(8): 793-7.

28- Fattibene P, Carosi A, De Coste V, Sacchetti A, Nucara A, Postorino P, Dore P. A comparative EPR, infrared and Raman study of natural and deproteinated tooth enamel and dentin. *Phys Med Biol.* 2005; 50(6): 1095-108. Epub 2005 Feb 23.

29- Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. *Dent Mater*. 2005; 21(10): 895-910.

30- Ferrari M, Gorraci G, Garcia-Godoy F. Bonding mechanism of three “one bottle”systems to conditionated and unconditionated enamel and dentin. Am J Dent. 1997;10: 224-230.

31- Tay FR, Gwinnett AJ, Pang KM, Wei SH. Structural evidence of a sealed tissue interface with a total-etch wet-bonding technique in vivo. J Dent Res. 1994 Mar;73(3):629-36.

32- Van Meerbeek B, Vargas M, Inoue S et al. Adhesive and cements to promote preservation dentistry. Oper Dent 2001. Supplement 6:119-144.

33- Nakabayashi N. Resin-reinforced dentin due to infiltration of monomers into the dentin at the adhesive interface. J Jap Dent Mater 1982; 1:78-81.

34- Kaaden C, Powers JM, Friedl KH, Schmalz G. Bond strength of self-etching adhesives to dental hard tissues. *Clin Oral Investig*. 2002; 6(3): 155-60. Epub 2002 Aug 9.

35- Jacobsen T, Söderholm KJ. Some effects of water on dentin bonding. *Dent Mater.* 1995; 11(2): 132-6.

36- Tay FR, Gwinnett AJ, Wei SH. The overwet phenomenon: a transmission electron microscopic study of surface moisture in the acid-conditioned, resin-dentin interface. *Am J Dent*. 1996; 9(4): 161-6.

37- Gwinnett A, Kanca J. Micromorphological relationship between resin and dentin in vivo and in vitro. *Am J Dent* 1992; 5: 19-23

38- Gregoire GL, Akon BA, Millas A. Interfacial micromorphological differences in hybrid layer formation between water- and solvent-based dentin bonding systems. *J Prosthet Dent.* 2002; 87(6): 633-41.

39- Toledano M, Osorio R, Perdigao J, Rosales JI, Thompson JY, Cabrerizo-Vilchez MA. Effect of acid etching and collagen removal on dentin wettability and roughness. *J Biomed Mater Res*. 1999; 47(2): 198-203.

40- Phrukkanon S, Burrow MF, Hartley PG, Tyas MJ. The influence of the modification of etched bovine dentin on bond strengths. *Dent Mater*. 2000; 16(4): 255-65.

Table 1 – Description of the groups according adhesive system, treatment and storage time.

Substrate treatment 0.5% NaOCl during 30 min	Storage time	Adhesive system		
		Adper Single Bond 2	Clearfil Protect Bond	Adper Prompt L-Pop
Without treatment	24h	G1	G2	G3
Without treatment	45 days	G4	G5	G6
Without treatment	90 days	G7	G8	G9
With treatment	24h	G10	G11	G12
With treatment	45 days	G13	G14	G15
With treatment	90 days	G16	G17	G18

Table 2 - Bonding Adhesive System, Composition, Manufacturer and 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	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 = 0.3 (etching acid) pH = 4.7 (primer + Bond)	a (15 s), b (30s), c, d, e, i (10 s)
Clearfil Protect Bond	Primer: water, MDP, MDPB, HEMA, Hidrophobic methacrylate, Bond: MDP, HEMA, Bis-GMA, Hydrophobic dimethacrylate, di-Camphorquinone, N-Diethanol-p-toluidine,silanated colloidal silica	Kuraray Medical, Kurashiki, Japan (Primer: 00017B) (Bond: 00027B)	pH = 1.9 (primer) pH = 2.8 (Bond)	f (20 s), e, g, i (10 s)
Adper Prompt L-Pop	Liquid 1 (red blister): Bis-GMA, Stabilizers Initiators based on camphorquinone Methacrylated phosphoric esters, Liquid 2 (yellow blister): Water 2-Hydroxyethyl methacrylate (HEMA) Polyalkenoic acid, Stabilizers	3M Dental Products, St. Paul, MN, USA (235117)	pH = 0.8 (mixed)	h (15s), e, I (10s)

*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-etching 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 3 - Microtensile bond strength values (MPa) in dentin treated or not with NaOCl irrigation and different storage times.

Treatment	Storage time		
	24h MS ± SD	45d MS ± SD	90d MS ± SD
With NaOCl	29.13 ± 6.71 Aa	28.17 ± 8.16 Aa	25.93 ± 4.31 Aa
Without NaOCl	30.63 ± 6.87 Aa	27.15 ± 4.48 Aa	20.73 ± 2.65 Ab

Similar capital letters means no statistical significant differences between averages, in column. Similar small letters means no statistical significant differences among averages, in row.

Table 4 - Microtensile bond strength values (MPa) of the adhesive systems used in dentin treated or not with NaOCl.

	Adhesive systems		
NaOCl irrigation	APL MS ± SD	CP MS ± SD	SB MS ± SD
With	20.67 ± 3.75 Ab	30.60 ± 2.89 Aa	31.96 ± 5.33 Aa
Without	23.21 ± 4.65 Aa	27.91 ± 4.30 Aa	27.39 ± 8.72 Aa

Similar capital letters means no statistical significant differences between averages, in column. Similar small letters means no statistical significant differences among averages, in row.

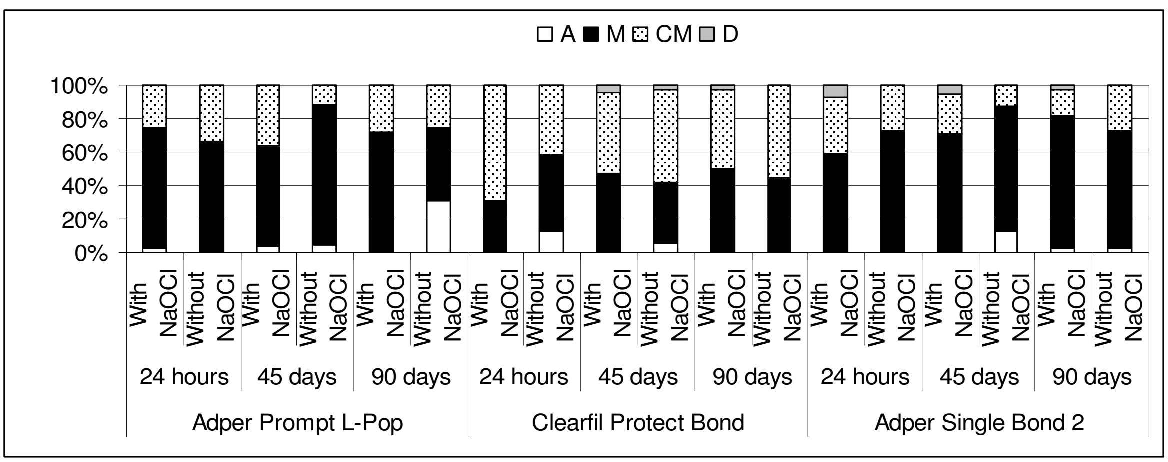

Figure 1 - Distribution of failure mode in dentin, considering adhesive systems, NaOCl irrigation and storage time. Adhesive failure (A), mixed failure (M), cohesive failure in material (CM), cohesive failure in dentin (D).

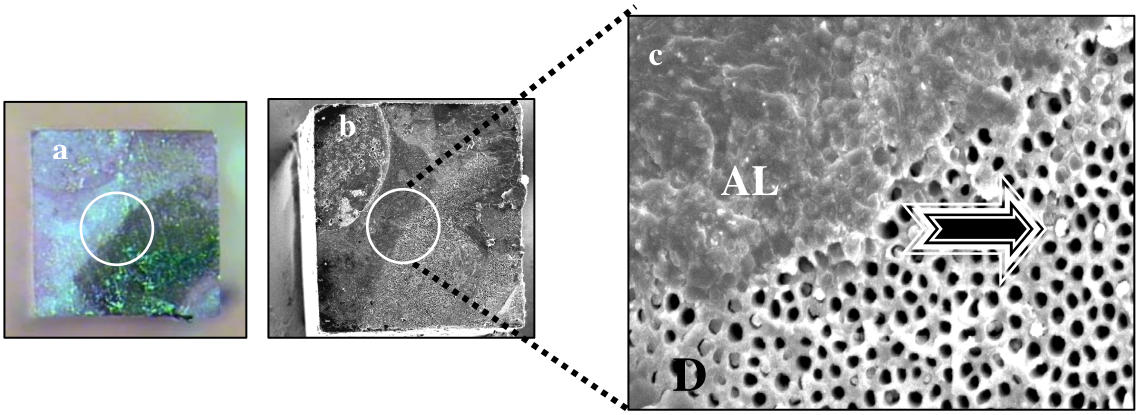

Figure 2 – a) Stereomicroscopy from Clearfil Protect Bond, stored for 45 days, without NaOCl irrigation (G5), 40x magnification; b) SEM electromicrography illustrating a mixed fracture from the same specimen, 70x magnification; c) a higher magnification (1,100x) of a cohesive/adhesive fracture– AL- adhesive layer; D – dentin; little bit dentinal tubules occluded by resin tags (*arrows).*

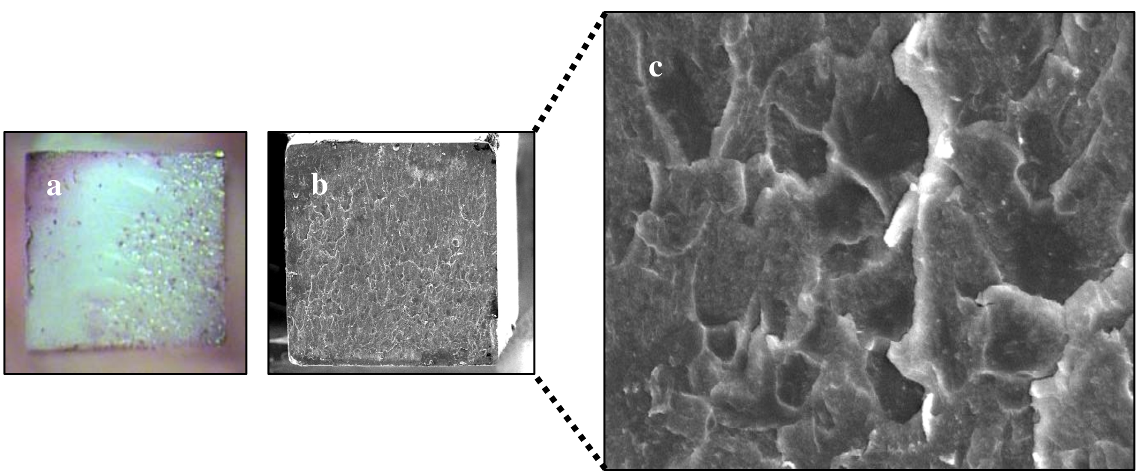

Figure 3 - a) Stereomicroscopy from Clearfil Protect Bond, stored for 45 days, without NaOCl irrigation (G5), 40x magnification; b) SEM electromicrography illustrating a cohesive fracture in material from the same specimen, 70x magnification; c) a higher magnification (1,100x) of a cohesive fracture in material. Note the stratificated composite layer.

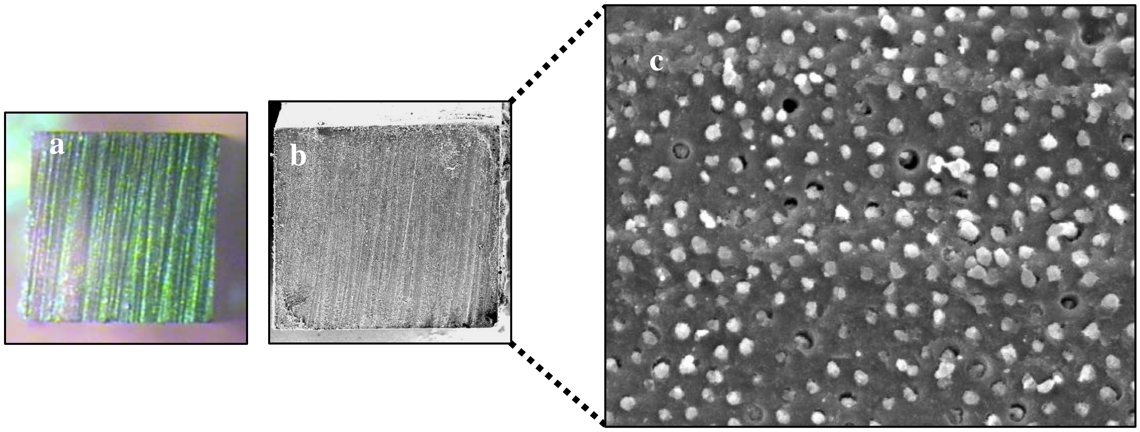

Figure 4 - a) Stereomicroscopy from Adper Prompt L-Pop, stored for 45 days, without NaOCl irrigation (G6), 40x magnification; b) SEM electromicrography illustrating a adhesive fracture under hybrid layer in the same specimen, 70x magnification; c) a higher magnification (1,100x) of a adhesive fracture. Note the plane surface with dentinal tubules occluded by resin tags.

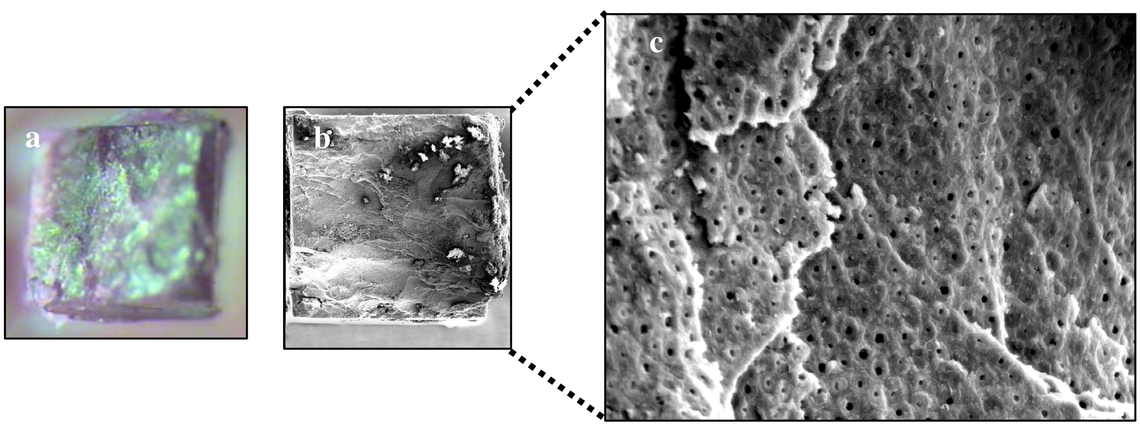

Figure 5 - a) Stereomicroscopy from Single Bond, stored for 24 hours, with NaOCl irrigation (G10), 40x magnification; b) SEM electromicrography illustrating a cohesive fracture in dentin in the same specimen, 70x magnification; c) a higher magnification (1,100x) of cohesive fracture in dentin. Note the stratificated dentin layer.

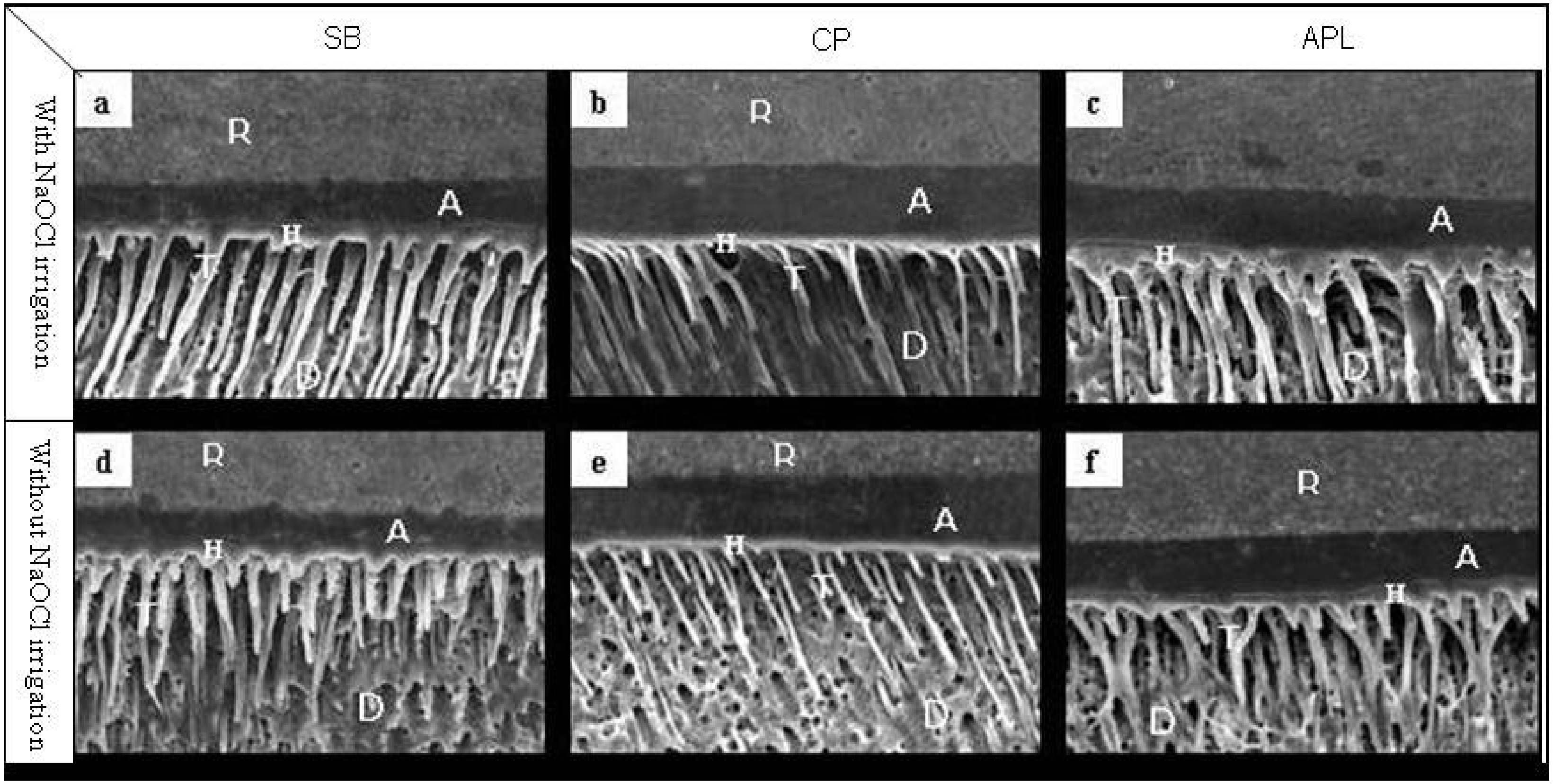

Figure 6 - Adhesive system/dentin interfaces analyzed by SEM (x 1200) in groups stored for 45 days: a- Adper Single Bond 2 interface, with NaOCl irrigation (G13); b- Clearfil Protect Bond interface, with NaOCl irrigation (G14); c-Adper Prompt L-Pop, with NaOCl irrigation (G15). d- Adper Single Bond 2 interface, without NaOCl irrigation (G4); e-Clearfil Protect Bond interface, without NaOCl irrigation (G5); f-Adper Prompt L-Pop, without NaOCl irrigation (G6). R – composite resin; A – adhesive; D – dentin; H- hybrid layer; T – resin tag. Note that CP group showed thinner hybrid layer than APL and SB; the thickness of resin tags in the opening tubules of the SB and APL are similar and larger than CP; the SB presented the highest number of resin tags.

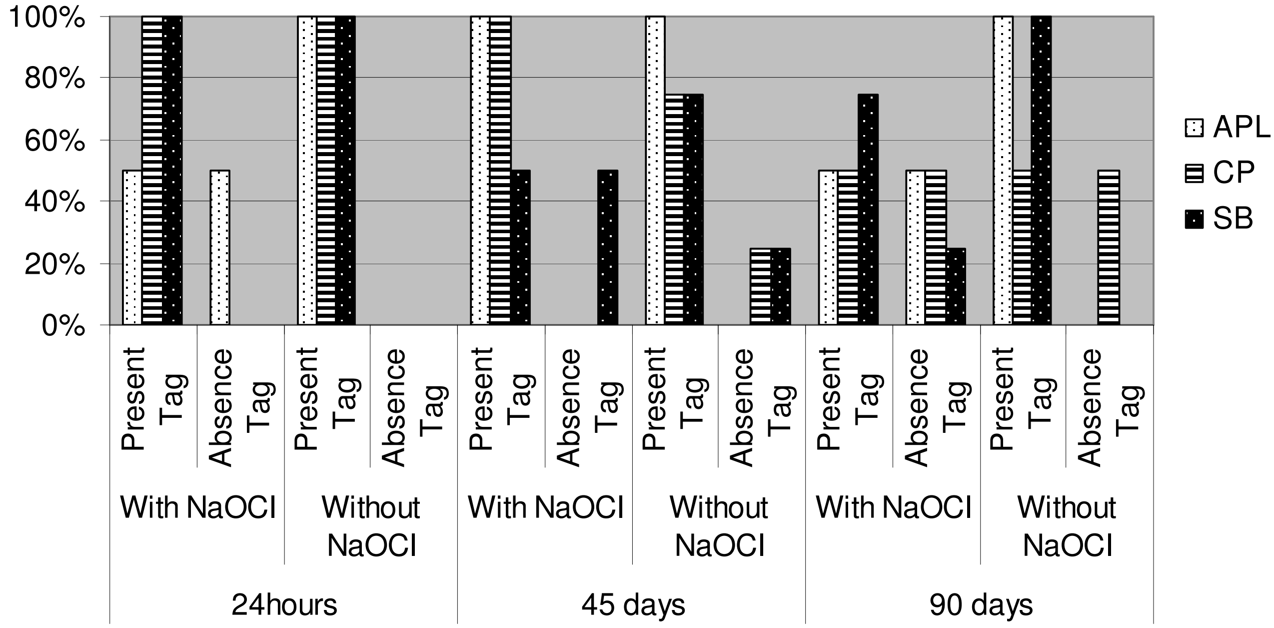

Figure 7 - Frequency of presence or absence of remaining tags in resin/dentin interface. Adper Prompt L-Pop (APL), Clearfil Protec Bond (CP), Adper Single Bond 2 (SB).

CAPÍTULO 2

Short period of bonding degradation and morphology of resin/dentin interface of primary teeth irrigated with NaOCL using different adhesive systems – An in vitro study[3]**.**

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Short period of bonding degradation and morphology of resin/dentin interface of primary teeth irrigated with NaOCL using different adhesive systems - An in vitro study

SUMMARY

Objectives: To evaluate NaOCl treated dentin/resin-bonding degradation during 90-day storage time, using three adhesive systems by microtensile bond strength (μTBS) and the resin/dentin interface morphology of primary teeth. ***Methods:*** Ninety sound primary molars were used. Eighteen groups were assigned according to adhesive system (Adper Single Bond 2 -SB, Clearfil Protect Bond -CP, Adper Prompt L-Pop -APL); dentin treatment (NaOCl 0.5%) and storage time (24h, 45d, 90d). The middle dentin was exposed; NaOCl irrigation was accomplished according to the group, and restored with composite (Charisma-Shade-A3.5). Sticks with $1mm^2$ section area were obtained and submitted to μTBS test. The obtained data were submitted to ANOVA and Tukey tests ($p<0.05$). The failure mode, resin tags presence and the resin/dentin interface were evaluated by Scanning Electron Microscopy (SEM) and obtained data were submitted to Chi-square test ($p<0.05$). ***Results:*** For primary teeth, APL showed the lowest μTBS value and it was statistically different from the others adhesive systems regardless treatment and storage time. There was significant decrease in μTBS after 90 days storage only when primary dentin was not irrigated with NaOCl. After 90 days of storage, the tags presence generally decreased, except to SB that it remains almost 100%. ***Conclusions:*** The NaOCl can be benefic for the microtensile bond strength, since at 90 days of storage the μTBS values did not decreased just in the irrigated groups. Adhesive system is one of the main facts to be considered when

primary dentin bonding is tested: etch & rinse and two-bottles adhesive systems produced the highest µTBS values.

Introduction

The high-quality restoration of endodontically treated teeth is important for clinical success.[1] One of the purposes of the restoration of endodontically treated teeth is to prevent bacteria leakage from oral cavity.[2] Adhesive restorations are based in micromechanical concept. [3]

In dentin, monomers interlocking the exposed collagen network of the demineralized superficial dentin, and upon polymerization, results in the formation of a hybrid layer.[4,5] Hybrid layer has been described as responsible for improving the bond strength between dentin and adhesive material.[6,7]

However, the quality of the substrate is an important factor for the appropriate formation of hybrid layer.[8,9] Clinical procedures can modify the substrate, like the irrigation with sodium hypochlorite (NaOCl) used for endontontic treatment.[10] Sodium hypochlorite solution is an oxidizing agent that can alter the configuration or removes the organic components of dentin, producing deproteinization of collagen fibrils.

In addition, it breaks down to sodium chloride and oxygen, which could provide oxidation of some components in the dentin matrix[11] and consequently decrease the elastic modulus and flexural strength of dentin.[12] It could also interfering in the restoration performance[12], affect the resin penetration into the dentin structure and/or the polymerization of monomers in the demineralized dentin.

The literature does not show a consensus on the bond strength values of teeth treated with NaOCl irrigation, some studies shows that NaOCl increase[13] and some studies show that NaOCl decrease bond strength values of resin/dentin bonding.[10,14] In addition, the long or even-short term effect of NaOCl irrigation on bonding adhesive performance has not been evaluated, mainly for primary teeth. The bonding degradation can lead to microleakage and determine the failure of the restoration and consequently of the endodontic treatment.[15]

Several studies have mentioned bond strength in permanent teeth but little is considered for primary teeth. Primary and permanent teeth have some morphological and constitutional difference that may lead different bonding characteristics.[16,17] Primary dentin has been assumed to be different for permanent dentin due to the variable amounts of mineral components, as well as morphological and structural differences.[18] Primary dentin has a mineral content arrangement different from the permanent one; conversely, the organic content is similar for both dentin types.[17] Then, application of NaOCl to that substrate would produce different changes compared with those noted in permanent teeth. In addition, primary teeth are more susceptible to acid etching[16], so, their bond behavior may differ.

Concerning the adhesive system, currently they can be divided in two categories: total-etching and self-etching systems. The technique application, the components and etching aggressiveness[3] of these two systems are supposed to be different. These factors can contribute for different microtensile bond strength values.[19] However, not too many studies have been done considering primary teeth as bonding substrate, when self-etching adhesive systems are used. In addition, there is no information concerning the NaOCl irrigated dentin as a substrate for adhesion even so, to consider this bonding performance during a water storage time.

The aim of this study was to analyze the bonding degradation after simulating of 0.5% NaOCl irrigation among three different adhesive systems in primary and permanent sound dentin, using microtensile bond strength test (μTBS), in three different storage time. The failure sites and bonded interfaces were also evaluated using Scanning Electron Microscopy (SEM). The hypothesis tested for primary dentin is that there is influence of storage time, adhesive systems and NaOCl irrigation on μTBS and at resin/dentin interfaces.

Materials and Methods

Specimen preparation and bonding procedures for Microtensile Bond Strength Test and SEM Evaluation of the Dentin/Resin Interface

This study was conducted after approval of the Ethical Committee of Piracicaba Dental School, University of Campinas (Protocol 083/2005). Ninety sound human primary molars teeth were selected, cleaned and stored in a saline solution at 4º C for no longer than 2 months after extraction. A total of forty primary molars were donated by the University of São Paulo Teeth Bank and the remainder teeth were donated for the patients at the clinical of Piracicaba Dental School, State University of Campinas. In order to reach large area in middle dentin the primary teeth were sectioned in the buccal-lingual direction in the center of the crown, and the middle dentin of both halves was visually determined. Each two halves from primary teeth were ground flat in the occlusal surface, until middle dentin be reached on a water-cooled mechanical polisher (Metaserv 2000, Buehler, UK LTD, Lake Bluff, IL, USA) using 600-grit abrasive papers.

The specimens were randomly assigned into 18 groups according the adhesive system, dentin treatment and period of storage in distilled water (Table1). The adhesive systems, the way that they were used in this study and components are described in Table 2. Charisma light-cured composite resin, shade A3.5 (Heraus Kulzer, Wehrheim, Germany-batch # 010201) was used to complete the bonding procedure.

Before the restorative treatment the pulp horns and enamel areas were identified with a pen marker. In all groups, the dentin was treated with the adhesive systems according to the manufacturers' directions. Additionally, according to each group, 0.5% sodium hypochlorite solution (NaOCl) was applied for thirty minutes in an ultrasound bath before the adhesive treatment to simulate the irrigation procedure during an endodontic treatment. After that, the adhesive systems were applied and a composite resin block (3 mm) was built on the bonding surface, using the incremental technique. Each layer of composite resin was individually light-cured for 20 seconds, with an Elipar Tri-light unit (ESPE – America Co., Seefeld 82229 - Germany). Light intensity was periodically measured (470mW/cm^2). The pulp chamber was equally filled up. The specimens were placed in distilled water at 37º C for 24 hours.

Microtensile Bond Strength Test

For the microtensile bond strength test, three teeth for each group were used (n=3). Each teeth(two halves)/restoration set was buccal-lingual and mesio-distal sectioned (Isomet, Buheler, Lake Bluff, IL, USA) in order to obtain sticks with a cross-sectional surface area of around 1.0 mm^2. The cross-section area was verified with electronic digital caliper (Mitutoyo, Japan) and the sticks were observed with a stereomicroscope (Model XLT30, Nova Optical Systems, SP, Brazil) at 25X magnification in order to verify if the stick didn't have any color mark that indicated enamel area or pulp horns. It was obtained a total of 8 sticks, and only four sticks in average per tooth were considered adequate and were used. The sticks were immersed in distilled water and stored according to the groups at 37º C. Distilled water was changed weekly.[20]

Each stick was attached to a microtensile jig with a cyanocrilate adhesive (Super Bond, Loctite, Itapevi, SP, Brazil) and then subjected to a tensile loading in a universal testing machine (Instron model 4411, Canton, MA, USA) at a cross-head speed of 0.5 mm/min. The tensile strength values (MPa) were calculated from the peak load at failure divided by a cross-section area of adhesion. Means and standard deviation were calculated.

Failure Mode Evaluation

A calibrated examiner evaluated all the sticks tested at 40X magnification to initially classify the failure sites as cohesive (in dentin or in the material), adhesive, or mixed failure. To facilitate this analyzes 2% basic fucsin was dropped and left at the bonding area for 10 seconds, then, washed and tissue paper dried. Five representative sticks of each group were mounted on aluminum stubs,[21,22] sputter-coated with gold (Balzers-SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL- JSM 5600LV, Tokyo, Japan) and with a magnification of 70X and 1100X.

SEM Evaluation of Dentin/Resin Interface

For the Scanning Electron Microscopy (SEM) evaluation of dentin/resin interface, two primary teeth were used for each group described above (n=4). The bonding procedure was done according the described groups (Table 1 and 2). Each teeth(two halves)/restoration set was buccal-lingual sectioned (Isomet, Buheler, Lake Bluff, IL, USA) in order to obtain slices. Four slices of each teeth, in the buccal-lingual direction were obtained. Two slices of each tooth had been randomly chosen for the SEM evaluation of dentin/resin interface.The slices were immersed in distilled water and stored according to the groups at 37º C. Distilled water was changed weekly.[20] Only one side of each slice was prepared for SEM and it was polished on a water-cooled mechanical grinder (Metaserv 2000, Buehler, UK LTD, Lake Bluff, IL, USA) using 320-, 400-, 600- and 1200-grit silicon carbide abrasive paper (Carbimet Disc Set, # 305178180, Buehler, UK LTD) and with a 3-µm and 1-µm diamond paste. The specimens were ultrasonically cleaned in distilled water for 3 min in each exchange of carbide abrasive paper and between each diamond paste with detergent solution. Then, they were demineralized with 35% phosphoric acid for 15 s, washed with distilled water for 30s and dried with a tissue paper. Next, they were deproteinized with 1% NaOCl for 10 min, washed in ultrasound bath and dried with a tissue paper. The specimens were left dried in a dissecator, for 24h at room temperature. After this period, the specimens were mounted on aluminum stubs, sputter-coated with gold (Balzers-SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL- JSM 5600LV, Tokyo, Japan) with a magnification of 600X, 1200X, 2500X, and 3300X. A calibrated examiner (86% coincidence intra-examiner) evaluated the interface resin/dentin. The electromicrographs were classified according presence or absence of remaining resin tags, since all they presented hybrid layer.

Statistical analysis

Bond strength data were submitted to ANOVA. When the F ratio of bond strength test was significant, Tukey test was used to compare specific mean values at $p<0.05$. SEM resin/dentin interface data and failure mode data were submitted to Chi-Square test $p< 0.05$.

Results

For primary, teeth ANOVA test showed that there was significant statistically difference among adhesive systems (p=0.0002) regardless the treatment and storage time. The lowest values μTBS were obtained with Adper Prompt L-Pop (Table 3). There was significant interaction between the NaOCl irrigation and storage time (p=0.0209). Only in the groups without NaOCl irrigation μTBS values were not influenced by storage time until 45 days, but in 90 days storage it decreased significantly (Table 4).

Concerning failure sites evaluation, in general, the mixed failure was the most frequent (Figure 1 and 2). There was no significant interaction among NaOCl irrigation, different times of storage and all adhesive systems (p<0.05). CP showed the highest percentage of cohesive failure in the material, compared with the others adhesive systems (Figure 1 and 3). Mixed failure was the most frequent in APL and SB (Figure 1 and 2). Adhesive failure mode was often when NaOCl was not used (Figure 1 and 4) and cohesive failure in dentin was more frequent when NaOCl was used (Figure 1 and 5).

SEM images of all adhesive systems showed adequade adaptation and well established and uniform hybrid layer formation in all groups. CP group showed thinner hybrid layer than APL and SB (Figure 6). The thickness resin tags in the opened tubules of the SB and APL groups were similar, but SB presented a higher number of resin tags and CP showed thinner and lower number than SB and APL (Figure 6).

The percentage of presence of resin tag at the interface resin/dentin primary teeth are shown in Figure 7. All the specimens of SB showed resin tags presence (100%) regardless the treatment and storage time, but when APL and CP were used some specimens showed lower percentages of resin tags presence, 70.8% and 79.2%, respectively. Time of storage and treatment did not influence the resin tags presence (presence of 100%).

Discussion

In order to simulate the irrigation procedure, this study used a 0.5% NaOCl solution to immerse the dentin samples for 30 minutes, simulating the clinical time.[23] Therefore, important features are accomplished when the irrigating solution is used in endodontic treatment: all canal length is bathing on the solution and also all dentin surfaces, from the apices throw the coronal dentin.

The first hypothesis tested was proved, concerning the adhesive types influence on μTBS. APL showed the lowest μTBS values. In addition, it was verified that there was no statistically significant difference between one-bottle etch & rinse adhesive system and self-etching adhesive system, Adper Single Bond 2 and Clearfil Protect Bond, respectively, providing the highest values of microtensile bond strength on primary dentin. This result corroborates those from Atash *et al.*[24] and Miranda *et al.*[25], although they have used a different test (shear bond strength). The SB combines the primer and adhesive in one solution to be applied after etching the substrate with 37% phosphoric acid (pH=0.3) for 15-20 seconds.[26] So, this adhesive system create a mechanical interlocking with etched dentin by means of resin tags, adhesive lateral branches and hybrid layer formation, and show high strength values to bond.[27] The higher values for CP can be explained by the mild potential of demineralization of the primer (pH=1.9) and adhesive (pH=2.8) of this adhesive system. The primer demineralizes dentine only up to a depth of 1 μm. Moreover this superficial demineralization occurs only partially. Keeping residual hydroxyapatite still attached to the collagen. The preservation of hydroxyapatite within the submicron hybrid layer may serve as receptor for additional chemical bonding. Furthermore CP contains MDP (methacryloxydecyl dihydrogen phosphate) which has a chemical bond potential to the calcium in the residual hydroxyapatite.[3] The reduced thickness of hybrid layer should not decrease the adhesive properties, a demineralization of approximately 2 µm is sufficient for good bonding.[28] However, when compared both self-etching adhesive systems, all-in-one (APL)- and two-bottles (CP) adhesive systems there was significant difference between both ones, corroborating the results obtained by Kaaden *et al*.[29] The difference in µTBS values for both self-etching adhesive systems can be due to some differences between them. APL has a high amount of water in the primer, raging from 70% to 80% wt as solvent and it is responsible for the lower µTBS values. Because of the high water amount and its low vapor pressure, the water does not evaporate easily and completely during the bonding procedure. The residual water can remain within the adhesive interface, and also in the hybrid layer, it compromises the monomer cure. This high water content may result in competition between the monomer (HEMA) and the remaining water inside the demineralized dentine. In this way, the bond strength performance may be affected by the residual water.[30] In addition, phase separation of the hydrophobic and hydrophilic monomer components causing blister-like spaces and globule formation of the resin within the hybrid layer has been observed in overly wet conditions.[31] The water excess may also dilute the primer and reduce its effectiveness, resulting in lower bond strength.[29]

In this study, there was no statistically significant difference on µTBS values for all storage time with NaOCl irrigation, and until 45 days of storage without NaOCl irrigation. Also, the µTBS values among treatments in the same periods were similar (Table 4), these data corroborate Uno & Finger[32], Correr *et al*.[22] and Arias *et al.,*[33] although the storage time of these studies were lower. There was significant decrease in μTBS values after 90 days storage only when primary dentin was not irrigated with NaOCl, these data corroborate others studies.[34,35] It seems that the NaOCl irrigation protected the bonding area from degradation. It is believed that inorganic content changes can be resulted of some hydroxyapatite molecules reaction with sodium hypochlorite resulting in calcium hypochlorite and sodium phosphate. This reaction can provide the solubilization of some hydroxyapatite molecules, and reveling a mineral surface rich in hydroxyl, carbonate and phosphate groups as seen by Di Renzo *et al*.[36] In this way, after dentin NaOCl irrigating, the etching acid would increase the area of adhesion by exposition of unbound hydroxyapatite crystals and resin monomers are capable of penetrating within this area. In addition, the hydroxyl, phosphate and carbonate groups can be available for chemical bonding when used adhesive systems containing acidic monomers.[37] All this facts would protect the bonding area from degradation, similar to the those observed in permanent teeth (unpublished results)[38]

The resin tags are infiltration of resin monomers polymerized into the tubule that forms an intratubular digitation and provides mechanical anchoring, which helps bonding. Mechanical anchoring is greater when the resin is in contact with the tubule walls over a large area. This mechanical property can be improved when the lateral branches are filled up with monomers.[39,40]

The configuration of the different hybrid layer and resin tags is due to the different values of pH of the adhesive systems. Van Meerbeeck *et al*., (2001)[3] subdivided the adhesive systems into "strong", "intermediary strong" and "mild" according to their etching aggressiveness. SB (pH=0.3) and APL (pH=0.8) are considering "strong" and CP (primer pH=1.9 and bond pH=2.8) is considering "mild" aggressiveness. The more aggressive the adhesive, the greater substrate the demineralization, and the higher hybrid layer and the large thickness resin tags in the opened tubules are formed.

Also the morphology of the resin tags results from their interaction with the organic elements of peritubular dentin after demineralization and is influenced by the resin composition. As showed in Figure 6, the etch & rinse adhesive system (SB) presented greater contact with the opening and wall tubules and greater thickness of hybrid layer. It can be owned to the acid conditioner that causing more demineralization, roughness and a higher wettability can interfere on the bond strength in dentin.[41,42] In addition, the NaOCl treatment not influenced the resin tag morphology and formation of hybrid layer.

Further studies concerning long-term degradation and *in vivo* studies are needed to clarify the interactions of self-etching primers and all-in-one- and two-bottles adhesive systems with dentin treated with NaOCl considering the esthetic restorations performance.

Conclusion

Based on the results obtained and considering the limitations of this study it can be concluded that the NaOCl cannot be deleterious for adhesive/dentin bonding, since at 90 days of storage the μTBS values did not decreased in the irrigated groups. Adhesive system is the main fact to be considered when primary dentin bonding is tested: etch & rinse and two-bottles adhesive systems produced the highest μTBS values than all-in-one adhesive system. The remaining resin tag and hybrid layer are affected just by the adhesive systems.

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References

1- Ray HA, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. Int Endod J. 1995 Jan;28(1):12-8.

2- Ausiello P, De Gee AJ, Rengo S, Davidson CL.Fracture resistance of endodontically-treated premolars adhesively restored.Am J Dent. 1997 Oct;10(5):237-41

3- Van Meerbeek B, Vargas M, Inoue S et al. Adhesive and cements to promote preservation dentistry. Oper Dent 2001. Supplement 6:119-144.

4- Nakabayashi N, Ashizawa M, Nakamura M.Identification of a resin-dentin hybrid layer in vital human dentin created in vivo: durable bonding to vital dentin. Quintessence Int. 1992; 23(2): 135-41.

5- Perdigao J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F. Effect of a sodium hypochlorite gel on dentin bonding. *Dent Mater*. 2000; 16(5): 311-23.

6- Gwinnett AJ. Quantitative contribution of resin infiltration/hybridization to dentin bonding. *Am J Dent*. 1993; 6(1): 7-9.

7- Ferrari M, Mason PN, Vichi A, Davidson CL. Role of hybridization on marginal leakage and bond strength. *Am J Dent*. 2000; 13(6): 329-36.

8- Pashley DH, Sano H, Ciucci B, Yoshiyama M, Carvalho RM. The microtensile bond test: a review. *J Adhes Dent.* 1999; 1(4): 299-309.

9- Cederlund A, Jonsson B, Blomlof J. Do intact collagen fibers increase dentin bond strength. *Swed Dent J.* 2002; 26(4): 159-166.

10- Nikaido T, Takano Y, Sasafuchi Y, Burrow MF, Tagami J. Bond strengths to endodontically-treated teeth. *Am J Dent*. 1999.; 12(4): 177-80.

11- Yui CK, Garcia-Godoy F, Tay FR, Pashley DH, Imazato S, King NM, Lai SC. A nanoleakage perspective on bonding to oxidized dentin. *J Dent Res* 2002; 81: 628-632.

12- Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J*. 2001; 34(2): 120-32.

13- Wachlarowicz AJ, Joyce AP, Roberts S, Pashley DH. Effect of endodontic irrigants on the shear bond strength of epiphany sealer to dentin. *J Endod*. 2007; 33(2): 152-5.

14- Ozturk B, Ozer F. Effect of NaOCl on bond strengths of bonding agents to pulp chamber lateral walls. *J Endod*. 2004; 30(5): 362-5.

15- Hommez GM, Coppens CR, De Moor RJ. Periapical health related to the quality of coronal restorations and root fillings. *Int Endod J*. 2002; 35(8): 680-9.

16- Nor JE, Feigal RJ, Dennison JB, Edwards CA. Dentin bonding: SEM comparison of the dentin surface in primary and permanent teeth. *Pediatr Dent.* 1997; 19(4): 246-252.

17- Borges AFS, Bitar RA, Kantovitz KR, Correr AB, Martin AA, Puppin-Rontani RM New perspectives about molecular arrangement of primary and permanent dentin. *Applied Surface Science*, In press.

18- Uekusa S, Yamaguchi K, Miyazaki M, Tsubota K, Kurokawa H, Hosoya Y.Bonding efficacy of single-step self-etch systems to sound primary and permanent tooth dentin.Oper Dent. 2006 Sep-Oct;31(5):569-76.

19- Paradella TC, Fava M. Bond strength of adhesive systems to human tooth enamel. Braz Oral Res. 2007 Jan-Mar;21(1):4-9.

20- Fraga RC, Andrade SJ, Martins LRM, Lovadino JR. Hidrólise e resistência ao cisalhamento de uma resina composta polimerizada em condições úmidas (simulação do isolamento relativo). *Rev Bras Odontol.* 1997; 54(1): 22-4.

21- el Kalla IH, Garcia-Godoy F. Bond strength and interfacial micromorphology of four adhesive systems in primary and permanent molars. *ASDC J Dent Child*. 1998; 65(3): 169-76.

22- Correr GM, Puppin-Rontani RM, Correr-Sobrinho L, Sinhoreti MAC, Consani S. Effect of sodium hypochlorite on dentin bonding in primary teeth. *J Adhes Dent*. 2004;

6(4): 307-12.

23- Borges AF, Correr GM, Sinhoreti MA, Consani S, Sobrinho LC, Rontani RM. Compressive strength recovery by composite onlays in primary teeth. Substrate treatment and luting agent effects. *J Dent.* 2006; 34(7): 478-84. Epub 2006 Jan 19.

24- Atash R, Van den Abbeele A. Bond strengths of eight contemporary adhesives to enamel and to dentine: an in vitro study on bovine primary teeth. *Int J Paediatr Dent*. 2005; 15(4): 264-73.

25- Miranda C, Prates LH, Vieira Rde S, Calvo MC. Shear bond strength of different adhesive systems to primary dentin and enamel. *J Clin Pediatr Dent*. 2006; 31(1): 35-40.

26- Ferrari M, Gorraci G, Garcia-Godoy F. Bonding mechanism of three "one bottle"systems to conditionated and unconditionated enamel and dentin. *Am J Dent*. 1997;10: 224-230.

27- Tay FR, Gwinnett AJ, Pang KM, Wei SH. Structural evidence of a sealed tissue interface with a total-etch wet-bonding technique in vivo. *J Dent Res*. 1994 Mar;73(3):629-36.

28- Nakabayashi N. Resin-reinforced dentin due to infiltration of monomers into the dentin at the adhesive interface. J Jap Dent Mater 1982; 1:78-81.

29- Kaaden C, Powers JM, Friedl KH, Schmalz G. Bond strength of self-etching adhesives to dental hard tissues. *Clin Oral Investig*. 2002; 6(3): 155-60. Epub 2002 Aug 9.

30- Jacobsen T, Söderholm KJ. Some effects of water on dentin bonding. *Dent Mater*. 1995; 11(2): 132-6.

31- Tay FR, Gwinnett AJ, Wei SH. The overwet phenomenon: a transmission electron microscopic study of surface moisture in the acid-conditioned, resin-dentin interface. *Am J Dent*. 1996; 9(4): 161-6.

32- Uno S, Finger WJ. Function of the hybrid zone as a stress-absorbing layer in resin-dentin bonding. *Quintessence Int.* 1995; 26(10): 733-8.

33- Arias VG, Bedran-de-Castro AK, Pimenta LA. Effects of sodium hypochlorite gel and sodium hypochlorite solution on dentin bond strength. *J Biomed Mater Res B Appl*

Biomater. 2005; 72(2): 339-44.

34- Sato H, Miyazaki M, Moore BK. Influence of NaOCl treatment of etched and dried dentin surface on bond strength and resin infiltration. *Oper Dent.* 2005; 30(3): 353-8.

35- Abo T, Asmussen E, Uno S, Tagami J. Short- and long-term in vitro study of the bonding of eight commercial adhesives to normal and deproteinized dentin. *Acta Odontol Scand.* 2006; 64(4): 237-43.

36- Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. *Deproteination.Biomaterials*. 2001; 22(8): 793-7.

37- Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. *Dent Mater*. 2005; 21(10): 895-910.

38- Sacramento PA, Carvalho FG, Pascon FM, Borges AFS, Alves MC, Puppin-Rontani. Bonding degradation and morphology of resin/dentin interface in teeth irrigated with NaOCL using different adhesive systems – An in vitro study. *Unpublished study.*

39- Gwinnett A, Kanca J. Micromorphological relationship between resin and dentin in vivo and in vitro. *Am J Dent* 1992; 5: 19-23

40- Gregoire GL, Akon BA, Millas A. Interfacial micromorphological differences in hybrid layer formation between water- and solvent-based dentin bonding systems. *J Prosthet Dent.* 2002; 87(6): 633-41.

42- Toledano M, Osorio R, Perdigao J, Rosales JI, Thompson JY, Cabrerizo-Vilchez MA. Effect of acid etching and collagen removal on dentin wettability and roughness. *J Biomed Mater Res*. 1999; 47(2): 198-203.

43- Phrukkanon S, Burrow MF, Hartley PG, Tyas MJ. The influence of the modification of etched bovine dentin on bond strengths. Dent Mater. 2000; 16(4): 255-65.

Table 1 – Description of the groups according adhesive system, treatment and storage time.

Substrate Treatment 0.5% NaOCl during 30 min	**Storage Time**	**Adhesive system**		
		Adper Single Bond 2	Clearfil Protect Bond	Adper Prompt L-Pop
Without treatment	24h	G1	G2	G3
Without treatment	45 days	G4	G5	G6
Without treatment	90 days	G7	G8	G9
With treatment	24h	G10	G11	G12
With treatment	45 days	G13	G14	G15
With treatment	90 days	G16	G17	G18

Table 2 - Bonding Adhesive System, Composition, Manufacturer and 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	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 = 0.3 (etching acid) pH = 4.7 (primer + Bond)	a (15 s), b (30s), c, d, e, i (10 s)
Clearfil Protect Bond	Primer: water, MDP, MDPB, HEMA, Hidrophobic methacrylate, Bond: MDP, HEMA, Bis-GMA, Hydrophobic dimethacrylate, di-Camphorquinone, N-Diethanol-p-toluidine,silanated colloidal silica	Kuraray Medical, Kurashiki, Japan (Primer: 00017B) (Bond: 00027B)	pH = 1.9 (primer) pH = 2.8 (Bond)	f (20 s), e, g, i (10 s)
Adper Prompt L-Pop	Liquid 1 (red blister): Bis-GMA, Stabilizers Initiators based on camphorquinone Methacrylated phosphoric esters, Liquid 2 (yellow blister): Water 2-Hydroxyethyl methacrylate (HEMA) Polyalkenoic acid, Stabilizers	3M Dental Products, St. Paul, MN, USA (235117)	pH = 0.8 (mixed)	h (15s), e, I (10s)

*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-etching 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 3 - Microtensile bond strength values (MPa) of the adhesive systems in primary dentin.

Adhesive systems	Means ± SD
APL	20.77 ± 5.37 A
CP	24.78 ± 5.76 B
SB	26.84 ± 4.64 B

Means with the different letters are significantly different ($p<0.05$)

Table 4 - Microtensile bond strength values (MPa) in primary dentin treated or not with NaOCl irrigation and different storage times.

Treatment	Storage time		
	24h MS ± SD	45d MS ± SD	90d MS ± SD
With NaOCl	24.00 ± 4.11 Aa	23.99 ± 3.71 Aa	21.36 ± 4.39 Aa
Without NaOCl	29.65 ± 5.41 Aa	26.51 ± 7.28 Aa	19.21 ± 3.52 Ab

Similar capital letters means no statistical significant differences between averages, in column. Similar small letters means no statistical significant differences among averages, in row.

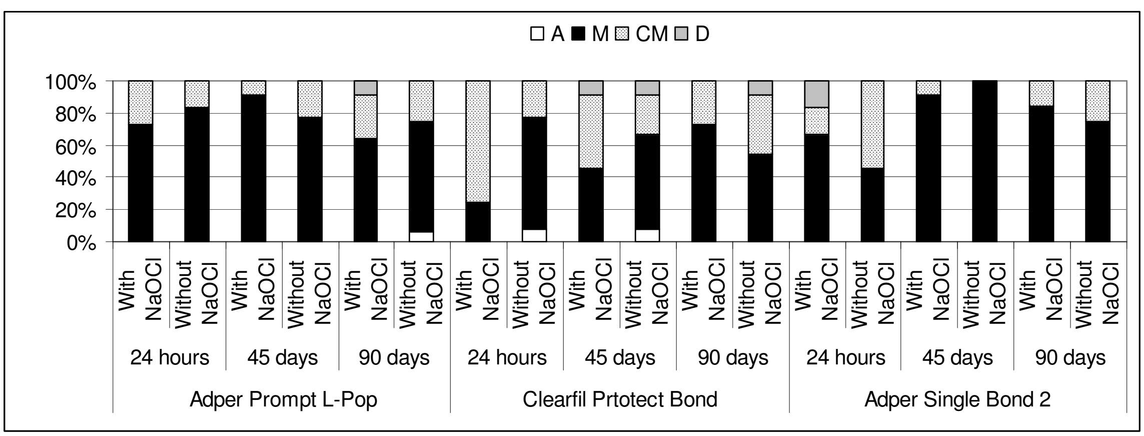

Figure 1 - Distribution of failure mode in primary dentin, considering adhesive systems, NaOCl irrigation and storage time. Adhesive failure (A), mixed failure (M), cohesive failure in material (CM), cohesive failure in dentin (D).

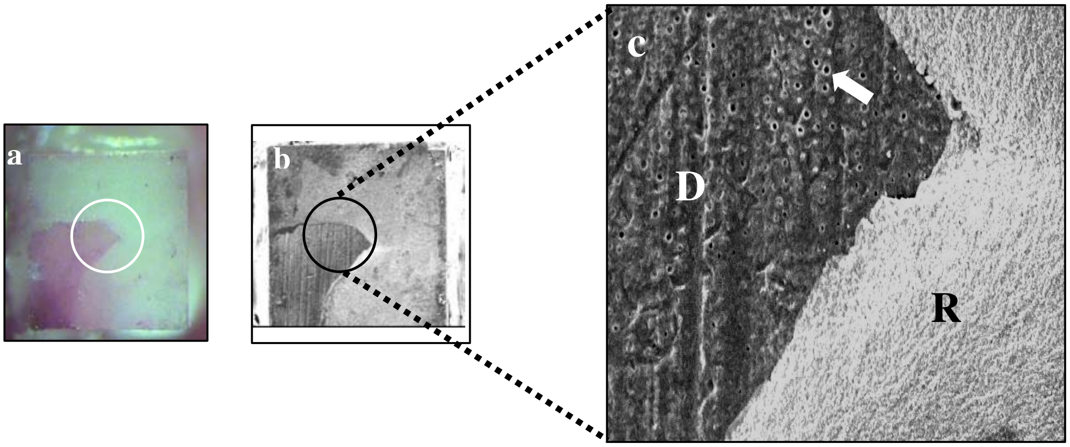

Figure 2 – a) Stereomicroscopy from Adper Single Bond 2, stored for 90 days, without NaOCl irrigation (G7), 40x magnification; b) SEM electromicrography illustrating a mixed fracture from the same specimen, 70x magnification; c) a higher magnification (1,100x) of a cohesive/adhesive fracture; R- composite resin (material); D – dentin; dentinal tubules (*arrows).*

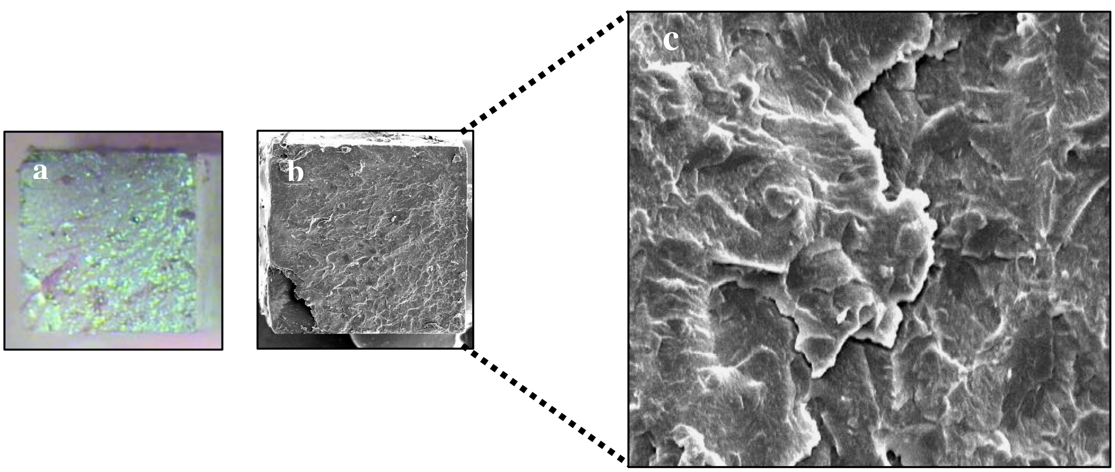

Figure 3 - a) Stereomicroscopy from Adper Single Bond 2, stored for 90 days, without NaOCl irrigation (G7), 40x magnification; b) SEM electromicrography illustrating a cohesive fracture in material from the same specimen, 70x magnification; c) a higher magnification (1,100x) of a cohesive fracture in material. Note the stratificated composite layer.

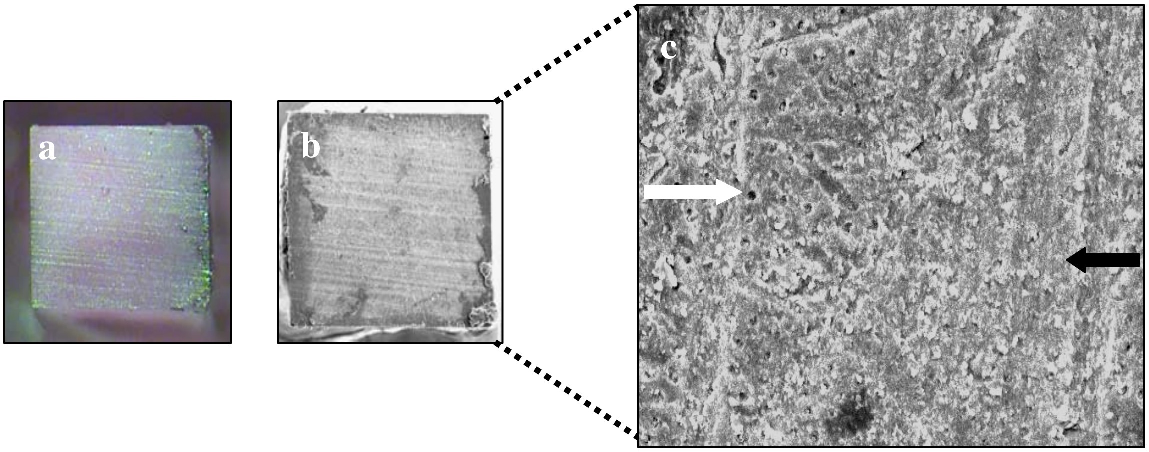

Figure 4 - a) Stereomicroscopy from Clearfil Protect Bond, stored for 45 days, without NaOCl irrigation (G5), 40x magnification; b) SEM electromicrography illustrating a adhesive fracture in the same specimen, 70x magnification; c) a higher magnification (1,100x) of a adhesive fracture. Note the plane surface with opened tubules (white arrow); some of then with remain full of adhesive (black arrow).

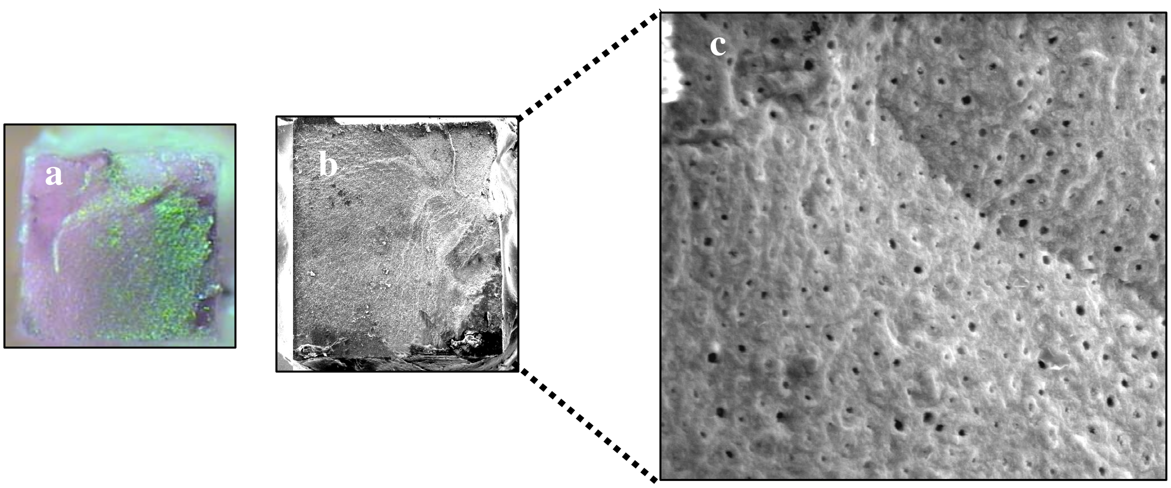

Figure 5 - a) Stereomicroscopy from Adper Single Bond 2, stored for 24 hours, with NaOCl irrigation (G10), 40x magnification; b) SEM electromicrography illustrating a cohesive failure in dentin in the same specimen, 70x magnification; c) a higher magnification (1,100x) of cohesive failure in dentin. Note the stratificated dentin layer.

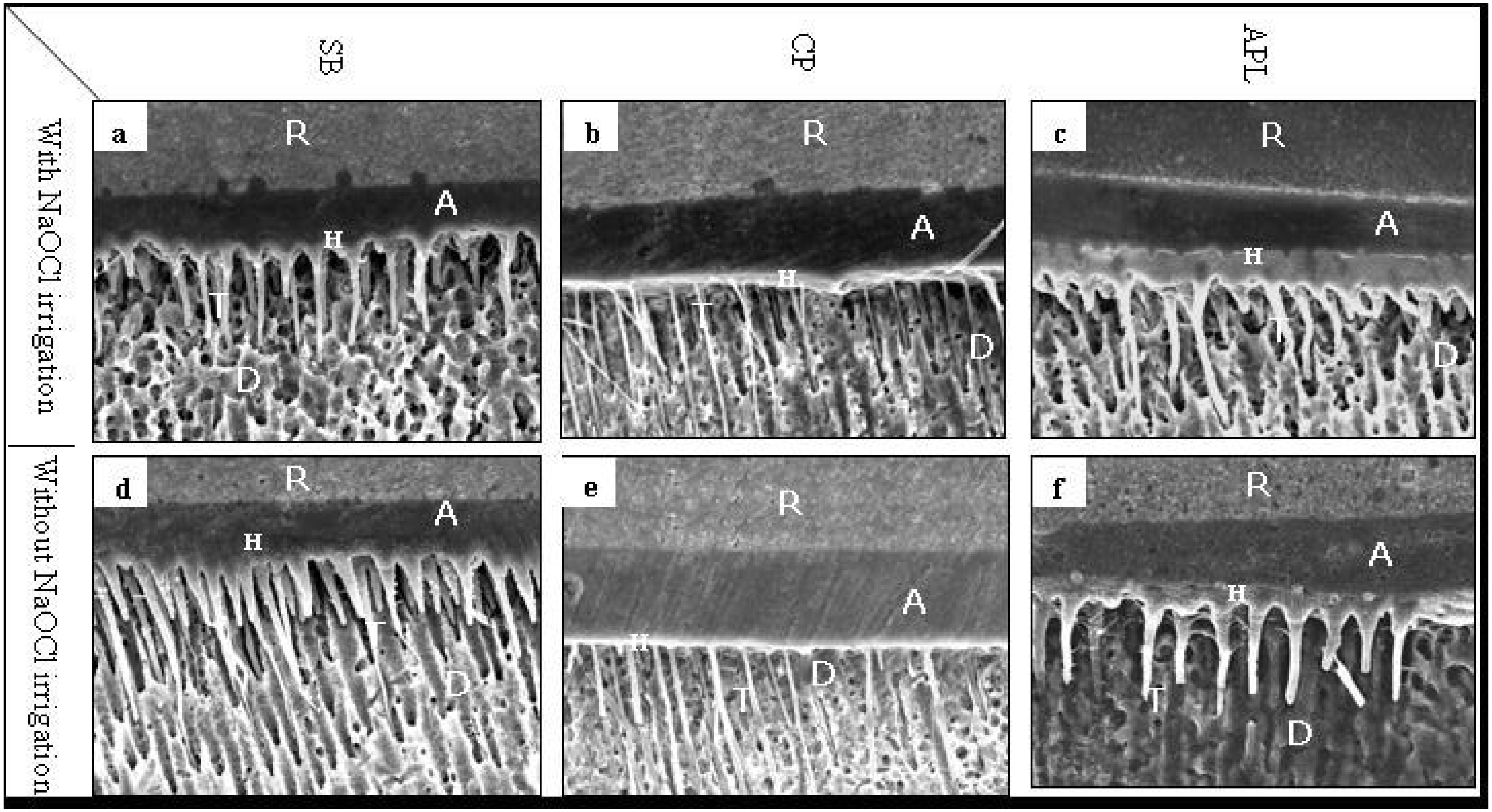

Figure 6 - Adhesive system/dentin interfaces analyzed by SEM (x 1200) in groups stored for 45 days: a- Adper Single Bond 2 interface, with NaOCl irrigation (G13); b- Clearfil Protect Bond interface, with NaOCl irrigation (G14); c- Adper Prompt L-Pop, with NaOCl irrigation (G15). d- Adper Single Bond 2 interface, without NaOCl irrigation (G4); e- Clearfil Protect Bond interface, without NaOCl irrigation (G5); f-Adper Prompt L-Pop, without NaOCl irrigation (G6). R – composite resin; A – adhesive; D – dentin; H- hybrid layer; T – resin tag. Note that CP group showed thinner hybrid layer than APL and SB; the thickness of resin tags in the opening tubules for SB and APL are similar and wider than CP; the SB presented the highest number of resin tags.

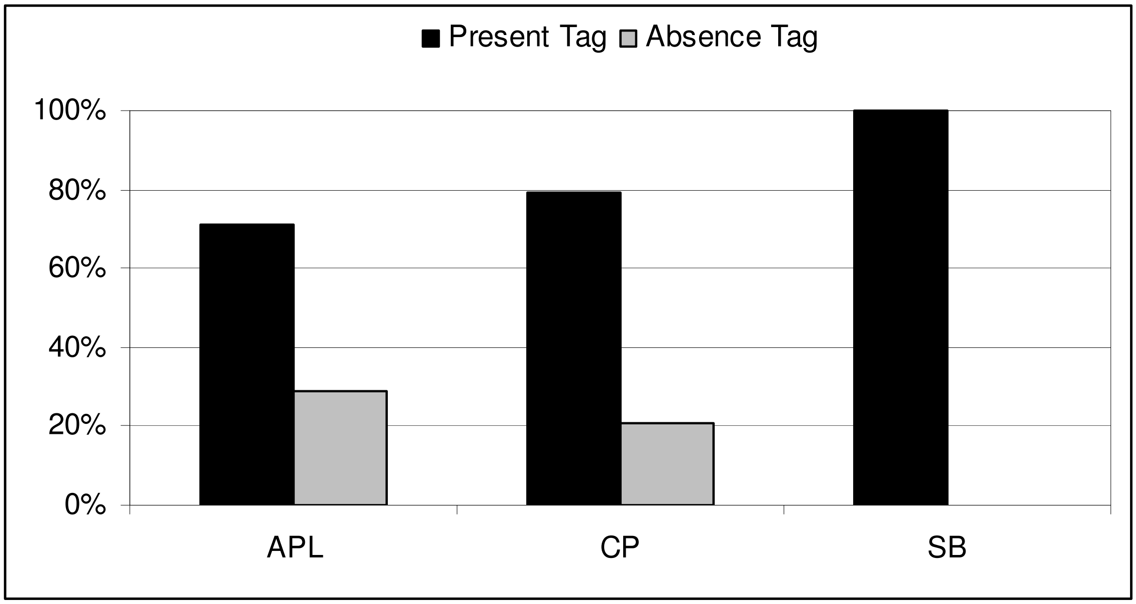

Figure 7 - Frequency of presence or absence of tags in resin/dentin interface in primary teeth. Adper Prompt L-Pop (APL), Clearfil Protec Bond (CP), Adper Single Bond 2 (SB).

CONCLUSÃO GERAL

Baseado nos resultados obtidos e considerando as limitações deste estudo, concluiu-se que:

1. O sistema adesivo é um importante fator a ser considerado quando é testada a adesão em dentes decíduos: *etch* & *rinse* e sistemas adesivos auto-condicionantes de dois frascos produziram os maiores valores de resistência de união.
2. Os valores de resistência de união para dentes decíduos e permanentes são afetados pelo tempo de armazenamento e irrigação com NaOCl: após 90 dias os valores de resistência de união diminuíram para os dentes não irrigados.
3. Os valores de resistência de união para a dentina de dentes permanentes são afetados pelo tipo de sistema adesivo e irrigação com NaOCl: Adper Prompt L-Pop aplicado na dentina de dentes permanentes previamente irrigada com NaOCl mostrou os menores valores de resistência de união.
4. Para dentes permanentes e decíduos o modo de fratura é afetado pelo sistema adesivo e tratamento do substrato; a configuração dos *resin tags* e da camada híbrida são influenciados somente pelos sistemas adesivos.
5. A porcentagem de *resing tags* remanescentes não é afetada por nenhum dos fatores de estudo para dentes permanentes, mas para dentes decíduos é afetada somente pelos sistemas adesivos.

REFERÊNCIAS BIBLIOGRÁFICAS[5]

1. Armstrong SR, Boyer DB, Keller JC. Microtensile bond strength testing and failure analysis of two dentin adhesives**.** ***Dent Mater***. 1998; 14(1): 44-50.
2. Berber VB, Gomes BPFA, Sena NT, Vianna ME, Ferraz CCR, Zaia AA, Souza-Filho FJ. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. ***Int Endod J***. 2006; 39: 10–17.
3. Borges AFS, Bitar RA, Kantovitz KR, Correr AB, Martin AA, Puppin-Rontani RM New perspectives about molecular arrangement of primary and permanent dentin. Applied Surface Science. *In press*
4. Burrow MF, Nopnakeepong U, Phrukkanon S. A comparison of microtensile bond strengths of several dentin bonding systems to primary and permanent dentin. ***Dent Mater.*** 2002; 18(3): 239-245.
5. Cederlund A, Jonsson B, Blomlof J. Do intact collagen fibers increase dentin bond strength. ***Swed Dent J.*** 2002; 26(4): 159-166.
6. Erdemir A, Ari H, Gungunes H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. *J Endod.* 2004; 30(2): 113-6.
7. Giannini M, Carvalho RM, Martins LR, Dias CT, Pashley DH. The influence of tubule density and area of solid dentin on bond strength of two adhesive systems to dentin. ***J Adhes Dent.*** 2001; 3(4): 315-24.
8. Gruverman A, Wu D, Rodriguez BJ, Kalinin SV, Habelitz S. High-resolution imaging of proteins in human teeth by scanning probe microscopy. ***Biochem Biophys Res Commun***. 2007; 352(1):142-6.
9. Gürgan S, Bollay S, Kiremitçi A. Effect of disinfectant application methods on the bond strength of composite to dentin. ***J Oral Rehabil.***1999; 26: 836-840.
10. Hommez GM, Coppens CR, De Moor RJ Periapical health related to the quality of coronal restorations and root fillings**.** ***Int Endod J.*** 2002; 35(8): 680-9.
11. Horiba N, Maekawa Y, Matsumoto T, Nakamura H.A study of the distribution

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

of endotoxin in the dentinal wall of infected root canals. ***J Endod.*** 1990; 16(7): 331-4.

12. Kugel G, Ferrari M. The science of bonding: from first to sixth generation***. J Am Dent Assoc.*** 2000; [Suppl 20S-5S]: 131.
13. Nakabaiashi N, Pashley DH. Hybridization of dental hard tissue. ***Tokyo:Quintessence Publishing Co***. 1998; 8-9.
14. Nakabayashi N, Ashizawa M, Nakamura M. Identification of a resin-dentin hybrid layer in vital human dentin created in vivo: durable bonding to vital dentin. ***Quintessence Int***. 1992; 23(2): 135-41.
15. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltation of monomers into tooth substrates. ***J. Biomed Mater Res***. 1982; 16: 265-273.
16. Nakabayashi N. Adhesive bonding with 4-META. ***Oper Dent.***1992; 5: 125-130.
17. Nakabayashi N. Resin-reinforced dentin due to infiltration of monomers into the dentin at the adhesive interface. ***J Jnp Dent Mater.*** 1982; 1: 78-81.
18. Nakajima M, Sano H, Burrow MF, Tagami J, Yoshiyama M, Ebisu S, Ciucchi B, Russell CM, Pashley DH. Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives. ***J Dent Res.*** 1995; 74(10): 1679-1688.
19. Nikaido T, Takano Y, Sasafuchi Y, Burrow MF, Tagami J. Bond strengths to endodontically-treated teeth. ***Am J Dent***. 1999.; 12(4): 177-80.
20. Nor JE, Feigal RJ, Dennison JB, Edwards CA. Dentin bonding: SEM comparison of the dentin surface in primary and permanent teeth. ***Pediatr Dent***. 1997; 19(4): 246-252.
21. Ozturk B, Ozer F. Effect of NaOCl on bond strengths of bonding agents to pulp chamber lateral walls. ***J Endod***. 2004; 30(5): 362-5.
22. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA, Tay F. The microtensile bond test: a review. ***J Adhes Dent***. 1999; 1(4): 299-309.
23. Pashley DH. The effects of acid etching on the pulpodentin complex. ***Oper***

Dent. 1992; 17: 229-242.

24. Ray HA, Trope M.Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. ***Int Endod J.*** 1995; 28(1): 12-8.
25. Sano H, Ciucchi B, Matthews WG, Pashley DH. Tensile properties of mineralized and demineralized human and bovine dentin. ***J Dent Res.*** 1994; 73(6): 1205-1211.
26. Spanó JCE. Estudo "in vitro" das propriedades físico-químicas das soluções de hipoclorito de sódio, em diferentes concentrações, antes e após a dissolução de tecido pulpar bovino. ***Dissertação (Mestrado) –Ffaculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo. Ribeirão Preto.*** 1999.
27. Sritharan A. Discuss that the coronal seal is more important than the apical seal for endodontic success – a review. ***Aust Dent J.*** 2002; 28(3): 112-115.
28. Yamauti M, Hashimoto M, Sano H, Ohno H, Carvalho R, Kaga M, Tagami J, Oguchi H, Kubota M. degradation of resin-dentin bonds using NaOCL storage. ***Dent Mater.***.2003; 19(5): 399-405.
29. Wachlarowicz AJ, Joyce AP, Roberts S, Pashley DH. Effect of endodontic irrigants on the shear bond strength of epiphany sealer to dentin. ***J Endod***. 2007; 33(2): 152-5.
30. Walshaw PR, McComb D.SEM evaluation of the resin-dentin interface with proprietary bonding agents in human subjects.***J Dent Res***. 1994 May;73(5):1079-87.
31. Wang T, Nakabayashi N.Effect of 2-(methacryloxy)ethyl phenyl hydrogen phosphate on adhesion to dentin.***J Dent Res***. 1991 Jan;70(1):59-66.

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

CERTIFICADO-2ª VIA

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Interação molecular da dentina decídua 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 14/09/2005, sendo esta via expedida em 11/12/2007.

Piracicaba, 11 de dezembro de 2007

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Molecular Interaction of deciduous 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 researching in human subjects and was approved by this committee at 14/09/2005.

Piracicaba, SP, Brazil, Dezemberr 11 2007

Profa. Cínthia Pereira Machado Tclhoury
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

Mensagem Original

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Figura 1: Materiais utilizados neste estudo[*]**.**

A: Adper Single Bond 2

B: Clearfil Protect Bond

C: Adper-Prompt-Lpop

D: Resina Charisma Cor A 3,5

[*] Informações quanto à composição, fabricantes e lotes dos materiais utilizados estão descritos nas página 24 e 48.


3M ESPE
Adper™
Adhesive
6g
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CLEARFIL
PROTECT BOND
PRIMER 6ml
KURARAY MEDICAL INC.
LOT 00017B
2008-01
CLEARFIL
PROTECT BOND
BOND 5ml
B
Press
LOT
C
CHARISMA
D

Figura 2: Ilustrações da metodologia

A: Seleção dos dentes permanentes

B: Seleção das dos dentes decíduos

C: Marcação para corte no sentido vestíbulo-lingual da coroa de dente decíduo

D: Hemi-coroas de dente decíduo, marcação da dentina média

E: Dentes permanentes posicionados para exame radiográfico

F: Delimitação da dentina média em permanentes após exame radiográfico

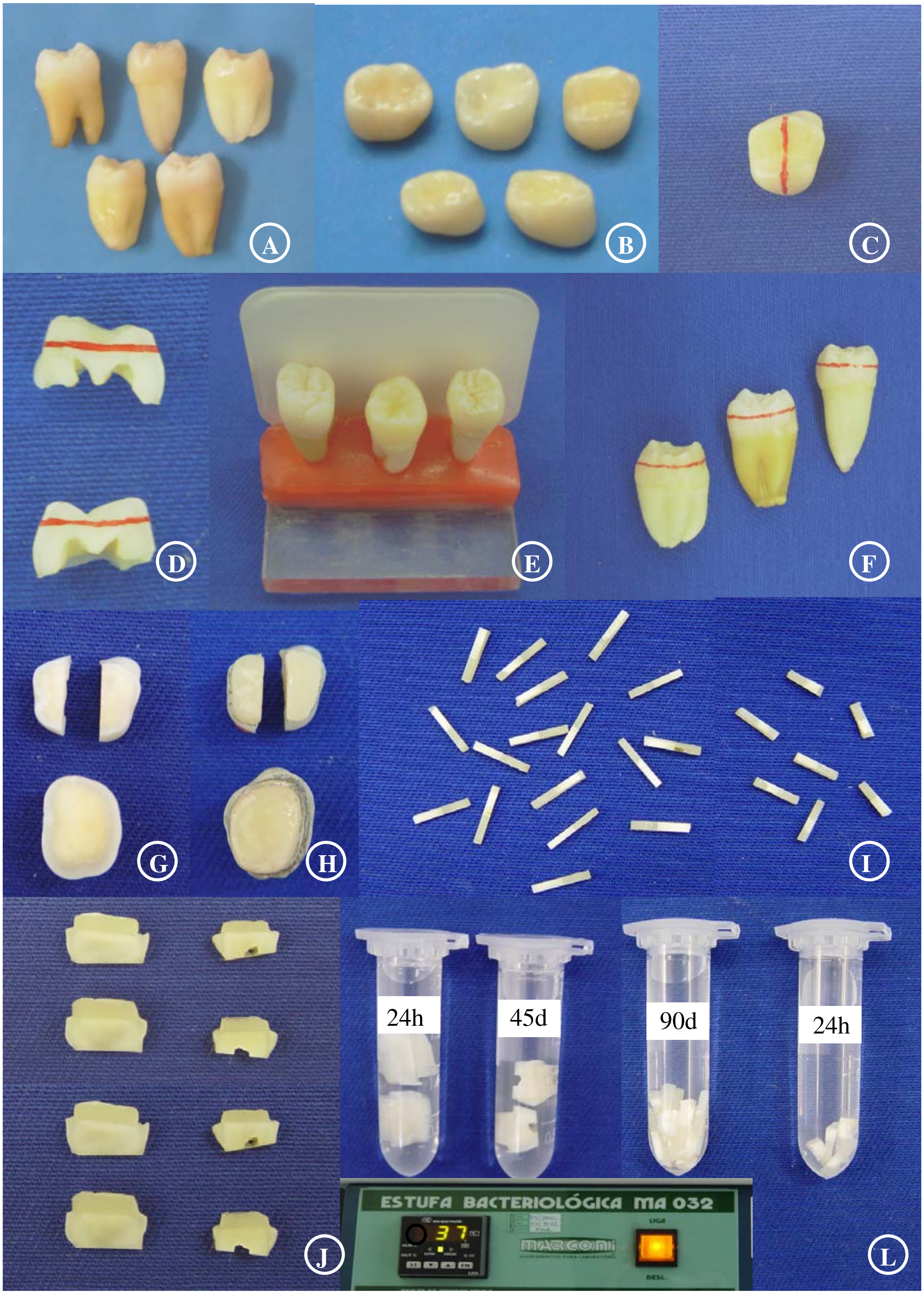
G: Dentina média exposta em dentes permanentes e hemi-coroas de dentes decíduos

H: Espécimes após procedimento de união e construção de bloco em resina foto-ativada

I: Palitos obtidos de dentes permanentes e decíduos

J: Espécimes (fatias) obtidas para estudo da interface de união em MEV

L: Palitos e espécimes (fatias) imersos em água destilada, armazenados em estufa a 37°C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ESTUFA BACTERIOLÓGICA MA 032
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Figure 3: Continuação de ilustrações da metodologia.

A: Máquina de Ensaio Universal (modelo 4411, Instron Corp., Canton, MA – USA)

B: Palito fixado ao dispositivo Bencor Multi T-Test

C: Palito após fratura

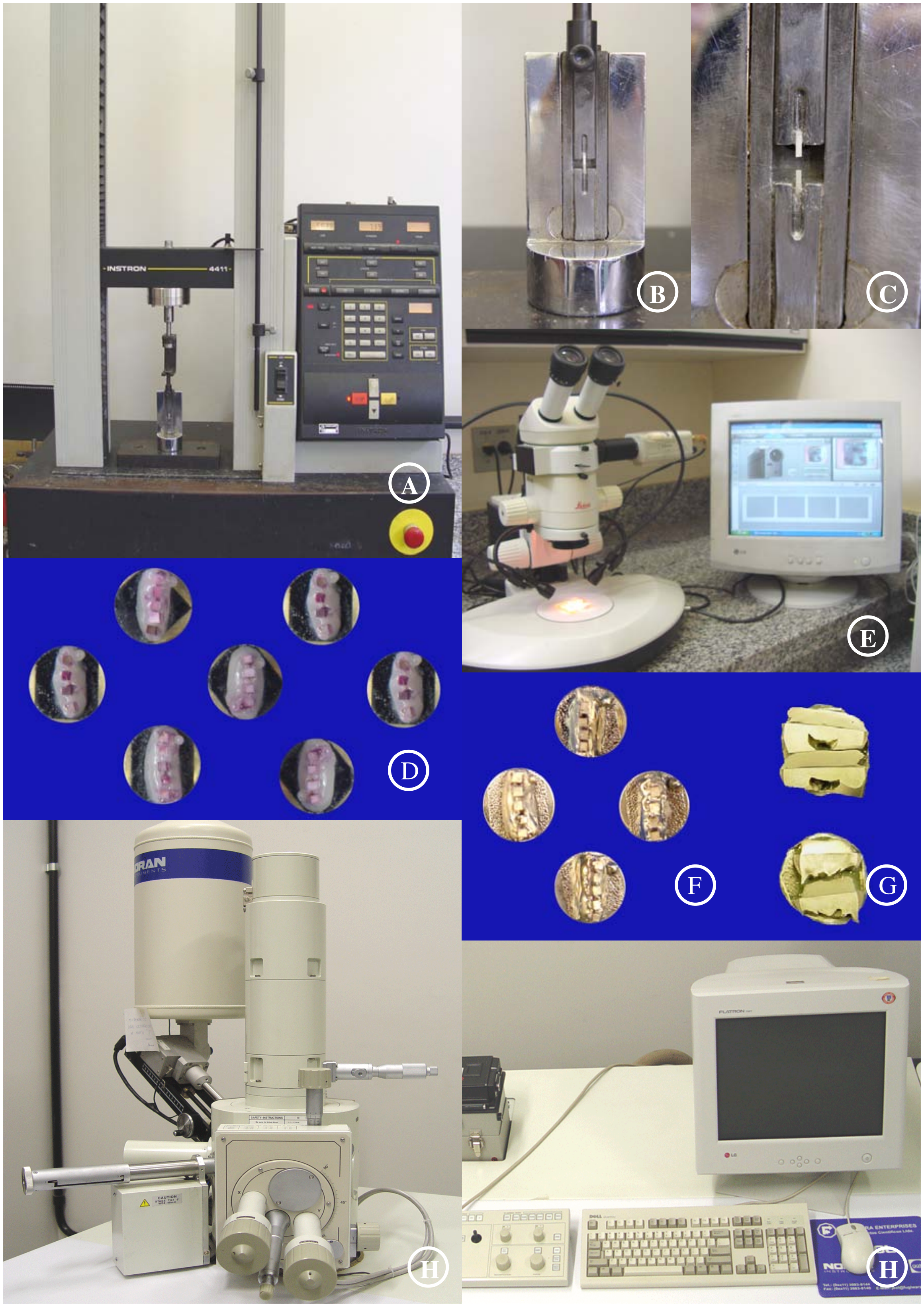
D: Palitos fraturados fixados em stubs de latão e corados com fucsina

E: Microssópio Estereoscópico acoplado ao computador

F: Palitos metalizados

G: Espécimes (fatias) metalizados

H: Microscópio Eletrônico de Varredura – JEOL – JSM 5600LV, Tókio, Japão.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Análises Estatísticas dos Capítulos 1 e 2

Análise estatística do ensaio de resistência à microtração de sistemas adesivos aplicados à dentina de dentes decíduos e permanentes tratadas ou não previamente com solução de NaOCL 0.5%.

Análise de Variância

Class Level Information

Class	Levels	Values
adesivo	3	APL CP SB
tratamento	2	com sem
tempo	3	24 h 45 dias 90 dias

Number of Observations Read 54
Number of Observations Used 54

Variável dependente: dente decíduo

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
adesivo	2	342.7233294	171.3616647	10.91	0.0002*
tratamento	1	55.6443679	55.6443679	3.54	0.0680 NS
tempo	2	415.3837206	207.6918603	13.22	<.0001*
adesivo*tratamento	2	59.8460814	29.9230407	1.90	0.1636 NS
adesivo*tempo	4	58.4850732	14.6212683	0.93	0.4572 NS
tratamento*tempo	2	135.6524989	67.8262495	4.32	0.0209*
adesiv*tratame*tempo	4	131.4410324	32.8602581	2.09	0.1022 NS
Error	36	565.666108	15.712947		
Corrected Total	53	1764.842212			

* significativo

NS Não significativo

R-Square	Coeff Var	Root MSE	deciduo Mean
0.679481	16.42388	3.963956	24.13533

Least Squares Means
Adjustment for Multiple Comparisons: Tukey

	tratamento	tempo	deciduo LSMEAN	LSMEAN Number
Ba	com	24 h	24.0062833	1
Aa	com	45 dias	23.9986306	2
Aa	com	90 dias	21.3557337	3
Aa	sem	24 h	29.6526939	4
Aa	sem	45 dias	26.5152944	5
Ab	sem	90 dias	19.2833280	6

Letras maiúsculas iguais não há diferença estatística significativa dentro do mesmo tempo

Letras minúsculas iguais não há diferença estatística significativa dentro do mesmo tratamento

Least Squares Means for effect tratamento*tempo
Pr > |t| for H0: LSMean(i)=LSMean(j)

Variável dependente: dente decíduo

i/j	1	2	3	4	5	6
1		1.0000	0.7159	0.0485	0.7596	0.1430
2	1.0000		0.7183	0.0480	0.7573	0.1442
3	0.7159	0.7183		0.0011	0.0877	0.8744
4	0.0485	0.0480	0.0011		0.5539	<.0001
5	0.7596	0.7573	0.0877	0.5539		0.0054
6	0.1430	0.1442	0.8744	<.0001	0.0054	

Variável dependente: dente decíduo

Tratamento	Tempo de armazenamento	N Obs	Mean	Std Dev	Upper 95% CL for Mean	Lower 95% CL for Mean
com	24 h	9	24.0062833	4.1150868	27.1694191	20.8431476
	45 dias	9	23.9986306	3.7177923	26.8563787	21.1408824
	90 dias	9	21.3557337	4.3902036	24.7303429	17.9811245
sem	24 h	9	29.6526939	5.4103797	33.8114799	25.4939079
	45 dias	9	26.5152944	7.2814169	32.1122870	20.9183019
	90 dias	9	19.2833280	3.5283361	21.9954471	16.5712088

Tukey's Studentized Range (HSD) Test for decíduo

Alpha	0.05
Error Degrees of Freedom	36
Error Mean Square	15.71295
Critical Value of Studentized Range	3.45676
Minimum Significant Difference	3.2297

Tukey Grouping	Mean	N	adesivo
A	26.844	18	SB
A	24.785	18	CP
B	20.777	18	APL

Médias com a mesma letra não são significativamente diferentes

Análise da variável : dente decíduo

	Adesivo	N Obs	Mean	Std Dev	Upper 95% CL for Mean	Lower 95% CL for Mean
B	APL	18	20.7767475	5.3735085	23.4489304	18.1045646
A	CP	18	24.7850306	5.7648039	27.6518001	21.9182610
A	SB	18	26.8442039	4.6418201	29.1525267	24.5358810

Médias com a mesma letra não são significativamente diferentes

Class Level Information

Class	Levels	Values
adesivo	3	APL CP SB
tratamento	2	com sem
tempo	3	24 h 45 dias 90 dias

Number of Observations Read	54
Number of Observations Used	54

Variável dependente: dente permanente – valores transformados

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
adesivo	2	1.03600636	0.51800318	20.82	<.0001 *
tratamento	1	0.04358538	0.04358538	1.75	0.1940 NS
tempo	2	0.53677326	0.26838663	10.79	0.0002 *
adesivo*tratamento	2	0.20787393	0.10393696	4.18	0.0234 *
adesivo*tempo	4	0.13629533	0.03407383	1.37	0.2638 NS
tratamento*tempo	2	0.18643727	0.09321864	3.75	0.0332 *
adesiv*tratame*tempo	4	0.00948803	0.00237201	0.10	0.9833 NS
Error	36	0.89564320	0.02487898		
Corrected Total	53	3.05210275			

* significativo

NS Não significativo

R-Square	Coeff Var	Root MSE	tpermanente Mean
0.706549	4.828670	0.157731	3.266546

Least Squares Means
Adjustment for Multiple Comparisons: Tukey

	adesivo	tratamento	tpermanente LSMEAN	LSMEAN Number
Aa	APL	com	3.01476957	1
Aa	APL	sem	3.12661816	2
Ab	CP	com	3.41714542	3
Aa	CP	sem	3.31794549	4
Ab	SB	com	3.45295309	5
Aa	SB	sem	3.26984352	6

Letras maiúsculas iguais não há diferença estatística significativa dentro do mesmo adesivo

Letras minúsculas iguais não há diferença estatística significativa dentro do mesmo tratamento

Least Squares Means for effect adesivo*tratamento

Pr > |t| for H0: LSMean(i)=LSMean(j)

Variável dependente: tpermanente

i/j	1	2	3	4	5	6
1		0.6638	<.0001	0.0030	<.0001	0.0176
2	0.6638		0.0049	0.1304	0.0012	0.4034
3	<.0001	0.0049		0.7644	0.9965	0.3726
4	0.0030	0.1304	0.7644		0.4689	0.9865
5	<.0001	0.0012	0.9965	0.4689		0.1625
6	0.0176	0.4034	0.3726	0.9865	0.1625	

Variável dependente: dente permanente

Adesivo	Tratamento	N Obs	Mean	Std Dev	Upper 95% CL for Mean	Lower 95% CL for Mean
APL	com	9	20.6745671	3.7535730	23.5598187	17.7893155
	sem	9	23.2161412	4.6512488	26.7914075	19.6408749
CP	com	9	30.6009064	2.8972766	32.8279504	28.3738625
	sem	9	27.9116100	4.3047237	31.2205135	24.6027064
SB	com	9	31.9675279	5.3384054	36.0709895	27.8640663
	sem	9	27.3970813	8.7251552	34.1038294	20.6903333

Least Squares Means
Adjustment for Multiple Comparisons: Tukey

tratamento	tempo	tpermanente LSMEAN	LSMEAN Number
Aa com	24 h	3.34318357	1
Aa com	45 dias	3.29958643	2
Aa com	90 dias	3.24209808	3
Aa sem	24 h	3.40257155	4
Aa sem	45 dias	3.28755503	5
Ab sem	90 dias	3.02428059	6

Letras maiúsculas iguais não há diferença estatística significativa dentro do mesmo tempo

Letras minúsculas iguais não há diferença estatística significativa dentro do mesmo tratamento

Least Squares Means for effect tratamento*tempo

Pr > |t| for H0: LSMean(i)=LSMean(j)

Variável dependente: tpermanente

i/j	1	2	3	4	5	6
1		0.9913	0.7501	0.9659	0.9743	0.0017
2	0.9913		0.9703	0.7354	1.0000	0.0086
3	0.7501	0.9703		0.2819	0.9895	0.0601
4	0.9659	0.7354	0.2819		0.6373	0.0002
5	0.9743	1.0000	0.9895	0.6373		0.0132
6	0.0017	0.0086	0.0601	0.0002	0.0132	

Variável dependente: dente permanente

Tratamento	Tempo de armazenamento	N Obs	Mean	Std Dev	Upper 95% CL for Mean	Lower 95% CL for Mean
com	24 h	9	29.1342253	6.7166828	34.2971248	23.9713258
	45 dias	9	28.1771607	8.1640546	34.4526086	21.9017129
	90 dias	9	25.9316154	4.3139142	29.2475834	22.6156474
sem	24 h	9	30.6385058	6.8711445	35.9201350	25.3568766
	45 dias	9	27.1543036	4.4848080	30.6016321	23.7069750
	90 dias	9	20.7320232	2.6585185	22.7755414	18.6885049

Análise estatística do modo de fratura de sistemas adesivos aplicados à dentina de dentes decíduos e permanentes tratadas ou não previamente com solução de NaOCL 0.5%.

Análise de variância

Class Level Information

Class	Levels	Values
adesivo	3	APL CP SB
tratamento	2	com sem
tempo	3	24 h 45 dias 90 dias

Number of Observations Read	54
Number of Observations Used	54

Variável dependente: dente decíduo

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
adesivo	2	68426.9614	34213.4807	2.46	0.1000 NS
tratamento	1	7506.5751	7506.5751	0.54	0.4676 NS
tempo	2	37861.0318	18930.5159	1.36	0.2698 NS
adesivo*tratamento	2	49147.8438	24573.9219	1.76	0.1858 NS
adesivo*tempo	4	53923.0703	13480.7676	0.97	0.4371 NS
tratamento*tempo	2	19906.1033	9953.0517	0.71	0.4962 NS
adesiv*tratame*tempo	4	107179.5093	26794.8773	1.92	0.1276 NS
Error	36	501448.3016	13929.1195		
Corrected Total	53	845399.3966			

NS Nao significativo

R-Square	Coeff Var	Root MSE	deciduo Mean
0.406850	26.42888	118.0217	446.5634

Análise de Variância

Class Level Information

Class	Levels	Values
adesivo	3	APL CP SB
tratamento	2	com sem
tempo	3	24 h 45 dias 90 dias

Number of Observations Read	54
Number of Observations Used	54

Variável dependente: dente permanente

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
adesivo	2	190257.6372	95128.8186	10.30	0.0003*
tratamento	1	28011.8454	28011.8454	3.03	0.0902 NS
tempo	2	13161.4314	6580.7157	0.71	0.4973 NS
adesivo*tratamento	2	94.5492	47.2746	0.01	0.9949 NS
adesivo*tempo	4	13752.8845	3438.2211	0.37	0.8269 NS
tratamento*tempo	2	10461.2609	5230.6305	0.57	0.5727 NS
adesiv*tratame*tempo	4	48522.3028	12130.5757	1.31	0.2837 NS
Error	36	332605.9276	9239.0535		
Corrected Total	53	636867.8390			

* significativo

NS Não significativo

R-Square	Coeff Var	Root MSE	permanente Mean
0.477747	21.02104	96.12000	457.2561

t Tests (LSD) for permanente

Alpha	0.05
Error Degrees of Freedom	36
Error Mean Square	9239.054
Critical Value of t	2.02809
Least Significant Difference	64.98

Médias com as mesmas letras não são significativamente diferentes

t Grouping	Mean	N	adesivo
A	541.01	18	CP
B	420.27	18	SB
B	410.49	18	APL

Análise da variável: dente permanente

Adesivo	N Obs	Mean	Std Dev	Upper 95% CL for Mean	Lower 95% CL for Mean
APL	18	410.4874373	102.7096470	461.5637431	359.4111314
CP	18	541.0097012	99.4805032	590.4801915	491.5392108
SB	18	420.2710327	76.3252662	458.2266946	382.3153708

Substrato:dente decíduo

The FREQ Procedure

Table of adesivo by fratura

adesivo(Adesivo)   fratura(Tipo de Fratura)

Frequency / Percent / Row Pct / Col Pct	a.Adesiva	b.Mista	c.Coesiva material	d.Coesiva dentina	Total
APL	1	57	16	1	75
	0.47	26.51	7.44	0.47	34.88
	1.33	76.00	21.33	1.33	
	33.33	38.78	27.12	16.67	
CP	2	38	27	3	70
	0.93	17.67	12.56	1.40	32.56
	2.86	54.29	38.57	4.29	
	66.67	25.85	45.76	50.00	
SB	0	52	16	2	70
	0.00	24.19	7.44	0.93	32.56
	0.00	74.29	22.86	2.86	
	0.00	35.37	27.12	33.33	
Total	3	147	59	6	215
	1.40	68.37	27.44	2.79	100.00

Statistics for Table of adesivo by fratura

Statistic	DF	Value	Prob
Chi-Square	6	10.9268	0.0907
Likelihood Ratio Chi-Square	6	11.5945	0.0716
Mantel-Haenszel Chi-Square	1	0.4807	0.4881
Phi Coefficient		0.2254	
Contingency Coefficient		0.2199	
Cramer's V		0.1594	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Summary Statistics for adesivo by fratura

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.4807	0.4881
2	Row Mean Scores Differ	2	5.9369	0.0514
3	General Association	6	10.8760	0.0923

Total Sample Size = 215

Substrato:dente decíduo

The FREQ Procedure

Table of tratamento by fratura

tratamento(Tratamento) fratura(Tipo de Fratura)

Frequency / Percent / Row Pct / Col Pct	a.Adesiva	b.Mista	c.Coesiva material	d.Coesiva dentina	Total
com	0 0.00 0.00 0.00	72 33.49 68.57 48.98	29 13.49 27.62 49.15	4 1.86 3.81 66.67	105 48.84
sem	3 1.40 2.73 100.00	75 34.88 68.18 51.02	30 13.95 27.27 50.85	2 0.93 1.82 33.33	110 51.16
Total	3 1.40	147 68.37	59 27.44	6 2.79	215 100.00

Statistics for Table of tratamento by fratura

Statistic	DF	Value	Prob
Chi-Square	3	3.6305	0.3042
Likelihood Ratio Chi-Square	3	4.8004	0.1870
Mantel-Haenszel Chi-Square	1	0.8876	0.3461
Phi Coefficient		0.1299	
Contingency Coefficient		0.1289	
Cramer's V		0.1299	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Summary Statistics for tratamento by fratura

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.8876	0.3461
2	Row Mean Scores Differ	1	0.8876	0.3461
3	General Association	3	3.6136	0.3063

Total Sample Size = 215

Substrato:dente decíduo

The FREQ Procedure

Table of tempo by fratura

tempo(Tempo de armazenamento)   fratura(Tipo de Fratura)

Frequency / Percent / Row Pct / Col Pct	a.Adesiva	b.Mista	c.Coesiva material	d.Coesiva dentina	Total
24 h	1	43	25	2	71
	0.47	20.00	11.63	0.93	33.02
	1.41	60.56	35.21	2.82	
	33.33	29.25	42.37	33.33	
45 dias	1	52	15	2	70
	0.47	24.19	6.98	0.93	32.56
	1.43	74.29	21.43	2.86	
	33.33	35.37	25.42	33.33	
90 dias	1	52	19	2	74
	0.47	24.19	8.84	0.93	34.42
	1.35	70.27	25.68	2.70	
	33.33	35.37	32.20	33.33	
Total	3	147	59	6	215
	1.40	68.37	27.44	2.79	100.00

Statistics for Table of tempo by fratura

Statistic	DF	Value	Prob
Chi-Square	6	3.6035	0.7302
Likelihood Ratio Chi-Square	6	3.5671	0.7350
Mantel-Haenszel Chi-Square	1	1.1053	0.2931
Phi Coefficient		0.1295	
Contingency Coefficient		0.1284	
Cramer's V		0.0915	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Summary Statistics for tempo by fratura

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.1053	0.2931
2	Row Mean Scores Differ	2	2.3373	0.3108
3	General Association	6	3.5867	0.7324

Total Sample Size = 215

Substrato: dente permanente

The FREQ Procedure

Table of adesivo by fratura

adesivo(Adesivo)   fratura(Tipo de Fratura)

Frequency / Percent / Row Pct / Col Pct	a.Adesiva	b.Mista	c.Coesiva material	d.Coesiva dentina	Total
APL	15	148	58	0	221
	2.17	21.42	8.39	0.00	31.98
	6.79	66.97	26.24	0.00	
	51.72	35.75	24.37	0.00	
CP	7	100	125	4	236
	1.01	14.47	18.09	0.58	34.15
	2.97	42.37	52.97	1.69	
	24.14	24.15	52.52	40.00	
SB	7	166	55	6	234
	1.01	24.02	7.96	0.87	33.86
	2.99	70.94	23.50	2.56	
	24.14	40.10	23.11	60.00	
Total	29	414	238	10	691
	4.20	59.91	34.44	1.45	100.00

Statistics for Table of adesivo by fratura

Statistic	DF	Value	Prob
Chi-Square	6	65.1668	<.0001
Likelihood Ratio Chi-Square	6	66.7335	<.0001
Mantel-Haenszel Chi-Square	1	1.0575	0.3038
Phi Coefficient		0.3071	
Contingency Coefficient		0.2936	
Cramer's V		0.2171	

WARNING: 25% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Summary Statistics for adesivo by fratura

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0575	0.3038
2	Row Mean Scores Differ	2	45.1748	<.0001
3	General Association	6	65.0725	<.0001

Total Sample Size = 691

Substrato: dente permanente

The FREQ Procedure

Table of tratamento by fratura

```
tratamento(Tratamento)     fratura(Tipo de Fratura)

Frequency|
Percent  |
Row Pct  |
Col Pct  |a.Adesiv|b.Mista |c.Coesiv|d.Coesiv|  Total
         |a       |        |a materi|a dentin|
         |        |        |al      |a       |
---------+--------+--------+--------+--------+
com      |      3 |    207 |    127 |      9 |    346
         |   0.43 |  29.96 |  18.38 |   1.30 |  50.07
         |   0.87 |  59.83 |  36.71 |   2.60 |
         |  10.34 |  50.00 |  53.36 |  90.00 |
---------+--------+--------+--------+--------+
sem      |     26 |    207 |    111 |      1 |    345
         |   3.76 |  29.96 |  16.06 |   0.14 |  49.93
         |   7.54 |  60.00 |  32.17 |   0.29 |
         |  89.66 |  50.00 |  46.64 |  10.00 |
---------+--------+--------+--------+--------+
Total          29      414      238       10      691
             4.20    59.91    34.44     1.45   100.00
```

Statistics for Table of tratamento by fratura

Statistic	DF	Value	Prob
Chi-Square	3	25.7156	<.0001
Likelihood Ratio Chi-Square	3	29.3484	<.0001
Mantel-Haenszel Chi-Square	1	12.9131	0.0003
Phi Coefficient		0.1929	
Contingency Coefficient		0.1894	
Cramer's V		0.1929	

Summary Statistics for tratamento by fratura

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	12.9131	0.0003
2	Row Mean Scores Differ	1	12.9131	0.0003
3	General Association	3	25.6784	<.0001

Total Sample Size = 691

Substrato: dente permanente

The FREQ Procedure

Table of tempo by fratura

```
tempo(Tempo de armazenamento)     fratura(Tipo de Fratura)

Frequency|
Percent  |
Row Pct  |
Col Pct  |a.Adesiv|b.Mista |c.Coesiv|d.Coesiv|  Total
         |a       |        |a materi|a dentin|
         |        |        |al      |a       |
---------+--------+--------+--------+--------+
24 h     |      6 |    138 |     93 |      3 |    240
         |   0.87 |  19.97 |  13.46 |   0.43 |  34.73
         |   2.50 |  57.50 |  38.75 |   1.25 |
         |  20.69 |  33.33 |  39.08 |  30.00 |
---------+--------+--------+--------+--------+
45 dias  |     10 |    141 |     71 |      5 |    227
         |   1.45 |  20.41 |  10.27 |   0.72 |  32.85
         |   4.41 |  62.11 |  31.28 |   2.20 |
         |  34.48 |  34.06 |  29.83 |  50.00 |
---------+--------+--------+--------+--------+
90 dias  |     13 |    135 |     74 |      2 |    224
         |   1.88 |  19.54 |  10.71 |   0.29 |  32.42
         |   5.80 |  60.27 |  33.04 |   0.89 |
         |  44.83 |  32.61 |  31.09 |  20.00 |
---------+--------+--------+--------+--------+
Total          29      414      238       10      691
             4.20    59.91    34.44     1.45   100.00
```

Statistics for Table of tempo by fratura

Statistic	DF	Value	Prob
Chi-Square	6	6.9866	0.3221
Likelihood Ratio Chi-Square	6	7.0474	0.3165
Mantel-Haenszel Chi-Square	1	3.3176	0.0685
Phi Coefficient		0.1006	
Contingency Coefficient		0.1000	
Cramer's V		0.0711	

WARNING: 25% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Summary Statistics for tempo by fratura

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	3.3176	0.0685
2	Row Mean Scores Differ	2	3.6266	0.1631
3	General Association	6	6.9765	0.3230

Total Sample Size = 691

Análise estatística da interface de união de sistemas adesivos aplicados à dentina de dentes decíduos e permanentes tratadas ou não previamente com solução de NaOCL 0.5%.

Substrato: dente decíduo

Variável dependente: sistema adesivo

```
Frequency|
Percent  |
Row Pct  |
Col Pct  |a.Tag pr|a.Tag au|  Total
         |esente  |sente   |
---------+--------+--------+
APL      |     17 |      7 |     24
         |  23.61 |   9.72 |  33.33
         |  70.83 |  29.17 |
         |  28.33 |  58.33 |
---------+--------+--------+
CP       |     19 |      5 |     24
         |  26.39 |   6.94 |  33.33
         |  79.17 |  20.83 |
         |  31.67 |  41.67 |
---------+--------+--------+
SB       |     24 |      0 |     24
         |  33.33 |   0.00 |  33.33
         | 100.00 |   0.00 |
         |  40.00 |   0.00 |
---------+--------+--------+
Total          60       12       72
            83.33    16.67   100.00
```

Estatística para tabela de sistemas adesivos

Statistic	DF	Value	Prob
Chi-Square	2	7.8000	0.0202
Likelihood Ratio Chi-Square	2	11.3427	0.0034
Mantel-Haenszel Chi-Square	1	7.2479	0.0071
Phi Coefficient		0.3291	
Contingency Coefficient		0.3126	
Cramer's V		0.3291	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	7.2479	0.0071
2	Row Mean Scores Differ	2	7.6917	0.0214
3	General Association	2	7.6917	0.0214

Total Sample Size = 72

Substrato: dente decíduo

Variável dependente: tratamento

Frequency / Percent / Row Pct / Col Pct	a.Tag presente	a.Tag ausente	Total
com	32	4	36
	44.44	5.56	50.00
	88.89	11.11	
	53.33	33.33	
sem	28	8	36
	38.89	11.11	50.00
	77.78	22.22	
	46.67	66.67	
Total	60	12	72
	83.33	16.67	100.00

Estatística para tabela de tratamento

Statistic	DF	Value	Prob
Chi-Square	1	1.6000	0.2059
Likelihood Ratio Chi-Square	1	1.6261	0.2022
Continuity Adj. Chi-Square	1	0.9000	0.3428
Mantel-Haenszel Chi-Square	1	1.5778	0.2091
Phi Coefficient		0.1491	
Contingency Coefficient		0.1474	
Cramer's V		0.1491	

Fisher's Exact Test

Cell (1,1) Frequency (F)	32
Left-sided Pr <= F	0.9443
Right-sided Pr >= F	0.1717
Table Probability (P)	0.1160
Two-sided Pr <= P	0.3434

Sample Size = 72

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.5778	0.2091
2	Row Mean Scores Differ	1	1.5778	0.2091
3	General Association	1	1.5778	0.2091

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control	Mantel-Haenszel	2.2857	0.6211	8.4121
(Odds Ratio)	Logit	2.2857	0.6211	8.4121
Cohort	Mantel-Haenszel	1.1429	0.9270	1.4090
(Col1 Risk)	Logit	1.1429	0.9270	1.4090
Cohort	Mantel-Haenszel	0.5000	0.1651	1.5138
(Col2 Risk)	Logit	0.5000	0.1651	1.5138

Total Sample Size = 72

Substrato: dente decíduo

Variável dependente: tempo

```
Frequency|
Percent  |
Row Pct  |
Col Pct  |a.Tag pr|a.Tag au|  Total
         |esente  |sente   |
---------+--------+--------+
24 h     |     21 |      3 |     24
         |  29.17 |   4.17 |  33.33
         |  87.50 |  12.50 |
         |  35.00 |  25.00 |
---------+--------+--------+
45 dias  |     22 |      2 |     24
         |  30.56 |   2.78 |  33.33
         |  91.67 |   8.33 |
         |  36.67 |  16.67 |
---------+--------+--------+
90 dias  |     17 |      7 |     24
         |  23.61 |   9.72 |  33.33
         |  70.83 |  29.17 |
         |  28.33 |  58.33 |
---------+--------+--------+
Total          60       12       72
            83.33    16.67   100.00
```

Estatística para tabela de tempo

Statistic	DF	Value	Prob
Chi-Square	2	4.2000	0.1225
Likelihood Ratio Chi-Square	2	4.0531	0.1318
Mantel-Haenszel Chi-Square	1	2.3667	0.1240
Phi Coefficient		0.2415	
Contingency Coefficient		0.2348	
Cramer's V		0.2415	

WARNING: 50% of the cells have expected counts less
than 5. Chi-Square may not be a valid test.

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.3667	0.1240
2	Row Mean Scores Differ	2	4.1417	0.1261
3	General Association	2	4.1417	0.1261

Total Sample Size = 72

Substrato: dente permanente

Variável dependente: sistema adesivo

```
Frequency|
Percent  |
Row Pct  |
Col Pct  |a.Tag pr|a.Tag au|  Total
         |esente  |sente   |
---------+--------+--------+
APL      |     20 |      4 |     24
         |  27.78 |   5.56 |  33.33
         |  83.33 |  16.67 |
         |  33.90 |  30.77 |
---------+--------+--------+
CP       |     19 |      5 |     24
         |  26.39 |   6.94 |  33.33
         |  79.17 |  20.83 |
         |  32.20 |  38.46 |
---------+--------+--------+
SB       |     20 |      4 |     24
         |  27.78 |   5.56 |  33.33
         |  83.33 |  16.67 |
         |  33.90 |  30.77 |
---------+--------+--------+
Total          59       13       72
            81.94    18.06   100.00
```

Estatística para tabela de sistema adesivo

Statistic	DF	Value	Prob
Chi-Square	2	0.1877	0.9104
Likelihood Ratio Chi-Square	2	0.1844	0.9119
Mantel-Haenszel Chi-Square	1	0.0000	1.0000
Phi Coefficient		0.0511	
Contingency Coefficient		0.0510	
Cramer's V		0.0511	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0000	1.0000
2	Row Mean Scores Differ	2	0.1851	0.9116
3	General Association	2	0.1851	0.9116

Total Sample Size = 72

Substrato: dente permanente

Variável dependente: tratamento

Frequency Percent Row Pct Col Pct	a.Tag presente	a.Tag ausente	Total
com	27 37.50 75.00 45.76	9 12.50 25.00 69.23	36 50.00
sem	32 44.44 88.89 54.24	4 5.56 11.11 30.77	36 50.00
Total	59 81.94	13 18.06	72 100.00

Estatística para tabela de tratamento

Statistic	DF	Value	Prob
Chi-Square	1	2.3468	0.1255
Likelihood Ratio Chi-Square	1	2.3978	0.1215
Continuity Adj. Chi-Square	1	1.5020	0.2204
Mantel-Haenszel Chi-Square	1	2.3142	0.1282
Phi Coefficient		-0.1805	
Contingency Coefficient		0.1777	
Cramer's V		-0.1805	

Fisher's Exact Test

Cell (1,1) Frequency (F)	27
Left-sided Pr <= F	0.1098
Right-sided Pr >= F	0.9684
Table Probability (P)	0.0782
Two-sided Pr <= P	0.2196

Sample Size = 72

Summary Statistics for tratamento by pati2

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.3142	0.1282
2	Row Mean Scores Differ	1	2.3142	0.1282
3	General Association	1	2.3142	0.1282

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control	Mantel-Haenszel	0.3750	0.1038	1.3546
(Odds Ratio)	Logit	0.3750	0.1038	1.3546
Cohort	Mantel-Haenszel	0.8438	0.6763	1.0526
(Col1 Risk)	Logit	0.8438	0.6763	1.0526
Cohort	Mantel-Haenszel	2.2500	0.7615	6.6482
(Col2 Risk)	Logit	2.2500	0.7615	6.6482

Total Sample Size = 72

Substrato: dente permanente

Variável dependente: tempo

```
Frequency|
Percent  |
Row Pct  |
Col Pct  |a.Tag pr|a.Tag au|  Total
         |esente  |sente   |
---------+--------+--------+
24 h     |     22 |      2 |     24
         |  30.56 |   2.78 |  33.33
         |  91.67 |   8.33 |
         |  37.29 |  15.38 |
---------+--------+--------+
45 dias  |     20 |      4 |     24
         |  27.78 |   5.56 |  33.33
         |  83.33 |  16.67 |
         |  33.90 |  30.77 |
---------+--------+--------+
90 dias  |     17 |      7 |     24
         |  23.61 |   9.72 |  33.33
         |  70.83 |  29.17 |
         |  28.81 |  53.85 |
---------+--------+--------+
Total          59       13       72
            81.94    18.06   100.00
```

Estatística para tabela de tempo

Statistic	DF	Value	Prob
Chi-Square	2	3.5671	0.1680
Likelihood Ratio Chi-Square	2	3.6322	0.1627
Mantel-Haenszel Chi-Square	1	3.4713	0.0624
Phi Coefficient		0.2226	
Contingency Coefficient		0.2173	
Cramer's V		0.2226	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Sample Size = 72

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	3.4713	0.0624
2	Row Mean Scores Differ	2	3.5176	0.1723
3	General Association	2	3.5176	0.1723

Total Sample Size = 72

Coeficiente de correlação de Spearman

Dentes Decíduos

1 With Variables: fratura
1 Variables: deciduo

Spearman Correlation Coefficients, N = 54
Prob > |r| under H0: Rho=0

	deciduo
fratura Tipo de Fratura	-0.05060 0.7163

Dentes Permanentes

1 With Variables: fratura
1 Variables: permanente

Spearman Correlation Coefficients, N = 54
Prob > |r| under H0: Rho=0

	permanente
fratura Tipo de Fratura	0.40896 0.0021

Estudo de associação entre classes
Dentes Decíduos

Table of classe_fra by classe_mpa

classe_fra (Frequency / Percent / Row Pct / Col Pct)	classe_mpa: a.<=20.0 856	b.<=23.0 811	c.<=29.6 637	d. >29.6 637	Total
a. <=1	4	4	5	4	17
	7.41	7.41	9.26	7.41	31.48
	23.53	23.53	29.41	23.53	
	28.57	30.77	35.71	30.77	
b. <=2	10	9	9	9	37
	18.52	16.67	16.67	16.67	68.52
	27.03	24.32	24.32	24.32	
	71.43	69.23	64.29	69.23	
Total	14	13	14	13	54
	25.93	24.07	25.93	24.07	100.00

Statistics for Table of classe_fra by classe_mpa

Statistic	DF	Value	Prob
Chi-Square	3	0.1774	0.9812
Likelihood Ratio Chi-Square	3	0.1758	0.9814
Mantel-Haenszel Chi-Square	1	0.0448	0.8324
Phi Coefficient		0.0573	
Contingency Coefficient		0.0572	
Cramer's V		0.0573	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Sample Size = 54

Estudo de associação entre classes
Dentes Permanentes

The FREQ Procedure

Table of classe_fra by classe_mpa

classe_fra / classe_mpa Frequency Percent Row Pct Col Pct	a.<=21.9 648	b.<=27.4 796	c.<=31.3 327	d. >31.3 327	Total
a. <=1	3 5.56 42.86 23.08	1 1.85 14.29 7.14	2 3.70 28.57 14.29	1 1.85 14.29 7.69	7 12.96
b. <=2	10 18.52 21.28 76.92	13 24.07 27.66 92.86	12 22.22 25.53 85.71	12 22.22 25.53 92.31	47 87.04
Total	13 24.07	14 25.93	14 25.93	13 24.07	54 100.00

Statistics for Table of classe_fra by classe_mpa

Statistic	DF	Value	Prob
Chi-Square	3	1.9407	0.5848
Likelihood Ratio Chi-Square	3	1.8693	0.6000
Mantel-Haenszel Chi-Square	1	0.8301	0.3623
Phi Coefficient		0.1896	
Contingency Coefficient		0.1863	
Cramer's V		0.1896	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Sample Size = 54