Victor Pinheiro Feitosa

Nanoinfiltração e resistência da união de sistemas adesivos avaliados sob pressão pulpar simulada

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Orientador: Prof. Dr. Américo Bortolazzo Correr

Co-Orientador: Prof. Dr. Mário Alexandre Coelho Sinhoreti

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Prof. Dr. AMERICO BORTOLAZZO CORRER

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Prof. Dr. ANDRÉ FIGUEIREDO REIS

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Prof. Dr. MARCELO GIANNINI

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"Ninguém é tão grande que não possa aprender e nem tão pequeno que não possa ensinar."

(Píndaro)

RESUMO

O objetivo neste estudo foi avaliar o efeito do tempo de fotoativação de adesivos de passo único e da pressão pulpar simulada na resistência da união e nanoinfiltração de compósitos resinosos à dentina humana. Este estudo foi separado em 2 Capítulos. No Capítulo 1 foi verificada a influência do aumento do tempo de fotoativação para um sistema adesivo autocondicionante de passo único (Clearfil S3 Bond) submetido ou não à pressão intrapulpar simulada. Foram obtidas superfícies planas em dentina profunda de terceiros molares, os quais foram separados em quatro grupos (n=5). O adesivo foi aplicado seguindo a recomendação do fabricante e fotoativado por 10 segundos (recomendação do fabricante) ou 40 segundos (tempo de fotoativação aumentado). A fotoativação foi realizada com o aparelho de luz de lâmpada halógena XL-2500 com irradiância de 600 mW/cm². Os dentes foram restaurados com o compósito nanoparticulado Filtek Z350. Metade dos grupos foi armazenada sob pressão pulpar simulada e a outra metade foi armazenada em água, sem pressão pulpar simulada. Após 24 horas, os dentes foram cortados em palitos e submetidos ao teste de resistência da união por microtração. Os dados foram submetidos à análise de variância dois fatores e teste de Tukey (p<0.05). Os resultados mostraram que a pressão pulpar diminuiu significativamente a resistência da união para ambos os tempos de fotoativação. O aumento do tempo de fotoativação de 40s resultou em significante aumento de resistência para os grupos com pressão pulpar; entretanto, não foi significativo para os grupos sem pressão. Pode ser concluído que a pressão pulpar simulada diminuiu a resistência da união, mas o aumento do tempo de fotoativação melhorou a resistência da união a dentina nos grupos armazenados sob pressão pulpar. No Capítulo 2 o objetivo foi avaliar *in vitro* uma nova metodologia para simular a pressão pulpar comparada à metodologia tradicional. Foram utilizados quatro sistemas adesivos (Clearfil S3 Bond, Clearfil SE Bond, Adper Single Bond Plus, and Scotchbond Multi-Purpose), que foram aplicados em superfícies planas de dentina profunda de terceiros molares. Após a restauração com compósito as amostras foram armazenadas em água sem pressão pulpar, submetidas à pressão pulpar convencional ou pela nova metodologia de pressão pulpar. Após 24 horas, os dentes foram cortados em palitos e submetidos ao teste de resistência da união por microtração. Os dados foram submetidos à análise de variância dois fatores e teste de Tukey (p<0.05). Os resultados mostraram que os adesivos simplificados (autocondicionante de passo único e de técnica úmida de dois passos) foram negativamente influenciados pela pressão pulpar, mas não houve diferença significativa entre as duas metodologias de simulação da pressão pulpar. Os adesivos autocondicionante de dois passos e de técnica úmida de três passos não foram afetados pela simulação da pressão pulpar (p>0,05). O padrão de nanoinfiltração mostrou similaridade entre as duas metodologias. Os maiores valores de resistência da união foram apresentados pelo Scotchbond Multi-Purpose e os menores pelo adesivo Clearfil S3 Bond. Pode ser concluído que ambas as metodologias diminuíram a resistência da união dos adesivos de técnica simplificada, sem haver diferença entre a metodologia experimental e a metodologia convencional para resistência da união e padrão de nanoinfiltração. Conclui-se que o aumento do tempo de fotoativação melhorou a união nos adesivos autocondicionante simplificados sob pressão pulpar simulada. Além disso, a metodologia experimental de simulação da pressão pulpar pode ser utilizada em substituição a metodologia tradicional.

Palavras-chave: fotopolimerização, adesivos dentinários.

ABSTRACT

The aim of this study was to evaluate the extended photoactivation time for one-step self-etch adhesives and the simulated pulpal pressure applied into two methods on bond strength of direct restoration of composite resin to human dentin. This work was divided into two Chapters. The Chapter 1 analyzed the influence of extended photoactivation time of one-step self-etch adhesive Clearfil S3 Bond with and without conventional simulated pulpal pressure. It was obtained flat surfaces in deep dentin from extracted third molars and they were divided randomly in four groups (n=5). The adhesive was used in agreement with manufacturer's recommendation (photoactivation time 10s) and with the extended photoactivation time to 40s, after the composite restoration was built up with nanofilled composite resin Filtek Z350. The photoactivation procedures were realized with quartz-tungsten halogen lamp XL-2500 with a standard irradiance of 600mW/cm². Half of the samples were submitted to simulated pulpal pressure and the other half was stored in water without pulpal pressure. After 24 hours, the samples were cut into sticks and the microtensile bond strength test was performed. The results were submitted to two-way ANOVA and Tukey's test (p<0.05). Pulpal pressure decreased bond strength for both photoactivation times and the extended photoactivation time showed significant increase in bond strength for groups with simulated pulpal pressure, however the increase was not statistically significant for groups without pulpal pressure. It can be concluded that simulated pulpal pressure decreased bond strength, however the extended photoactivation time improved the bonding under pulpal pressure simulation. In Chapter 2, a new methodology to simulate pulpal pressure was tested in comparison with the traditional methodology and control groups without pulpal pressure. One adhesive system of each of the four approaches was applied in flat surfaces in deep dentin of extracted molars as in Chapter 1. After the composite was built up, samples were stored in water storage without pulpal pressure, under conventional simulated pulpal pressure or under the new methodology to simulate pulpal pressure. After microtensile test, the results showed no differences between two methodologies of simulated pulpal pressure (p<0.05) and nanoleakage patterns were similar for both methods. Simplified adhesives were more influenced by pulpal pressure than multi-step adhesives, showing significant decrease in bond strength. The highest bond strength values were obtained by the three-step adhesive Scotchbond Multi-Purpose and the lowest were obtained by the one-step adhesive Clearfil S3 Bond. In conclusion, the extended photoactivation time improves bonding for one-step self-etch adhesives under simulated pulpal pressure. In addition, the experimental methodology to simulate pulpal pressure can be used in replacing the conventional methodology.

Key words: light-curing, dentin-bonding agents.

SUMÁRIO

INTRODUÇÃO GERAL

Os materiais restauradores adesivos passaram por grande desenvolvimento. Devido à utilização destes materiais, não é necessário o desgaste de estrutura sadia para a retenção das restaurações, pois eles propiciam união efetiva e relativamente duradoura ao esmalte e dentina. O avanço dos sistemas adesivos em conjunto da melhoria nas técnicas restauradoras tem facilitado a prática clínica e aumentado a durabilidade das restaurações de compósitos resinosos restauradores.

No intuito de diminuir o tempo clínico e simplificar a técnica restauradora, surgiram os sistemas adesivos autocondicionantes, os quais concentram os tradicionais três passos para adesão (condicionamento ácido, aplicação do *primer* e aplicação do agente de união) em dois ou até mesmo em um passo. Esses sistemas adesivos não utilizam a técnica úmida, não necessitando da etapa inicial do condicionamento com ácido fosfórico e lavagem. Portanto, a técnica adesiva é mais padronizada entre os diferentes operadores. Nos adesivos autocondicionantes, a desmineralização e penetração dos monômeros no substrato dentinário ocorrem simultaneamente, que diminui a quantidade de fibrilas colágenas não envoltas por polímero e susceptíveis à degradação. Também ocorre a modificação e a incorporação da lama dentinária à camada híbrida, mantendo os *smear plugs* e impedindo aumento excessivo de permeabilidade do substrato (40).

A simplificação dos passos para uma única aplicação ainda não tem demonstrado resultados satisfatórios *in vitro* há alguns anos atrás (16). A tentativa de manter em um único frasco todos os componentes dos sistemas adesivos fez com que a solução tivesse que ser excessivamente hidrófila (5,40). Nestes sistemas a camada de adesivo, mesmo depois de polimerizada permanece hidrófila, mostrando altas taxas de permeabilidade e absorção de água e, com isso, aumento na velocidade de degradação da região de união (15,37). A permeabilidade e absorção de água por esses adesivos após a polimerização são resultantes da grande quantidade de monômeros mais ácidos e hidrófilos e do solvente residual (8). Esse fato também ocorre com adesivos de técnica úmida, principalmente com os de dois frascos, onde *primer* e adesivo encontram-se em uma única solução (15). Nos sistemas

adesivos autocondicionantes de dois passos, após a aplicação do *primer*, que é mais ácido e hidrófilo, é aplicada uma solução sem solvente, composta na maioria por monômeros hidrófobos, havendo diminuição da permeabilidade e da absorção de água (30,31).

Após a aplicação dos adesivos autocondicionantes de frasco único é notada a distribuição heterogênea dos monômeros ácidos hidrófilos e hidrófobos na camada híbrida e na camada de adesivo (12,42). Isto ocorre devido à hidrofilia, sendo um problema intrínseco destes adesivos. Ao observar esses adesivos em microscopia de luz, é possível notar a distribuição heterogênea com separação de fase e formação de gotículas (39). Isso promove a formação de sítios hidrófilos na região de adesão (36), o que aumenta a absorção de água e, consequentemente, diminui as propriedades mecânicas do adesivo. A captação e transdução de água através da camada de adesivo e camada híbrida (34) assim como os sítios polares formados podem resultar em plastificação dos polímeros com característica mais hidrófila resultando na degradação da interface de união e na redução da durabilidade da união (11,27). Os sítios hidrófilos, a captação e a transudação da água (árvores de água) são notados microscopicamente na superfície do adesivo e camada híbrida, principalmente após simulação de pressão pulpar (1,18,31).

Para a mistura de monômeros de diferentes características (muito ácidos, pouco ácidos, hidrófilos e hidrófobos) é necessária grande quantidade de solvente. Normalmente, esse solvente é a água (16), pois ela é essencial para a ionização dos monômeros funcionais (40), além de prevenir a polimerização dos monômeros dentro do frasco (6). No entanto, a água conduz à separação de fase quando não está associada a outros solventes ou a monômeros hidrófilos como o 2-hidroxietilmetacrilato (HEMA) (39), que são utilizados para aumentar a solubilidade dos monômeros hidrófobos.

Melhorias em relação aos monômeros e ao balanceamento das soluções estão sendo feitas pelos fabricantes, e isso tem demonstrado melhores resultados nos últimos anos (41). Contudo, clinicamente há maneiras de melhorar o desempenho dos adesivos autocondicionantes de passo único encontrados atualmente no mercado. Uma delas é a dupla aplicação do adesivo, recomendada por alguns fabricantes, e muitos trabalhos têm mostrado promover considerável melhoria na resistência da união e menor nanoinfiltração (2,15,27). Outra forma de melhorar as propriedades e diminuir a permeabilidade do adesivo é a fotoativação por um tempo prolongado. Geralmente é recomendado pelos fabricantes tempos de fotoativação de 10 ou 20 segundos; entretanto, foi demonstrado que o aumento

do tempo para 40 segundos ou 60 segundos promove maior grau de conversão e diminuição de permeabilidade do adesivo (4,6,21). No entanto, todos esses procedimentos demandam maior tempo clínico para a aplicação do adesivo.

Clinicamente, além dos fatores relacionados à técnica de aplicação dos adesivos, outros fatores interferem no desempenho destes materiais, como a pressão intrapulpar. Com testes *in vitro* de permeabilidade (30) e simulação de pressão intrapulpar (18,31) pode-se observar maior nanoinfiltração, que degrada a união em condições próximas ao que acontece *in vivo*. As duas avaliações simulam a pressão que os fluidos da polpa promovem dentro dos túbulos dentinários e que é transmitida para a camada híbrida e camada de adesivo, sendo mais evidente em cavidades profundas. Tanto a micropermeabilidade como a simulação de pressão intrapulpar têm demonstrado eficiência em avaliar e promover degradação acelerada de sistemas de união à dentina (1,30).

Alguns trabalhos analisam os sistemas adesivos sob efeito da pressão pulpar, simulando a pressão hidrostática dentro dos túbulos dentinário, desde a aplicação do adesivo (1,10). A pressão intratubular é significantemente reduzida em razão do vasoconstritor da anestesia local, a qual é realizada frequentemente na prática clínica antes do procedimento restaurador (9,25). Por essa razão, muitos estudos utilizam essa metodologia simulada da pressão pulpar reduzida a zero durante a adesão (7,17,31,38). Após o tempo necessário para passar o efeito da anestesia, as cavidades restauradas são expostas a pressão de 20 cm de H_2O (1,31), que corresponde à pressão pulpar normal da polpa não inflamada, que é de 7,5 a 22 cm H_2O (10).

Diante dos problemas dos adesivos simplificados, em especial dos de passo único, é importante estudar métodos para melhor o desempenho desses adesivos sobre a dentina. Um método simples que pode realizar essa melhoria é o aumento do tempo de fotoativação. Com as dificuldades na execução da pressão pulpar simulada atualmente em laboratório, o objetivo neste estudo foi avaliar o efeito do tempo de fotoativação de adesivos de passo único e de uma nova metodologia de pressão pulpar simulada, na resistência da união e nanoinfiltração de compósitos resinosos à dentina humana. A hipótese testada foi que não haveria diferença entre nos padrões de nanoinfiltração e na resistência da união entre os grupos submetidos às duas metodologias para simulação de pressão pulpar. Além disso, o aumento do tempo de fotoativação aumentaria a resistência da união do adesivo autocondicionante de passo único.

CAPÍTULO 1

Effect of pulpal pressure and extended photoactivation time on bond strength of one-step self-etch adhesive

Abstract

Purpose: The aim of this study was to evaluate the microtensile bond strength (µTBS) of a one-step self-etch adhesive (1-SEA), photoactivated for two different time intervals and subjected to simulated pulpal pressure.
Materials and Methods: Flat surfaces of deep dentin were obtained from 20 third molars, and divided into four groups (n=5). Clearfil S3 Bond (S3) and Filtek Z350 were used to build up restorations. The groups were divided as follow: C1- S3 was photoactivated for 10s and stored in distilled water for 24h without pulpal pressure; C2- S3 was photoactivated for 40s and stored in distilled water for 24h without pulpal pressure; P1- S3 was photoactivated for 10s and the samples were subjected to simulated pulpal pressure, the samples were subjected to 20cm water pressure for 24h; P2- S3 was photoactivated for 40s and the samples were subjected to simulated pulpal pressure for 24h. After this, the samples were cut into sticks and then subjected to µTBS. The data were submitted to two-way ANOVA and Tukey's test (p<0.05).
Results: There was no significant difference between C1 (41.5 ± 6.2 MPa) and C2 (44.2 ± 8.8 MPa). However, P1 (31.2 ± 6.9 MPa) showed significantly lower µTBS than P2 (40.8 ± 7.9 MPa). Samples subjected to pulpal pressure (P1 and P2) presented lower µTBS than samples that were not subjected (C1 and C2) (p<0.05).
Conclusion: The µTBS of the 1-SEA was adversely affected by simulated pulpal pressure. Nevertheless, photoactivation time extended to 40s raised the µTBS of the group subjected to simulated pulpal pressure.

Key Words: pulpal pressure, self-etch adhesives, polymerization time.

Introduction

Simplified dental bonding agents (DBAs), one-step self-etch adhesive (1-SEA) and two-step etch-and-rinse adhesives have reduced the number of clinical steps and technique sensitivity; however, they show a relevant increase in permeability during and after bonding, especially under pulpal pressure.[14] Multi-step DBAs, two-step self-etch adhesive (2-SEA) and three-step etch-and-rinse adhesives have shown low permeability and have

maintained bond strength due to the subsequent presence of a hydrophobic adhesive layer.[27,30]

Exposure to water is a known degradation factor in resin-dentin bonding.[13] Water transudation through the hybrid and adhesive layers is increased when there is physiological hydrostatic pulpal pressure, which leads to a faster decrease in adhesive bond strength.[7,23] Simulated pulpal pressure (PP) is a reliable and effective method for testing dentin-biomaterial and provides laboratory studies with a relevant clinical variable.[22,26] Under simulated PP, water sorption is enhanced; it plasticizes the polymer chains and promotes hybrid and adhesive layer degradation, decreasing the mechanical properties of DBAs, and contributing to reduced long-term durability of resin based materials.[16, 21]

One-step self-etch adhesives are reported to be permeable membranes[31] and present fluid transudation with a consequent decrease in bond strength and increase in nanoleakage.[18] A large quantity of solvent and hydrophilic monomers decrease the degree of conversion[24] and increase adhesive permeability;[29] however it is necessary to solvate the monomers with different characteristics mixed in 1-SEAs in order to make them durable in a simple solution.

The literature has shown some ways of improving the adhesive performance of 1-SEAs, such as double application and the application of an extra hydrophobic resin layer.[2,20,27] Although these procedures have shown great improvements, they convert these simplified DBAs into multi-step adhesives. Other clinical procedures for improving the performance of 1-SEAs are agitation during application,[3,4] use of a warm air-stream and extended drying time to increase solvent evaporation.[28] The extended photoactivation time is useful to increase the degree of conversion and decrease permeability.[5,6]

The aim of this study was to evaluate the effect of simulated pulpal pressure and extended photoactivation time on the microtensile bond strength (μTBS) of a 1-SEA. It was hypothesized that pulpal pressure and extended photoactivation time would be similar in μTBS in comparison with control groups (no pulpal pressure and photoactivation time recommended by manufacturers).

Materials and Methods

Sample preparation

Twenty extracted human third molars of a similar size and shape, free of lesions, were taken from patients between the ages of 18 and 30 years, after obtaining approval from the Research Ethics Committee of the Piracicaba Dental School - University of Campinas (protocol 167/2009). The teeth were stored in 0.5% chloramine and water for a period not exceeding 2 months at a temperature of 4°C.

For each tooth, a remaining dentin thickness (RDT) of 0.9-1.0 mm was obtained. The roots were removed 1.5 mm below cementoenamel junction (CEJ) and a parallel cut was made on the occlusal surface 1.5 mm above CEJ using a slow-speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) to expose a flat surface on deep dentin. The exposed dentin was wet-polished for 30s with 600 grit SiC papers to create a standard smear layer. A pincer-type caliper was used to measure the RDT, which was set between 0.9-1.0 mm.

Subsequently, the teeth were randomly divided into 4 groups (n= 5), according to DBA photoactivation time (10s and 40s) and pulpal pressure (no pressure 24h-control and 20 cm H_2O simulated PP 24h - experimental groups). The material compositions and application procedures are described in Table 1.

Table 1. **Materials composition and application procedures used in restorations.**

Materials	Composition	Application Procedure	Batch no.	Manufacturer
Clearfil S3 Bond *(1-SEA)*	10-MDP, BISGMA, HEMA, dimethacrylates, photoinitator	Apply adhesive for 20s. Air-dry for 5s to evaporate solvent. Light cure for 10s or 40s.	127A	Kuraray Medical, Tokyo, Japan
Filtek Z350 Shade A3 *(composite resin)*	Bis-GMA, TEGDMA, UDMA. Silica and zirconia nanofiller	Apply in 1-2 mm increments. Light cure for 40s.	N124853	3M ESPE, St. Paul, MN, USA

10-MDP: methacryloloxydecyl dihydrogenphosphate; HEMA: 2-hydroxyethyl methacrylate; TEGDMA: triethylene glycol dimethacrylate; BIS-GMA: 2,2-bis[p-(3-methacryloxy-2-hydroxypropoxy)phenyl]propane; UDMA: urethane dimethacrylate.

Bonding procedures

The 1-SEA was applied on dentin for 20s and air-dried for 5s to evaporate solvent. The photoactivation was performed for 10s (manufactures recommendation) or with an extended curing time (40s). Photoactivation of the DBA and composite resin was performed using a quartz-tungsten-halogen lamp XL-2500 (3M ESPE, St Paul, MN, USA) with an irradiance 600mW/cm², at a standardized distance of 3 mm from the bonding surface. Composite build-ups were made in 3 or 4 layers (each layer 1-2 mm thick) to a height 5-6 mm. Following, the samples from control groups were stored in distilled water at 37° C for 24h until µTBS test. Samples from simulated PP groups were restored and PP was induced after 1 hour, this period acts as the time to elapse effect from local anesthesia on decreasing pulpal pressure to simulate this clinical effect.[19,25]

Simulated pulpal pressure

The crown segments were fixed using cyanoacrylate glue to a Plexiglas plate through which an 18-gauge stainless steel tube had been inserted. The tube permitted communication with the pulp chamber and was connected to a hydraulic pressure device.

All samples were bonded and restored with 0 cm H_2O water pressure. For samples in intrapulpal pressure groups, the hydraulic pressure device was filled with water in order to be reproducing a pressure of 20 cm H_2O at bonded dentin surface (Figure 1) and the water pressure was implemented after 1 hour and maintained for 24 hours. After simulated PP, samples were cut into sticks and µTBS test was carried out.
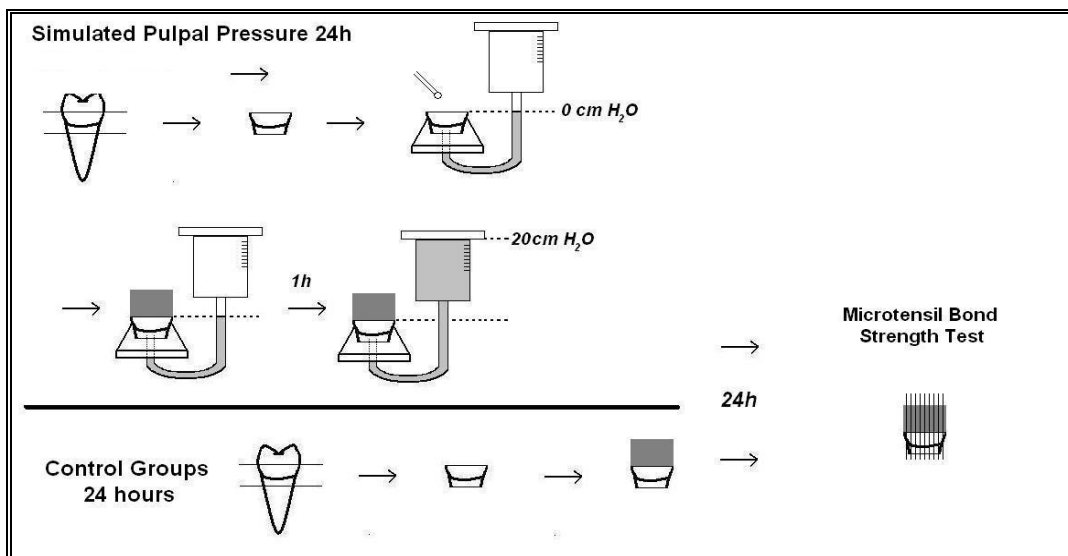

Figure 1. Control and simulated pulpal pressure groups.

Microtensile Bond Strength Testing

The restored teeth were sectioned occluso-gingivally direction into approximately 0.9 mm thick slabs with a diamond saw (Isomet saw, Buehler, Lake Bluff, IL, USA). Each slab was further sectioned to produce resin-dentin sticks with approximately 0.9 mm^2 in cross section, according to a protocol previously described.[9] Five teeth were used for each group, yielding 8-11 sticks from the central area of each specimen. The sticks from the most peripheral area were excluded to test adhesion just to dentin.

The beams were affixed to a jig with cyanoacrylate glue (Super Bonder gel, Loctite, Henkel Corp., Rocky Hill, CT, USA) in a universal testing machine (EZ-test, Shimadzu Co., Kyoto, Japan) and tested until failure under tensile tension at 1.0 mm/minute. The cross-sectional area of each tested beam was measured with a digital micrometer after bond failure. Means and standard deviations were calculated and expressed in MPa. The μTBS data were statistically analyzed using two-way ANOVA (adhesive curing time and simulated pulpal pressure) to identify differences among groups, if they were found, they would be compared using Tukey's test ($p < 0.05$).

Analysis of fracture type

After μTBS test, the failure pattern was verified with stereomicroscopy at 60x magnification. Representative fractured dentin and composites surfaces, exhibiting the most frequently observed failure pattern and the μTBS close to mean, were processed for scanning electron microscopy (SEM). Fractured samples were paired and placed in aluminum stubs and coated with gold (Balzers model SCD 050 sputter coater, Balzers Union Aktiengesellschaft, Fürstentum Liechtenstein, FL-9496, Germany) and examined by SEM, JSM-5600LV (JEOL, Tokyo, Japan), operated at 15 kV. The failures were classified as follows:

Type A: Adhesive failure.

Type M: Mixed failure.

Type C: Total cohesive failure in resin composite.

Type D: Total cohesive failure in the dentin.

Results

Two-way ANOVA showed significant interaction between pulpal pressure and curing time (p<0.001) and differences inside the factors (p<0.05). Mean values of μTBS (MPa) and the standard deviation values are shown in Table 2. Groups without simulated PP obtained the highest μTBS among groups with same photoactivation time. Under simulated PP for 24h, the photoactivation time extended to 40s produced higher μTBS than the 10s recommended by the manufacturers.

The failure mode of debonded specimens is shown in Table 3 and some representative images are presented in Figure 2. Groups without simulated PP presented more mixed failures and groups with simulated PP showed more adhesive failures. Between the groups subjected to hydrostatic PP (10s and 40s), failures in 10s group mainly occurred between the adhesive layer and composite resin. The 40s group presented failures between the hybrid layer/dentin and adhesive layer (Figure 2).

Table 2. Mean (Standard deviations) of μTBS (MPa).

Curing Time	No Pulpal Pressure	20cm H_2O simulated PP
Clearfil S3 - 10s	41.06 (6.33) [A, a]	31.19 (6.83) [B, b]
Clearfil S3 - 40s	44.20 (8.77) [A, a]	40.82 (7.87) [A, b]

Different upper case letters represent statistical significant difference within each column (p>0.05). Different lower case letters represent statistical significant difference within each row (p>0.05).

Table 3. Fracture mode after microtensile bond strength test.

Pulpal pressure/Curing time	Fracture type			
	A	M	C	D
C1-Clearfil S3/ no PP/10s	30%	37%	26%	7%
P1- Clearfil S3/PP/10s	46%	31%	23%	0%
C2-Clearfil S3/no PP/40s	23%	47%	24%	6%
P2- Clearfil S3/ PP/40s	55%	39%	5%	1%

*Type A means adhesive failure, type M means mixed failure, type C cohesive failure in composite resin and type D cohesive failure in dentin. PP – simulated pulpal pressure.
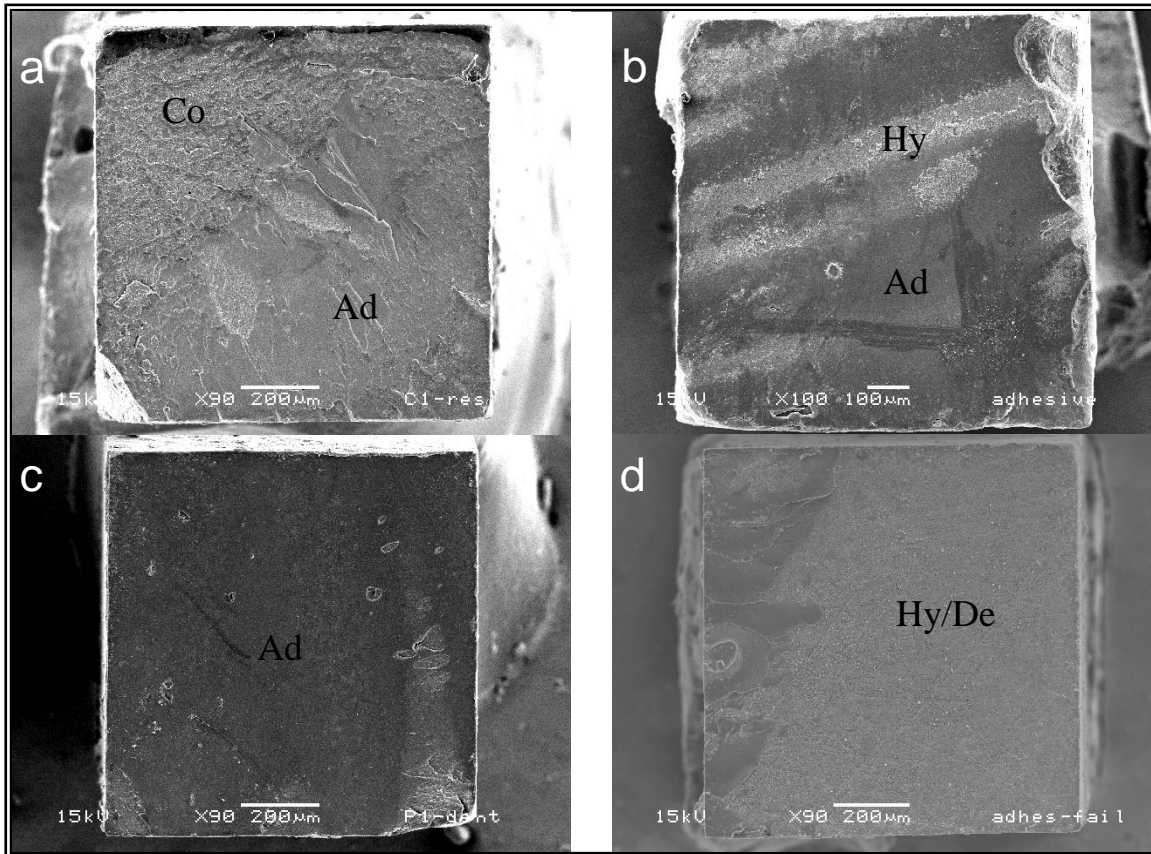
Figure 2. Representative SEM images of failure mode. (a) Group C1- Mixed failure among hybrid layer, adhesive layer and composite resin. (b) Group C2- Mixed failure between hybrid layer and adhesive layer. (c) Group P1- adhesive failure between adhesive layer and composite resin. (d) Groups P2- adhesive failure between dentin and hybrid layer, with slight vestiges of adhesive layer.

Ad-Adhesive resin. Hy-Hybrid layer. Co-Composite resin. De-Dentin.

Discussion

The aim of this study was to evaluate the effect of simulated pulpal pressure and photoactivation time on the microtensile bond strength (μTBS) and failure pattern of the 1-SEA Clearfil S3 Bond. ANOVA showed significant differences for the factors adhesive photoactivation time and simulated pulpal pressure (Table 2), and for the interaction between factors ($p<0.001$). All-in-one adhesives have an intrinsic instability in a water

environment, even after polymerization; because they have a variety of different monomers (hydrophobic and hydrophilic) and consequently a high amount of solvent is useful (usually water and ethanol) to ensure a homogeneous mixture. The high solvent content is necessary to maintain a durable solution with different solvated monomers.[34] However, their hydrophilic characteristic induces absorption and passage of extrinsic water.[29] The seepage of additional extrinsic water contributes to the denuding of collagen bundles, within the hybrid layer.

Hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA) are capable of imbibing large amounts of water, but in simplified adhesives such as 1-SEAs, HEMA becomes a substantial component to increase the solubility of hydrophobic monomers in water[34] and prevent phase separation of the adhesive solution.[32] Clearfil S3 Bond is a HEMA-rich 1-SEA and the consequent presence of residual water within adhesive film and extrinsic water sorption may degrade the mechanical properties of the polymers,[10] such as the modulus of elasticity[17] and the ultimate tensile strength.[35] This could be responsible for the reduced bond strength reported in this study. This process is more evident in deep dentin, which is a highly permeable substrate and can supply excessive amounts of water to polymerized adhesives after the vasoconstrictions effect of local anesthetic solutions.[11] Therefore, deep dentin with a mean thickness of 0.9mm was chosen as the remaining dentin thickness, in agreement with other studies, because there is higher tubules concentration and their diameter is greater.[8,29,30]

The groups subjected to simulated pulpal pressure showed an adverse effect on bond strength, especially when 1-SEA was photoactivated for 10 s. Nowadays, the majority of studies uses 15-20cm H_2O as the simulated PP, which have shown that normal human physiological PP corresponded to a hydrostatic pressure ranging between 8-22cm H_2O.[1,12,29,30] Thus, simulated pulpal pressure was used to expedite the degradation process and water seepage, since it produces extra water on the surface and creates more microchannels for water movement. This is easier for simplified etch-and-rinse DBAs, because of the more permeable surface created by phosphoric acid; however, it is more difficult to seal open tubules than partially sealed smeared tubules in a self-etch approach.[7,8]

Failure pattern analysis showed predominantly mixed failures in groups without simulated PP, especially between the adhesive layer and composite resin (Figure 2a). Pulpal pressure in group P1 (with photoactivation time of 10s) promoted water seepage through

the adhesive layer up to the composite resin, showing more failures between the adhesive layer and composite resin (Figure 2c). Accumulation of water between the composite resin and adhesive layer is common, whereas the oxygen inhibition layer is located on top of the adhesive layer, and after DBA polymerization this area is hypertonic with uncured monomers. As 1-SEAs behave like permeable membranes,[31] the transmission of small molecules, such as water is allowed. The water is transmitted from dentin to the adhesive layer/ composite resin interface by a diffusion process.[33] This can explain the predominance of adhesive failures between the adhesive layer and composite resin in the group that underwent simulated PP and the photoactivation time was set at 10 seconds.

The 1-SEA Clearfil S3 Bond photoactivated with an extended photoactivation time (40 seconds) showed significantly higher bond strength after 24h simulated hydrostatic PP than adhesive photoactivated for 10 seconds. These results confirms that the photoactivation of the 1-SEAs for an extended photoactivation time (40s and 60s) improves the degree of conversion and consequently creates a more homogeneous and less porous polymer, decreasing adhesive permeability and nanoleakage.[5,6]

Group P2, in which simulated PP was performed and the photoactivation time was extended to 40 seconds, showed few failures between adhesive layer and composite resin, but presented adhesive failures between the hybrid layer and dentin or the hybrid layer and adhesive layer (Figure 2d). This fact confirms that an extended photoactivation time for 1-SEAs diminishes adhesive permeability due to the higher degree of conversion.[5,6] Although water transudation through hybrid and adhesive layers occurs under simulated PP, the 40s photoactivation time decreases the fluid accumulation between the adhesive layer and composite resin, resulting in more water degradation on the bottom of the hybrid layer under PP. Furthermore, the μTBS in group with a photoactivation time extended to 40s was significantly higher than in group photoactivated for the time (10s) recommended by the manufacturers, after 24 hours simulated PP (Table 2). Additionally, simulated PP decreased significantly the bond strength for both photoactivation times. Therefore, the hypothesis has to be rejected because significant differences were found in μTBS and failure pattern among the groups tested.

The results of this study suggest that for adhesive polymerization, an extended polymerization time should be recommended as a simple way to improve the bond performance of 1-SEAs after simulated PP, and are in agreement with others studies that

have shown a higher degree of conversion and lower adhesive permeability and nanoleakage with extended photoactivation time.[5,6]

Within the limitations of this *in vitro* study, it can be concluded that simulated pulpal pressure reduced the microtensile bond strength after 24 hours for both photoactivation time intervals. When the photoactivation process is extended to 40 seconds, it was observed a benefic effect in bond strength of Clearfil S3 Bond after simulated pulpal pressure. An extended photoactivation time would be recommended for improve bonding with one-step self-etch adhesives, when simulated PP was considered.

Acknowledgments

Clinical Relevance

Extended photoactivation time for simplified self-etch adhesives is a useful way to improve bond strength after clinical variables like pulpal pressure.

References

1. Abdalla AI, Elsayed HY, Garcia-Godoy F. Effect of hydrostatic pulpal water pressure on microtensile bond strength of self-etch adhesives to dentin. Am J Dent 2008; 21:233-238.

2. Albuquerque M, Pegoraro M, Mattei G, Reis A, Loguercio AD. Effect of Double-application or the application of a hydrophobic layer for improved efficacy of one-step self-etch systems in enamel and dentin. Oper Dent 2008;33:564-70.

3. Amaral RC, Stanislawczuc R, Zander-Grande C, Gagler D, Reis A, Loguercio AD. Bond strength and quality of the hybrid layer o fone-step self-etch adhesives applied with agitation on dentin. Oper Dent 2010;35:211-219.

4. Amaral RC, Stanislawczuz R, Zander-Grande C, Michel MD, Reis A, Loguercio AD. Active application improves the bonding performance of self-etch adhesives to dentin. J Dent 2009;37:82-90.

5. Breschi L, Cadenaro M, Antoniolli F, Sauro S, Biasotto M, Prati C, Tay FR, Di Lenarda R. Polymerization kinetics of dental adhesives cured with LED: correlation between extent of conversion and permeability. Dent Mater 2007;23:1066-1072.

6. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C. Degree of conversion and permeability of dental adhesives. Eur J Oral Sci 2005;113:525-530.

7. Campos EA, Correr GM, Leonardi DP, Barata-Filho F, Gonzaga CC, Zielak JC. Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing. J Dent 2009;37:108-114.

8. Cardoso MV, Moretto SG, Carvalho RC, Russo EM. Influence of intrapulpal pressure simulation on the bond strength of adhesive systems to dentin. Braz Oral Res 2008;22:170-175.

9. Carrilho MR, Geraldeli S, Tay F, De Goes MF, Carvalho RM, Tjaderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86:529-33.

10. Chersoni S, Acquaviva GL, Prati C, Ferrari M, Grandini S, Pashley DH, Tay FR. In vivo fluid movement through dentin adhesives in endodontically treated teeth. J Dent Res 2005;84:223-227.

11. Chng HS, Pitt Ford TR, Mcdonald F. Effect of prilocaine local anaesthetic solutions on pulpal blood flow in maxillary canines. Endod Dent Traumatol 1996;12:89-95.

12. Ciucchi B, Bouillaguet S, Holz J, Pashley DH. Dentinal fluid dynamics in human teeth, in vivo. J Endodontics 1995;21:191-194.

13. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003;82:136-140.

14. Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res 2004; 83:843-848.

15. Hosaka K, Masatoshi N, Monticelli F, Carrilho M, Yamauti M, Aksornmuang J, Nishitani Y, Tay FR, Pashley DH, Tagami J. Influence of hydrostatic pulpal pressure on the microtensile bond strength of all-in-one self-etching adhesives. J Adhes Dent 2007; 9:437-442.

16. Hosaka K, Nakajima M, Takahashi M, Itoh S, Ikeda M, Tagami J, Pashley DH. Relationship between mechanical properties of one-step self-etch adhesives and water sorption. Dent Mater 2010;26:360-367.

17. Ito S, Hashimoto M, Wadgaonkar B, Svizero M, Carvalho RM, Yiu C, Rueggeberg F, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, Pashley DH. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005;26:6449-6459.

18. Itthagarun A, Tay FR, Pashley DH, Wefel JS, Garcia-Godoy F, Wei SHY. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part III. Evidence from fluid conductance and artificial caries inhibition. Am J Dent 2004;17:394-400.

19. Kim S, Edwall L, Trowbridge H, Chien S. Effect of local anaesthetics on pulpal blood flow in dogs. J Dent Res 1984;63:650-652.

20. Loguercio AD, Costenaro A, Silveira AP, Ribeiro NR, Rossi TR, Reis A. A six-month clinical study of a self-etching and na etch-and-rinse adhesive applied as recommended and after doubling the number of adhesive coats. J Adhes Dent 2006;8:255-261.

21. Manso AP, Bedran-Russo AK, Suh B, Pashley DH, Carvalho RM. Mechanical stability of adhesives under water storage. Dent Mater 2009;25:744-749.

22. Mazzitelli C, Monticelli F, Osorio R, Casucci A, Toledano M, Ferrari M. Effect of simulated pulpal pressure on self-adhesive cements bonding to dentin. Dent Mater 2008;24:1156-1163.

23. Nakajima M, Hosaka K, Yamauti M, Foxton RM, Tagami J. Bonding durability of self-etching primer system to normal and caries-affected dentin under hydrostatic pulpal pressure in vitro. Am J Dent 2006;19:147-150.

24. Navarra C, Cadenaro M, Codan B, Mazzoni A, Sergo V, Dorigo ES, Breschi L. Degree of conversion and interfacial nanoleakage expression of three one-step self-etch adhesives. Eur J Oral Sci 2009;117:463-469.

25. Odor TM, Pitt Ford TR, Mcdonald F. Adrenaline in local anaesthesia: the effect of concentration on dental pulpal circulation and anaesthesia. Endodontic and Dental Traumatology 1994;10:167-173.

26. Pereira PNR, Sano H, Ogata M, Zheng L, Nakajima M, Tagami J, Pashley DH. Effect of region and dentin perfusion on bond strengths of resin-modified glass ionomer cements. J Dent 2000;28:347-354.

27. Reis A, Albuquerque M, Pegoraro M, Mattei G, Bauer JRO, Grande RHM, Klein-Junior CA, Baumhardt-Neto R, Loguercio AD. Can the durability of one-step self-etch adhesives be improved by double application or by an extra layer of hydrophobic resin? J Dent 2008;36:309-315.

28. Reis A, Klein-Junior CA, Coelho-De-Souza FH, Stanislawczuc R, Loguercio AD. The use of warm air-stream for solvent evaporation: effects on the durability of resin-dentin bonds. Oper Dent 2010;35:29-36.

29. Sauro S, Mannocci F, Toledano M, Osorio R, Thompson I, Watson TF. Influence of hydrostatic pulpal pressure on droplets formation in current etch-and-rinse and self-etch adhesives: a video rate/TSM microscopy and fluid filtration study. Dent Mater 2009;25,1392-1402.

30. Sauro S, Pashley DH, Montanari M, Chersoni S, Carvalho RM, Toledano M, Osorio R, Tay FR, Prati C. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater 2007;23:705-713.

31. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002; 30:371-382.

32. Van Landuyt K, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P, Van Meerbeek B. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res 2005; 84:183-8.

33. Van Landuyt KL, Snauwaert J, De Munck J, Coutinho E, Poitevin A, Yoshida Y, Suzuki K, Lambrechts P, Van Meerbeek B. Origin of interfacial droplets in one-step adhesives. J Dent Res 2007;86:739-744.

34. Van Landuyt KL, Snauwaert J, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. The role of HEMA on one-step self-etch adhesives. Dent Mater 2008;24:1412-1419.

35. Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. Biomaterials 2004;25:5789-5796.

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CAPÍTULO 2

A new method to simulate pulpal pressure: Bond strength and nanoleakage to dentin

Abstract

Objective: To evaluate a new methodology of simulating pulpal pressure after 24 hours in comparison with conventional simulated pulpal pressure using microtensile bond strength (µTBS) and nanoleakage.
Methods: One adhesive from each category was analyzed: a three-step etch-and-rinse (Scotchbond Multi Purpose - SBMP, 3M ESPE), a two-step etch-and-rinse (Adper Single Bond Plus - SB, 3M ESPE), a two-step self-etch (Clearfil SE Bond - SE, Kuraray) and a one-step self-etch (Clearfil S3 – Tri-S, Kuraray). Direct restorations were built up on flat deep dentin from extracted third molars with nanofilled composite resin. After two methods of simulated pulpal pressure or no pulpal pressure (control groups), the samples were cut into sticks and submitted to µTBS testing and nanoleakage evaluation. Results were analyzed with two-way ANOVA and Tukey's test ($p < 0.05$).
Results: For control groups, µTBS showed SBMP>SB=SE>Tri-S ($p < 0.05$). For both methods of simulated pulpal pressure µTBS from SB and Tri-S showed statically lower values than their control groups. For SBMP and SE the µTBS was preserved. Conventional and experimental methods to simulate pulpal pressure resulted in similar µTBS ($p < 0.05$) and nanoleakage patterns. Silver impregnation was increased for SB and Tri-S, especially after both simulated pulpal pressure methods.
Significance: The results of nanoleakage and µTBS were similar affected for the two methods of simulated pulpal pressure.

Key Words: pulpal pressure, dental adhesives, nanoleakage.

Introduction

Four categories of dentin-enamel bonding agents (DBAs) are available on the market [1]. Simplified DBAs, one-step self-etch adhesive (1-SEA) and two-step etch-and-rinse adhesives (2-E&R) reduce the number of clinical steps and technique sensitivity; however, they show relevant increase in permeability and consequent loss of bond strength after water storage [2,3]. Whereas the non-simplified DBAs, two-step self-etch adhesive (2-SEA) and three-step etch-and-rinse adhesive (3-E&R) exhibit low permeability and retain their bond strength [4-7], due to subsequent hydrophobic layer application [8-10].

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Water transduation through the hybrid and adhesive layer is increased with hydrostatic pulpal pressure [11-13]. Simulated pulpal pressure (PP) became a reliable and significant manner of testing dentin-biomaterial behavior [14-21]. Water sorption is enhanced and it plasticizes the polymer chains and promotes degradation of the bond area, contributing to the reduced long-term durability of dental material [22-24]. The influence of PP on dentin bonding and durability is so remarkable that many studies perform tubular occlusion with potassium oxalate to optimize bonding and sealing ability and decrease the deleterious effects of PP [25,26].

Some studies have measured physiological PP *in vivo*, in human teeth [27,28], from cats [29], monkeys [30] and dogs [31]. The study by Wynn [31] indicates that there is a direct relationship between PP and arterial blood pressure, which is important when treating patients with hypertension. However, local anesthesia significantly reduces pulpal blood circulation [32-36] and several studies have applied DBAs without simulated PP [4,19,37,38] and after the restorative procedure, PP had increased. Dentin permeability and PP is also a useful way to test the *in vitro* cytotoxicity of resin-based materials [39,40].

Nowadays, the majority of studies have tested simulated PP with 15-20cm H_2O, in agreement with Ciucchi et al. 1995 [28], who showed that normal human physiological PP corresponded to a hydrostatic pressure of 8-22 cm H_2O. *In vitro*, this procedure is performed with a water column connected to a Plexiglas or acrylic plate, through which an 18-gauge stainless steel tube was inserted [4,5,10]. Because of the difficulties inherent to gluing samples onto acrylic/Plexiglas plates, preventing the glue from penetrating into the pulp chamber and maintaining a closed system without water escaping, a different method to simulate PP was developed and used in the present study.

Moreover, the aim of this *in vitro* study was to evaluate the microtensile bond strength and the nanoleakage of four adhesives (1-SEA, 2-SEA, 2-E&R and 3-E&R), comparing the traditional method to simulate PP with a new method to simulate PP. The two hypothesis tested were that there would be no significant differences on microtensile bond strength (µTBS) for all the tested adhesives and among groups in pulpal pressure approach; for nanoleakage analysis, it was hypothesized that both methods to simulate PP would show higher silver penetration for all the DBAs in comparison with control groups.

Materials and methods

Sample preparation

It was used sixty extracted human third molars, with similar size, shape and free of lesions, taken from people between 18 and 30 years under the approval protocol of the Research Ethics Committee of the Dentistry School of Piracicaba - University of Campinas (167/2009). The teeth were stored in 0.5% chloramine/water for a period not exceeding 4 months at a temperature of 4°C.

Table 1. **Materials used and their composition.**

Materials	Composition	Application Procedure	Batch no.
Clearfil S3 Bond *(1-SEA, Kuraray Medical, Tokyo, Japan)*	MDP, BisGMA, HEMA, dimethacrylates, photoinitator	Apply adhesive for 20s. Air-dry for 5s to evaporate solvent. Light cure for 10s.	127A
Clearfil SE Bond *(2-SEA, Kuraray Medical, Tokyo, Japan)*	-Primer: MDP, HEMA, water, photoinitator -Bond: MDP, BisGMA, HEMA, hydrophobics dimethacrylates, photoinitator	Apply *primer* for 20s, gently air-dry; apply bond. Light cure for 10s.	896A 1321A
Adper Single Bond Plus *(2-E&R, 3M ESPE, St. Paul, MN, USA)*	-Etchant: 37% phosphoric acid -Adhesive: HEMA, BisGMA, polyalkenoic acid copolymer, dimethacrylates, ethanol, water and camphorquinone	Acid-etch for 15s, rinse with water for 15s leaving the dentin moist. Bond was applied in two coats and gently air-dried. Light cure for 10s.	7KK 9WP
Scotchbond Multi-Purpose *(3-E&R, 3M ESPE, St. Paul, MN, USA)*	-Etchant: 37% phosphoric acid -Primer: HEMA, polyalkenoic acid copolymer, water. -Adhesive: HEMA, BisGMA, amines.	Acid-etch for 15s, rinse with water for 15s and blot dry with excess of water. Apply *primer* and gently air dry. Apply bond. Light cure for 10s.	7KK N124653 5PJ
Filtek Z350 Shade A3 *(nanofilled composite resin, 3M ESPE, St. Paul, MN, USA)*	Matrix: BisGMA, TEGDMA, UDMA. Filler: Silica and zirconia nanofiller	Apply in 1-2 mm increments and light cure for 40s.	9XN

10-MDP: methacryloloxydecyl dihydrogenphosphate; HEMA: 2-hydroxyethyl methacrylate; TEGDMA: triethylene glycol dimethacrylate; BisGMA: 2,2-bis[p-(3-methacryloxy-2-hydroxypropoxy)phenyl]propane; UDMA: urethane dimethacrylate.

For each tooth, a mean remaining dentin thickness (RDT) of 0.9 mm were obtained, removing the roots 1.5 mm below cementoenamel junction (CEJ) and the occlusal surface with a parallel cut at 1.5 mm above CEJ using a slow-speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) to expose a flat surface on deep dentin. Pulpal tissue was removed from the pulp chamber with small forceps, taking care to avoid touching the pulp chamber walls and preserving predentin surface. A pincer-type caliper was used for measurements of RDT that was between 0.9-1.0 mm, if the RDT was not inside this range, flat dentin surface was abraded with SiC papers and water until RDT be standardized in the interval. The exposed dentin was polished just before bonding with SiC papers, #600 grids, for 30s with water, to create a standard smear layer.

Subsequently the teeth were divided randomly into 12 groups (n = 5), four DBAs and three pulpal pressure groups (no pressure-control, conventional simulated PP and experimental simulated PP). The materials used in each group, composition and application procedure are described in Table 1.

Bonding procedures

Light activation of the resin-based materials was performed using a quartz-tungsten-halogen lamp XL-2500 (3M ESPE, St Paul, MN, USA) with an output power intensity of 600mW/cm², at a standardized distance of 2 mm from the bonding surface. All materials were used following their manufacturers' recommendations (Table 1). Composite build-ups were made in 3 or 4 layers (each layer 1-2 mm thick) to a height of 5-6 mm. After this, the samples randomly divided in three storage groups. The control groups were stored in distilled water (level 2 cm above samples) at 37°C for 24h until microtensile bond strength testing. The simulated PP groups were restored and PP was induced in experimental or conventional methodology during 24 hours in lab temperature (25-27ºC).

Simulated pulpal pressure

The simulated intrapulpar pressure was executed in two techniques (Figure 1 and 2), and after 24 hours the samples were tested by microtensile bond strength.

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Conventional simulated pulpal pressure

All samples were bonded and restored without water pressure. For samples in intrapulpal pressure groups, the water pressure was implemented after 1 hour which acts as the time it takes for the effect of local anesthesia on decreasing pulpal pressure to wear off [20,33,34,37] and maintained for 24 hours. Samples were cut into sticks and microtensile bond strength testing was carried out after 24h of simulated PP (all groups with water pressure) or 24h water storage in distilled water (control groups – represented by letter "C").

The crown segments were fixed to a Plexiglas plate with cyanoacrylate glue, and an 18-gauge stainless steel tube was inserted through a hole in the plate (Figure 1). The tube allowed communication with the pulp chamber and was connected to a hydraulic pressure device filled with water in order to reproduce a pressure of 20 cm H_2O at the bottom dentin bonded to the composite (Figure 1). The conventional pulpal pressure groups were represented by letter "P".
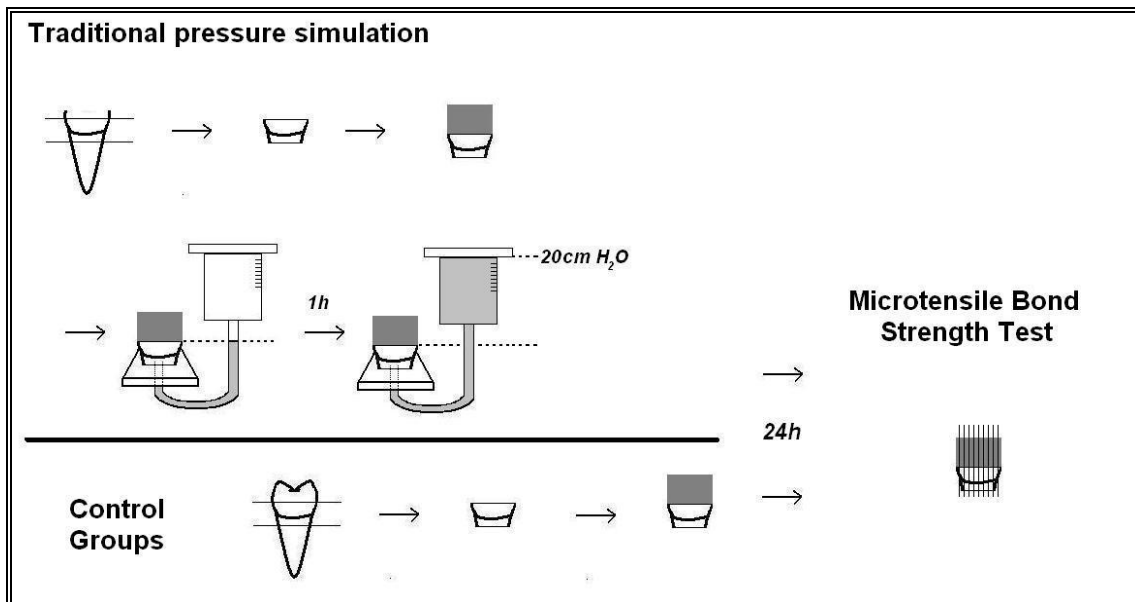

Figure 1. Groups which conventional simulated pulpal pressure was performed and control groups.

Experimental simulated pulpal pressure

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The new methodology to perform simulated hydrostatic PP uses only nail varnish, wax and a cylindrical container with a lid (25 cm high and 12 cm in diameter). The teeth were prepared in a similar way to that used for conventional PP simulation, with the RDT about 0.9 mm and open pulp chambers without roots. The teeth were restored without simulated PP and the resin-enamel interfaces were covered with two coats of nail varnish to avoid water seepage through resin-tooth interface, thus the passage of water and pressure was possible only through dentin tubules. After 1 hour, each sample was laid on its side and attached to the inside of the lid of a cylindrical receptacle (Figure 2) with wax. The pulp chamber was open and faced the container wall. After this, the receptacle was filled with distilled water up to a height of 20 cm, capped with the lid that had the sample fixed to it, and turned upside down. Thus, the samples had a 20 cm water column over them and the pressure within the pulp chamber was 20 hPa (according to the hydrostatic pressure equation. $P = g.d.h$, p-hydrostatic pressure, g- gravity, d- liquid density, h- liquid height) just as in the conventional simulated pulp pressure. The experimental pulpal pressure groups were represented by letter "E".

After 24 hours of simulated PP or distilled water storage (control groups), samples were cut into sticks and then taken for microtensile bond strength testing.
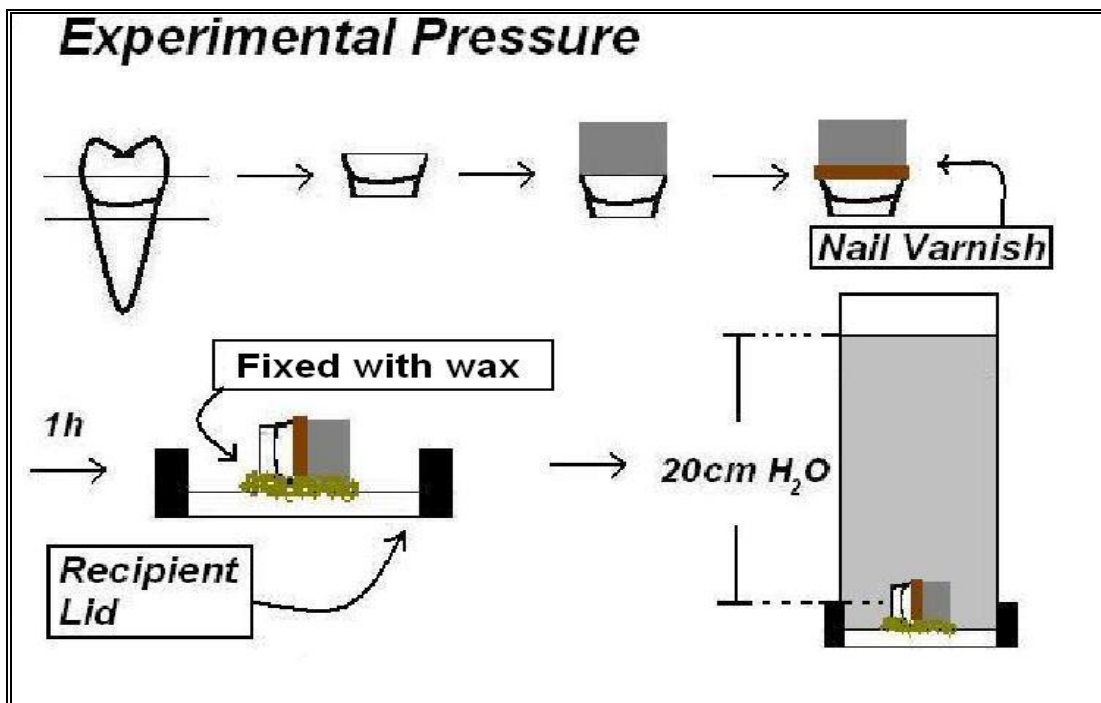

Figure 2. New method to perform simulated pulpal pressure.

Microtensile Bond Strength Testing

To obtain the beam specimens, the restored teeth were sectioned occluso-gingivally in serial slabs approximately 0.9 mm thick; using Isomet saw (Buehler, Lake Bluff, IL, USA). The slabs were then sectioned to create beams approximately 0.9 x 0.9mm in cross section. Five teeth were used for each adhesive and PP group (n=5), yielding 8-10 sticks from the central area of each specimen and the beams from the most peripheral area were excluded.

The beams were affixed to a jig with a cyanoacrylate glue (Super Bonder gel, Loctite, Henkel Corp., Rocky Hill, CT, USA) and tested to failure under tension in a universal testing machine EZ-test (Shimadzu Co., Kyoto, Japan) with a 500-N load cell, at a crosshead speed of 1.0 mm/minute. The exact cross-sectional area of each tested beam was measured with a digital micrometer after bond failure. Means and standard deviation were calculated and expressed in MPa. The microtensile bond strength (µTBS) data were statistically analyzed using two-way ANOVA (adhesive type and pulpal pressure) to identify differences among groups. When significant differences were found among groups, they were compared using Tukey's test ($p<0.05$).

Analysis of fracture type

After tensile test, the mode of failure was determined by stereomicroscopy at 60x magnification. Representative fractured dentin and composites surfaces, exhibiting the most frequently observed failure pattern and the µTBS close to mean, were processed for scanning electron microscopy (SEM). The parts of the fractured samples were paired and placed in samples of aluminum stubs and coated with gold (Balzers model SCD 050 sputter coater, Balzers Union Aktiengesellschaft, Fürstentum Liechtenstein, FL-9496, Germany) and examined by SEM, JSM-5600LV (JEOL, Tokyo, Japan), operated at 15 kV. The fractures were classified as follows:

Type A: Adhesive failure at the interface among adhesive resin, hybrid layer, composite resin and/or dentin.

Type M: Mixed failure.

Type C: Cohesive failure in resin composite.

Type D: Cohesive failure in the dentin.

Nanoleakage evaluation

One central stick from each tooth (n=5) was selected to nanoleakage evaluation. The protocol previously described by Vachiramon et al. 2008 [26] was used to prepare 50 wt% ammoniacal silver-nitrate solution. Bonded sticks were coated with two layers of nail varnish applied up to within 1 mm of bonded interfaces. The sticks were placed in ammoniacal silver nitrate in total darkness for 24h, rinsed thoroughly in distilled water and immersed in photodeveloping solution for 8h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface. Following, the silver impregnated sticks were embedded in epoxy resin, ground and polished using wet #600, #1200, #2000 silicone-carbide papers and diamond pastes 6, 3, 1 and 0.25 μm and ultrasonically cleaned for 20 minutes after use of each abrasive paper and polishing paste. Finally, they were air dried, coated with carbon and observed under a SEM by means of backscattered electron mode images at a standardized magnification (1000x, except for SBMP that was set in 500x).

Results

Two-way ANOVA showed interaction between adhesive type and pulpal pressure (p=0.01). Mean values (MPa) obtained in the microtensile test are shown in Table 2. Scotchbond Multi Purpose (SBMP) obtained the highest μTBS values among same pulpal pressure groups and Clearfil S3 (Tri-S) the lowest. The simplified DBAs (Single Bond-SB and Tri-S) presented reduced bond strength with simulated pulpal pressure. However, Clearfil SE Bond (SE) and SBMP preserved their μTBS under simulated PP. For all DBAs, conventional simulated PP and experimental simulated PP showed statistically equal μTBS.

Table 2. Mean (Standard deviations) of µTBS (MPa).

DBA	No Pulpal Pressure	Conventional PP	Experimental PP
Clearfil S3 Bond	41.47 (6.16) [C, a]	34.29 (10.49) [C, b]	33.26 (9.13) [C, b]
Clearfil SE Bond	44.02 (8.38) [BC, a]	42.36 (9.39) [B, a]	42.14 (8.53) [B, a]
Adper Single Bond	47.86 (7.09) [AB, a]	40.12 (9.79) [B, b]	41.07 (7.59) [B, b]
Scotchbond MP	51.27 (10.71) [A, a]	49.35 (10.71) [A, a]	49.69 (9.80) [A, a]

Same upper case letter represent no statistical significant difference within each column (p>0.05). Same lower case letter represent no statistical significant difference within each row (p>0.05).

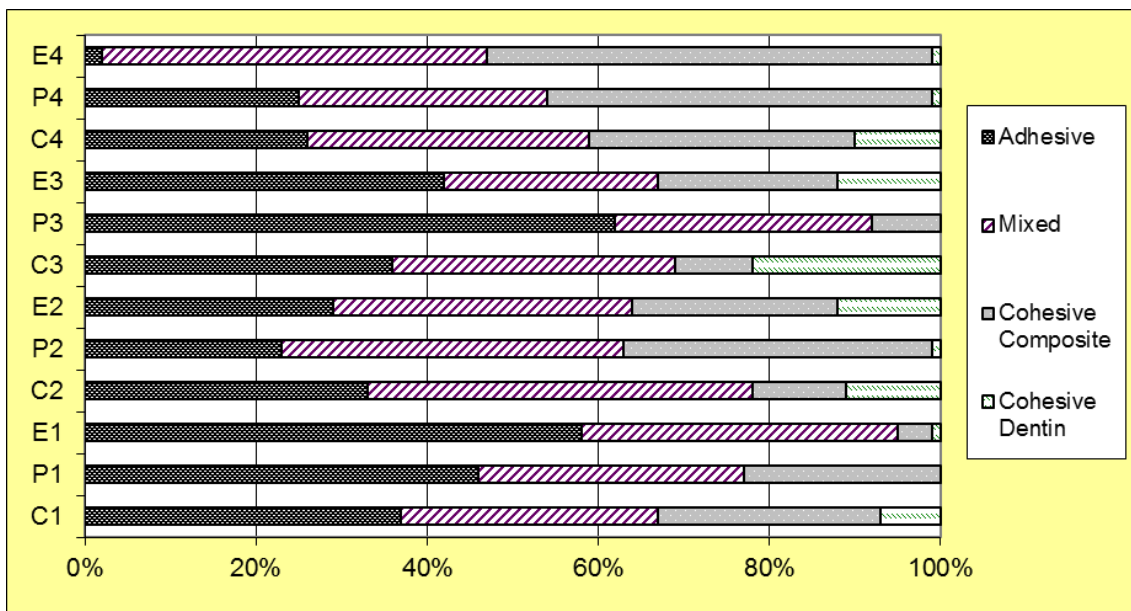**Figure 3. Fracture type (%) after microtensile bond strength test.**

"C" – Control; "P" - conventional simulated PP; "E" – experimental simulated PP. "1" - Tri-S, "2" - SE, "3" - SB and "4" - SBMP.

The failure patterns of specimens are shown in Figure 3. For groups C1, P1, E1, C3, P3 and E3, the failure pattern was predominantly type A (adhesive failure at the composite/adhesive/dentin interface). Groups C2, P2, E2 and C4 had mainly type M fractures (mixed failure, partially adhesive and cohesive). For group P4 and E4 the fracture type C (cohesive failure in composite resin) was the most common.

Qualitative nanoleakage evaluation showed predominantly more silver leakage after both simulated PPs than the control groups, especially for the simplified DBAs, 1-SEA Clearfil S3 Bond and for 2-E&R Adper Single Bond Plus. The simplified DBAs presented a striking increase in silver grains and silver channels ("water-trees") under pulpal pressure

(Figure 5C and 7B) and in some areas the leakage between adhesive layer and composite resin could be seen (Figure 4B). The control groups presented few silver deposits located only at the bottom and the top of hybrid layers (Figure 5A and 7A).

The multi-step DBAs Clearfil SE Bond and Scotchbond Multi Purpose showed high resistance to silver penetration, irrespective of the type of simulated PP. They exhibited only a little silver accumulation at the bottom of the hybrid layer and some silver grains in the adhesive layer after two types of simulated PP. Hybrid and adhesive layers almost free of silver penetration could be seen (Figures 5A and 7A) without simulated PP (control groups, C2 and C4). For the same DBA, experimental PP and conventional PP differed only slightly in the amount of silver impregnation and generally both modes of simulated PP had a similar nanoleakage pattern.
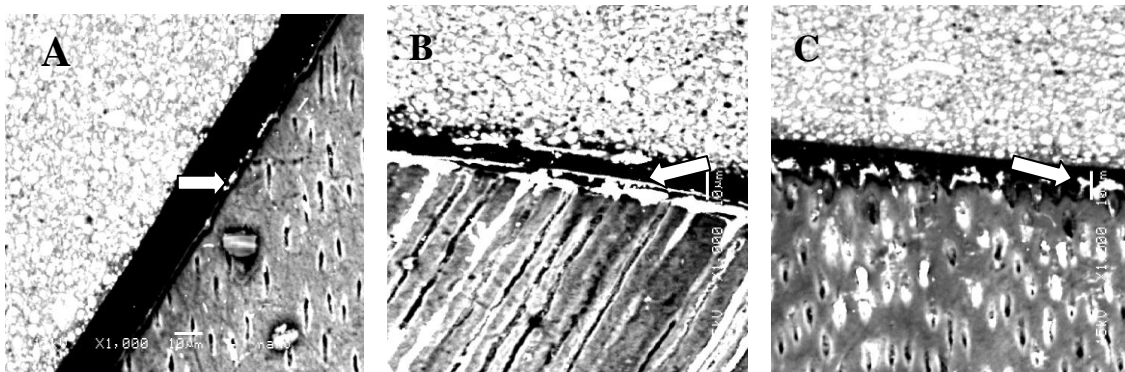

Figure 4. Nanoleakage illustrations for Clearfil S3. *Figure 4A shows nanoleakage in group C1 (control - no PP), Figure 4B in group E1 (experimental PP), and Figure 4C in group P1 (conventional PP). White arrows show the most silver penetration. Pulpal pressure promoted a large increase in silver impregnation, presenting the formation of many water channels (water trees).
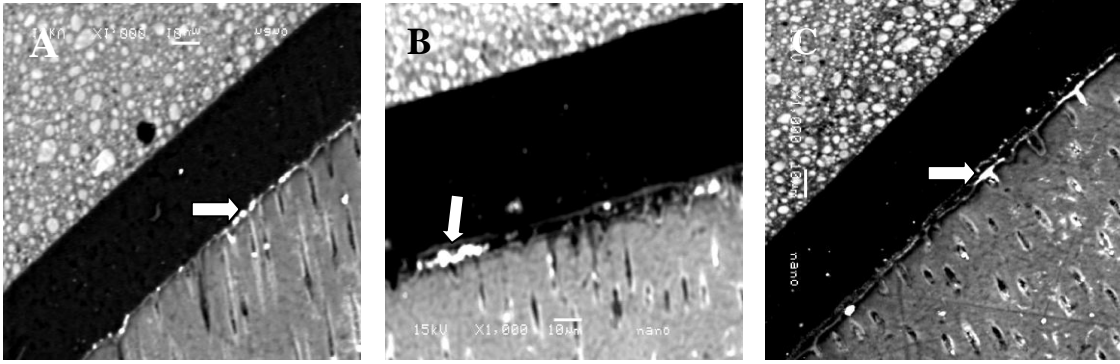
Figure 5. Nanoleakage illustrations for Clearfil SE Bond.
*Figure 5A presents nanoleakage in group C2 (control - no PP), Figure 5B in group E2 (experimental PP) and Figure 5C in group P2 (conventional PP). White arrows show the most silver penetration. With or without pulpal pressure simulation, silver impregnation was slight with small, isolated silver accumulations in the bottom of the hybrid layer.
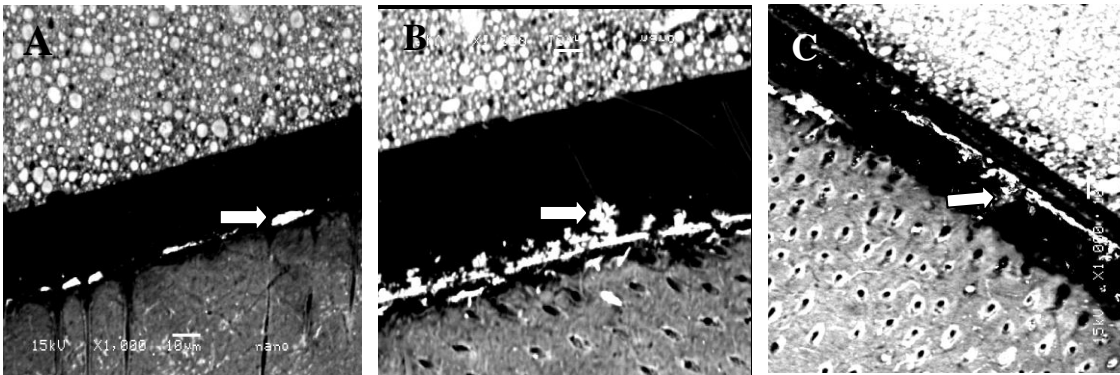

Figure 6. Nanoleakage illustrations for Adper Single Bond Plus. Figure 6A shows nanoleakage in group C3 (control - no PP), Figure 6B in group E3 (experimental PP), and Figure 6C in group P3 (conventional PP). White arrows show the most silver penetration. Silver impregnation was significantly increased with pulpal pressure, showing the formation of water channels (water trees).
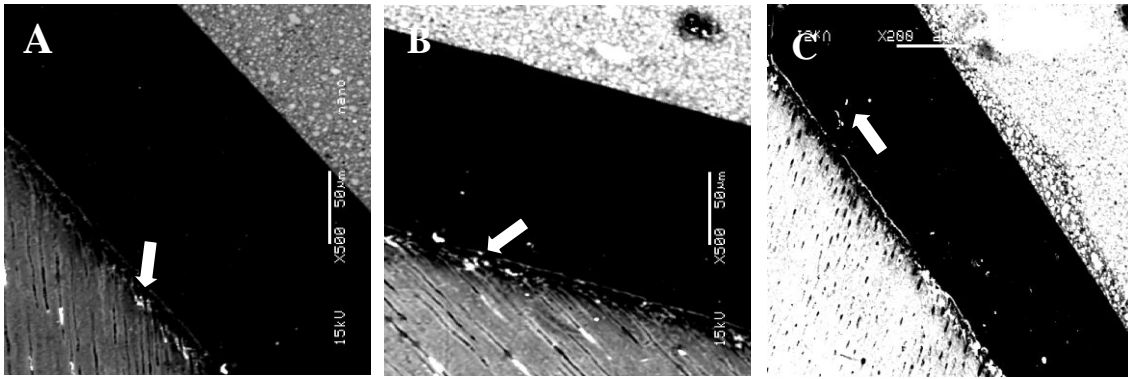
Figure 7. Nanoleakage illustrations for Scotchbond Multi Purpose. Figure 7A presents nanoleakage in group C4 (control - no PP), Figure 7B in group E4 (experimental PP) and Figure 7C in group P4 (conventional PP). White arrows show the most silver penetration. Silver impregnation was hardly seen, even after pulpal pressure with only slight spots of silver penetration.

Discussion

Several studies have shown the high permeability of simplified DBAs [5,11,41] even after polymerization. This is linked to the high amount of hydrophilic monomers and non-evaporated solvent, which may explain the results of the present study in the groups with 1-SEA (C1, P1 and E1). The striking decrease in µTBS of this type of DBA after 24 hours of simulated PP is in agreement with other studies [4,5]. 1-SEAs application on endodontically treated teeth presented significant increase in silver impregnation [42]. These teeth had pulpal tissue removed and PP is absent; so that obvious increase is expected with the hydrostatic pressure coming through the dentin tubules with this type of DBA in vital teeth. A similar reduction on bond strength after simulated PP is seen for 2-E&R adhesive Adper Single Bond Plus. In micropermeability tests, it has often been reported that these simplified DBAs have highly permeable films after polymerization [10,13]. The decreased microtensile bond strength and higher silver penetration for Adper Single Bond Plus [43] and for Clearfil S3 Bond after simulated pulpal pressure are in agreement with others studies [5,12].

Multi-step DBAs apply a subsequent hydrophobic adhesive resin layer without solvent after the priming procedure. This layer contributes to reduced permeability and high

resistance to water degradation even after simulated pulpal pressure [10,13], and consequently the microtensile bond strength is preserved [5,12]. In addition, the multi-step DBAs used in this study, Clearfil SE Bond (2-SEA) and Scotchbond Multi Purpose (3-E&R) are gold-standard DBAs and related to low permeability. This explains the preservation of µTBS for Groups P2, E2, P4 and E4 in comparison with Groups C2 and C4 (see Figure 3).

Nanoleakage is a useful method to predict the long-term stability of a restoration. Silver impregnation in the bonding area with 50% ammoniacal silver nitrate is at present a test to evaluate the quality of hybrid and adhesive layers by SEM or TEM [26,43,44]. The increase in silver impregnation means an increase in polymer degradation in the hybrid and adhesive layers, which represents more water penetration from dentin tubules and unaffected dentin. The high amount of silver penetration after simulated PP in the groups with 1-SEA and 2-E&R has a potential relationship with the decrease in µTBS for these DBAs. In contrast, 2-SEA and 3-E&R presented only a slight increase in silver impregnation, which accompanies no statically significant decrease in µTBS. Therefore, the two hypotheses have to be rejected, as nanoleakage evaluation showed only a slight increase in silver penetration for some adhesives and large increase for others; and bond strengths differed among DBAs and the approach to pulpal pressure.

Simulated pulpal pressure plays an substantial hole in adhesive dentistry development and *in vitro* evaluation of composite resins, DBAs and resin cements [5,19]. This clinical variable revealed the difficulties and boundaries for dentin sealing and restoration stability during and after bonding [23]. It expedites water penetration, polymer degradation and droplet formation in the tooth/restoration zone [4,13] with a positive physiological hydrostatic pressure through the dentin tubules. Thus, µTBS is soon shown to decrease and new DBAs, bonding techniques and resin cements can be tested in short-term experiments [12,24,26,38] with an *in vitro* study closer to the *in vivo* situation. However, a low number of studies have performed simulated pulpal pressure as a methodology to approximate *in vitro* studies to clinical condition. Simple water storage and thermal cycling are the most frequently used methods for this purpose, but both are more time-consuming methodologies. Water storage needs at least three or six months to allow differences to be discriminated, and thermal cycling needs up to 100,000 cycles for similar degradation [45].

One explanation for the low number of studies using conventional simulated PP is the more laborious methodology and devices required. Conventional PP *in vitro* requires a closed system with an 18-gauge stainless steel tube, plexiglass or acrylic plate, water column 15-20 cm above the sample [28] and sample cementation usually performed with cyanoacrylate glue. When the water column is in function, hydrostatic pressure is created inside the pulp chamber and the cyanoacrylate cementation frequently allows water seepage through the glue. Accordingly, the closed system is compromised and the pressure inside pulp chamber is reduced. Therefore, the conventional simulated PP device requires different components; it is necessary to use one device for each sample [17] and the closed system with complete cyanoacrylate sealing is more laborious. These are some of the disadvantages of traditional simulated PP [5,13,17].

The experimental methodology developed for this study to simulate PP transports the sample into the water column, so that it does not require cyanoacrylate glue cementation, plexiglass plate and stainless steel tube. It is easier to achieve and maintain the closed system with a stable and constant hydrostatic pressure in the pulp chamber. The samples are attached to a cylindrical receptacle lid with wax, and the receptacle is filled with distilled water until the water level reaches a height of 20 cm [4,20]. The receptacle is closed and turned upside down. This is a less laborious procedure that ensures a closed system without water seepage and many samples can be included in the same receptacle (in this study it was possible to place fifteen). The only disadvantage of this methodology is that simulated PP cannot be performed during bonding procedure. The two ways to perform simulated PP theoretically resemble each other, exactly following the hydrostatic pressure equation (p=g.d.h, mentioned in methods and materials). The µTBS and nanoleakage results of this *in vitro* study ratify the similarity between the two methods.

Conclusions

It can be concluded that simulated pulpal pressure had no significant effect on the multi-step adhesives (3-step etch-and-rinse and 2-step self-etch), which preserved µTBS and presented low increase in silver impregnation. However, the simplified adhesives (2-step etch-and-rinse and 1-step self-etch) had the opposite behavior, showing an adverse

effect on μTBS and nanoleakage. The experimental methodology to simulate pulpal pressure produced similar outcomes in comparison with the conventional methodology for all adhesives tested.

Acknowledgments

References

[1] Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin:current status and future challenges. Oper Dent 2003;28:215-35.

[2] Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, Huang C, Prati C, Breschi L, Ferrari M, Pashley DH, Tay FR. In vivo and in vitro permeability of one-step self etch adhesives. J Dent Res 2004;83:459-464.

[3] Itthagarun A, Tay FR, Pashley DH, Wefel JS, Garcia-Godoy F, Wei SHY. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part III. Evidence from fluid conductance and artificial caries inhibition. Am J Dent 2004;17:394-400.

[4] Sauro S, Pashley DH, Montanari M, Chersoni S, Carvalho RM, Toledano M, Osorio R, Tay FR, Prati C. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater 2007;23:705-713.

[5] Hosaka K, Masatoshi N, Monticelli F, Carrilho M, Yamauti M, Aksornmuang J, Nishitani Y, Tay FR, Pashley DH, Tagami J. Influence of hydrostatic pulpal pressure on the microtensile bond strength of all-in-one self-etching adhesives. J Adhes Dent 2007; 9:437-442.

[6] Rosales-Leal JI, De La Torre-Moreno FJ, Bravo M. Effect of pulp pressure on the micropermeability and sealing ability of etch&rinse and self-etching adhesives. Oper Dent 2007;32(3):242-250.

[7] Cardoso MV, Moretto SG, Carvalho RC, Russo EM. Influence of intrapulpal pressure simulation on the bond strength of adhesive systems to dentin. Braz Oral Res 2008;22:170-175.

[8] Bracket WW, Ito S, Tay FR, Haisch LD, Pashley DH. Microtensile bond strength of self-etching resins: effect of a hydrophobic layer. Oper Dent 2005;30:733-8.

[9]   Reis A, AlbuquerquE M, Pegoraro M, Mattei G, Bauer JRO, Grande RHM, Klein-Junior CA, Baumhardt-Neto R, Loguercio AD. Can the durability of one-step self-etch adhesives be improved by double application or by an extra layer of hydrophobic resin? J Dent 2008;36:309-315.

[10]   Silva SMA, Carrilho MRO, Marquezini Junior L, Garcia FCP, Manso AP, Alves MC, Carvalho RM. Effect of an additional hydrophilic versus hydrophobic coat on the quality of dentinal sealing provided by two-step etch-and-rinse adhesives. J Appl Oral Sci 2009;17:184-189.

[11]   Sauro S, Pashley DH, Mannocci F, Tay FR, Pilecki P, Sherriff M, Watson TF. Micropermeability of current self-etching and etch-and-rinse adhesives bonded to deep dentine: a comparison study using a double-staining/confocal microscopy technique. Eur J Oral Sci 2008;116:184-193.

[12]   Campos EA, Correr GM, Leonardi DP, Barata-Filho F, Gonzaga CC, Zielak JC. Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing. J Dent 2009;37:108-114.

[13]   Sauro S, Mannocci F, Toledano M, Osorio R, Thompson I, Watson TF. Influence of hydrostatic pulpal pressure on droplets formation in current etch-and-rinse and self-etch adhesives: a video rate/TSM microscopy and fluid filtration study. Dent Mater 2009;25,1392-1402.

[14]   Zheng L, Pereira PNR, Somphone P, Nikaido T, Tagami J. effect of hydrostatic pressure on regional bond strengths of compomers to dentine. J Dent 2000;28:501-508.

[15]   Pereira PNR, Sano H, Ogata M, Zheng L, Nakajima M, Tagami J, Pashley DH. Effect of region and dentin perfusion on bond strengths of resin-modified glass ionomer cements. J Dent 2000;28:347-354.

[16]   Sengun A, Ozturk Bozer F. The effect of simulated intrapulpal pressure on bond strength to enamel and dentin. J Oral Rehabil 2003;30:550-555.

[17]   Gupta R, Tewari S. Effect of Rotary instrumentation on composite bond strength with simulated pulpal pressure. Oper Dent 2006;31:188-196.

[18]   Mazzitelli C, Monticelli F, Osorio R, Casucci A, Toledano M, Ferrari M. Effect of simulated pulpal pressure on self-adhesive cements bonding to dentin. Dent Mater 2008;24:1156-1163.

[19]   Hiraishi N, Yiu CK, King NM, Tay FR. Effect of pulpal pressure on the microtensile bond strength of luting resin cements to human dentin. Dent Mater 2009;25:58-66.

[20]   Kim SY, Ferracane J, Kim HY, Lee IB. Real-time measurement of dentinal fluid flow during amalgam and composite restoration. J Dent 2010;38:343-351.

[21]   Lin J, Mehl C, Yang B, Kern M. Durability of four composite resin cements bonded to dentin under simulated pulpal pressure. Dent Mater 2010;26:1001-1009.

[22] De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003;82:136-140.

[23] Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res 2004; 83:843-848.

[24] Abdalla AI, Elsayed HY, Garcia-Godoy F. Effect of hydrostatic pulpal water pressure on microtensile bond strength of self-etch adhesives to dentin. Am J Dent 2008; 21:233-238.

[25] Sadek FT, Pashley DH, Ferrari M, Tay FR. Tubular occlusion optimizes bonding of hydrophobic resins to dentin. J Dent Res 2007;86:524-528.

[26] Vachiramon V, Vargas MA, Pashley DH, Tay FR, Geraldeli S, Qian F, Armstrong SR. Effects of oxalate on dentin bond after 3-month simulated pulpal pressure. J Dent 2008;36:178-185.

[27] Beveridge EE, Brown AC. The measurement of human intrapulpal pressure and its response to clinical variables. Oral Surg Oral Med Oral Pathol 1965;19;655-668.

[28] Ciucchi B, Bouillaguet S, Holz J, Pashley DH. Dentinal fluid dynamics in human teeth, in vivo. J Endodontics 1995;21:191-194.

[29] Vongsavan N, Matthews B. Fluid flow through cat dentine in vivo. Arch Oral Biol 1992;37:175-185.

[30] Stenvik A, Iversen J, Mjor IA. Tissue pressure and histology of normal and inflamed tooth pulps in macaque monkeys. Arch Oral Biol 1972;17:1501-1511.

[31] Wynn W, Haldi J, Hope MA, John K. Pressure within the pulp chamber of the dog's tooth relative to arterial blood pressure. J Dent Res 1963;42:1169-1177.

[32] Olgart L, Gazelius B. Effect of adrenalin and felypressine (octapressin) on blood flow and sensory nerve activity on the tooth. Acta Odontol Scand 1977;35:69-75.

[33] Kim S, Edwall L, Trowbridge H, Chien S. Effect of local anaesthetics on pulpal blood flow in dogs. J Dent Res 1984;63:650-652.

[34] Odor TM, Pitt Ford TR, Mcdonald F. Adrenaline in local anaesthesia: the effect of concentration on dental pulpal circulation and anaesthesia. Endodontic and Dental Traumatology 1994;10:167-173.

[35] Premdas CE, Pitt Ford TR. Effect of palatal injections on pulpal blood flow in premolars. Endod Dent Tramatol 1995;11:274-8.

[36] Chng HS, Pitt Ford TR, Mcdonald F. Effect of prilocaine local anaesthetic solutions on pulpal blood flow in maxillary canines. Endod Dent Traumatol 1996;12:89-95.

[37] King NM, Tay FR, Pashley DH, Hashimoto M, Ito S, Brackett WW, Garcia-Godoy F, Sunico M. Conversion of one-step to two-step self-etch adhesives for improved efficacy and extended application. Am J Dent 2005 18:126-134.

[38] Bakry AS, Nakajima M, Otsuki M, Tagami J. Effect of Er:YAG laser on dentin bonding durability under simulated pulpal pressure. J Adhes Dent 2009;11:361-368.

[39] Camps J, Tardieu C, Dejou J, Franquin JC, Ladaique P, Rieu R. In vitro cytotoxicity of dental adhesive systems under simulated pulpal pressure. Dent Mater 1997;13:34-42.

[40] Bouillaguet S, Virgillito M, Wataha J, Ciucchi B, Holz J. The influence of dentine permeability on cytotoxicity of four dentine bonding systems, in vitro. J Oral Rehabil 1998;25:45-51.

[41] Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002; 30:371-382.

[42] Chersoni S, Acquaviva GL, Prati C, Ferrari M, Grandini S, Pashley DH, Tay FR. In vivo fluid movement through dentin adhesives in endodontically treated teeth. J Dent Res 2005;84:223-227.

[43] Reis A, Klein-Junior CA, Accorinte M de L, Grande RH, Dos Santos CB, Loguercio AD. Effects of adhesive temperature on the early and 6-month dentin bonding. J Dent 2009;37:791-798.

[44] Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81(7):472-476.

[45] Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond strength tests and clinical outcomes. Dent Mater 2010;26:100-121.

CONSIDERAÇÕES GERAIS

Foi encontrada diferença estatisticamente significante nos resultados dos dois capítulos, com e sem pressão pulpar simulada, porém esse fato não ocorreu entre as duas metodologias para simulação da pressão pulpar nos testes de resistência da união e para a avaliação da nanoinfiltração, assim considera-se que a hipótese do trabalho deve ser parcialmente aceita. Adicionalmente, o aumento do tempo de fotoativação (para o primeiro capítulo) e o controle (para o segundo capítulo) mostraram diferentes resistências de união, o que corrobora para a parcial aceitação da hipótese do trabalho.

A simulação pressão pulpar é um método eficaz para testar a permeabilidade de sistemas adesivos em diferentes técnicas restauradoras. Um dos materiais que apresenta a característica de alta permeabilidade é o adesivo autocondicionante de passo único (15,34). Esse adesivo é mais prático e simples no manuseio e aplicação, permitindo maior padronização da técnica. Devido ao grande número de estudos, algumas técnicas têm sido implementadas para a melhoria da durabilidade da união e diminuição da permeabilidade das películas. A dupla aplicação e a aplicação posterior de uma camada de adesivo hidrófobo não solvatado são métodos a serem utilizados na tentativa de diminuir a passagem de fluidos e melhorar a resistência da união à dentina (2,27). Outras maneiras de melhorar o desempenho adesivo dos sistemas autocondicionantes de passo único são o aumento do tempo de jato de ar e o uso de jato de ar aquecido, para aumentar a evaporação de solvente (13,28).

O grau de conversão dos sistemas autocondicionantes simplificados é relativamente baixo em comparação com o de outras classes de adesivos (21). A explicação para este fato é a alta taxa de monômeros com características hidrófilas e a grande quantidade de solvente residual presente após a polimerização (37). O aumento do tempo de fotoativação para além do recomendado pelos fabricantes (normalmente de 10 a 20 segundos) é um método simples e efetivo para elevar o grau de conversão e diminuir a permeabilidade dos adesivos autocondicionantes de passo único (4,6). Entretanto, estudos da resistência da união após aumento do tempo de exposição são escassos na literatura. No estudo do primeiro capítulo desta dissertação foi comprovado o aumento da resistência da união após o aumento do tempo de exposição, o que pode ser correlacionado com o aumento do grau de conversão

(6). Adicionalmente, após simulação por 24 horas da pressão pulpar com coluna de 20 cm de água, ocorreu significativa preservação da resistência da união para o grupo com maior tempo de fotoativação em comparação ao tempo de exposição estabelecido pelo fabricante. Tal fato está de acordo com a diminuição da permeabilidade após o aumento do tempo de fotoativação (4) e demonstra que o aumento do tempo de exposição para esses adesivos simplificados seria clinicamente recomendável devido à melhora da resistência da união e do selamento da dentina.

Das classes de sistemas adesivos (40), os simplificados (autocondicionantes de passo único e de técnica úmida de dois passos) são os mais afetados pela pressão pulpar, pois são os adesivos que demonstram mais permeabilidade (18,26,31,33). Já os adesivos de vários passos (autocondicionantes de dois passos e os de técnica úmida de três passos) promovem melhor selamento e com isso são menos afetados pela pressão hidrostática proveniente dos túbulos dentinários (7,18,26,30). Uma explicação para esse melhor desempenho dos sistemas de vários passos é a aplicação separada de uma camada de adesivo não solvatada e com predominância de monômeros com características hidrófobas (3,33). Essa camada propicia aumento da espessura do adesivo e a formação de uma região não hidrófila melhor polimerizada, considerando que não possui solvente; assim, a penetração e transudação de água são dificultadas (imagens de nanoinfiltração do segundo capítulo) e a durabilidade de união significantemente aumentada. Os resultados encontrados no segundo capítulo podem ser fundamentados por essas explicações e estão de acordo com outros trabalhos encontrados na literatura (18,26).

Sob efeito da pressão pulpar, a captação e passagem de água são notavelmente aumentadas, tanto na camada híbrida como na camada de adesivo. Esse processo promove maior degradação dos polímeros, prejudicando as propriedades do material, como a resistência coesiva (43) e o módulo de elasticidade (19). Com a piora das propriedades do material, consequentemente ocorre também diminuição da capacidade adesiva e da durabilidade da adesão da restauração ao dente.

A adesão ao esmalte já está consolidada e pode ser tratada como uma adesão estável, mesmo por adesivos autocondicionantes mais atuais (41). No entanto, a união à dentina ainda necessita de melhorias e os esforços por parte dos fabricantes e pesquisadores têm sido focados neste objetivo. Por ser um substrato mais heterogêneo e permeável, a dentina propicia certas dificuldades para a adesão do material restaurador e para a

manutenção de uma união estável em longo prazo (5). A pressão hidrostática intrapulpar, por sua vez, mostra consequências significantes sobre os sistemas adesivos em dentina, sendo o seu efeito praticamente nulo em esmalte (32). A ação da pressão exerce efeito diferente e depende da região em que o sistema adesivo é aplicado. Deste modo, em dentina média e profunda ela promove maior degradação que em dentina mais superficial e em relação à proximidade dos cornos pulpares não demonstrou influência marcante (24). Assim, para o trabalho foi selecionada uma espessura de dentina pequena (0.9-1.0 mm), para ampliar os efeitos da pressão pulpar.

A pressão pulpar simulada exerce papel importante em estudos laboratoriais e promove maior proximidade entre os trabalhos *in vitro* e *in vivo*, considerando que a pressão é uma variável clínica de grande importância. A remoção da cárie geralmente é realizada após anestesia infiltrativa ou com bloqueio anestésico da região onde o dente se encontra (9,22,23,25). Durante o procedimento adesivo restaurador, o dente permanece anestesiado e a anestesia diminui o fluxo sanguíneo dentro da câmara pulpar. Essa redução da circulação sanguínea na polpa propicia diminuição da pressão intrapulpar e dentro dos túbulos dentinários, chegando próxima de zero. Por este motivo, muitos trabalhos não simulam a pressão pulpar durante a aplicação do adesivo, mas somente após o procedimento restaurador (17). Já foi estabelecido que a pressão pulpar fisiológica normal *in vivo* apresenta-se entre 8 e 22 cm de água (10) e normalmente os trabalhos utilizam uma coluna de 15 ou 20 cm de água para simular a pressão intrapulpar.

Os estudos que utilizam a pressão pulpar simulada mostram que em curto período de tempo ela consegue mostrar diferenças significantes para vários adesivos, cimentos resinosos e materiais restauradores (17,20,24,31).

É evidente que a pressão pulpar simulada é importante para o desenvolvimento e teste de novos materiais adesivos e técnicas restauradoras; entretanto, ela não é amplamente utilizada nos trabalhos. O principal motivo para isto é que a metodologia é mais trabalhosa e necessita de dispositivo especial para aplicá-la. Com um dispositivo tradicional é possível simular a pressão pulpar para apenas uma amostra, sendo necessários muitos dispositivos para aplicar em várias amostras (14). Adicionalmente, é essencial a fixação das amostras em plataformas acrílicas com cola de cianoacrilato, o que ocorrer em duas dificuldades técnicas. A primeira é a aplicação e fixação com cola sem obstruir a câmara pulpar ou a agulha que penetra a plataforma acrílica por onde passa a água destilada. Muitas vezes esta

obstrução ocorre, sendo notada somente quando a amostra for retirada da plataforma, o que representa incorreta aplicação da pressão pulpar hidrostática, assim sendo necessária a repetição do ensaio. A segunda dificuldade é o deficiente vedamento da cola que permite extravasamento de água através da interface dente/cola/plataforma. Uma significante desvantagem da técnica convencional é a dificuldade em manter o sistema fechado e a manutenção correta da pressão hidrostática.

Visando a sanar essas dificuldades e simplificar o dispositivo para simulação da pressão pulpar, foi criada a metodologia experimental apresentada no segundo capítulo, na qual não há necessidade de fixação com cola de cianoacrilato. As amostras ficam submersas abaixo da coluna de água, fixadas com cera à tampa de um recipiente. Para o primeiro capítulo, a pressão pulpar foi utilizada para promover maiores diferenças entre os grupos e simular um "envelhecimento" *in vitro* da união. Já no segundo capítulo ela foi utilizada nas duas metodologias (convencional e experimental) em comparação com grupos não submetidos à pressão pulpar simulada.

CONCLUSÃO GERAL

Diante dos resultados encontrados neste estudo, pode ser concluído que:

1. A pressão pulpar diminuiu a resistência da união dos adesivos simplificados, mas o aumento do tempo de fotoativação melhorou o desempenho dos adesivos autocondicionantes de passo único diante desta situação.

2. As duas metodologias para simular a pressão hidrostática intrapulpar demonstraram resultados similares tanto para resistência da união como na avaliação da nanoinfiltração, para todos os sistemas adesivos testados. Deste modo, a técnica convencional para aplicação de pressão intrapulpar simulada pode ser substituída pela metodologia experimental desenvolvida neste trabalho, que é mais simples de ser realizada.

REFERÊNCIAS[*]

1. Abdalla AI, Elsayed HY, Garcia-Godoy F. Effect of hydrostatic pulpal water pressure on microtensile bond strength of self-etch adhesives to dentin. Am J Dent 2008; 21:233-238.

2. Albuquerque M, Pegoraro M, Mattei G, Reis A, Loguercio AD. Effect of Double-application or the application of a hydrophobic layer for improved efficacy of one-step self-etch systems in enamel and dentin. Oper Dent 2008;33:564-70.

3. Brackett WW, Ito S, Tay FR, Haisch LD, Pashley DH. Microtensile bond strength of self-etching resins: effect of a hydrophobic layer. Oper Dent 2005;30:733-8.

4. Breschi L, Cadenaro M, Antoniolli F, Sauro S, Biasotto M, Prati C, Tay FR, Di Lenarda R. Polymerization kinetics of dental adhesives cured with LED: correlation between extent of conversion and permeability. Dent Mater 2007;23:1066-1072.

5. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, Dorigo EDS. Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008;24:90-101.

6. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C. Degree of conversion and permeability of dental adhesives. Eur J Oral Sci 2005;113:525-530.

7. Campos EA, Correr GM, Leonardi DP, Barata-Filho F, Gonzaga CC, Zielak JC. Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing. J Dent 2009;37:108-114.

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[*] *De acordo com a norma da UNICAMP/FOP, baseado na norma do International Committe of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline*

8. Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR, Pashley DH. Adhesive permeability affects coupling of resin cements that utilize self-etching primers to dentin. J Dent 2004;32:55-65.

9. Chng HS, Pitt Ford TR, Mcdonald F. Effect of prilocaine local anaesthetic solutions on pulpal blood flow in maxillary canines. Endod Dent Traumatol 1996;12:89-95.

10. Ciucchi B, Bouillaguet S, Holz J, Pashley DH. Dentinal fluid dynamics in human teeth, in vivo. J Endodontics 1995;21:191-194.

11. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003;82:136-140.

12. Furukawa M, Shingetani Y, Finger WJ, Hoffmann M, Kanehira M, Endo T, Komatsu M. All-in-one self-etch model adhesives: HEMA-free and without phase separation. J Dent 2008;36:402-408.

13. Garcia FCP, Almeida JCF, Osorio R, Carvalho RM, Toledano M. Influence of drying time and temperature on bond strength of contemporary adhesives to dentine. J Dent 2009;37:315-320.

14. Gupta R, Tewari S. Effect of Rotary instrumentation on composite bond strength with simulated pulpal pressure. Oper Dent 2006;31:188-196.

15. Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res 2004; 83:843-848.

16. Hiraishi N, Nishiyama N, Ikemura K, Yau JYY, King NM, Tagami J, Pashley DH, Tay FR. Water concentration in self-etching primers affects their aggressiveness and bonding efficacy to dentin. J Dent Res 2005;84:653-658.

17. Hiraishi N, Yiu CK, King NM, Tay FR. Effect of pulpal pressure on the microtensile bond strength of luting resin cements to human dentin. Dent Mater 2009;25:58-66.

18. Hosaka K, Masatoshi N, Monticelli F, Carrilho M, Yamauti M, Aksornmuang J, Nishitani Y, Tay FR, Pashley DH, Tagami J. Influence of hydrostatic pulpal pressure on the microtensile bond strength of all-in-one self-etching adhesives. J Adhes Dent 2007; 9:437-442.

19. Ito S, Hashimoto M, Wadgaonkar B, Svizero M, Carvalho RM, Yiu C, Rueggeberg F, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, Pashley DH. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005;26:6449-6459.

20. Nakajima M, Hosaka K, Yamauti M, Foxton RM, Tagami J. Bonding durability of self-etching primer system to normal and caries-affected dentin under hydrostatic pulpal pressure in vitro. Am J Dent 2006;19:147-150.

21. Navarra C, Cadenaro M, Codan B, Mazzoni A, Sergo V, Dorigo ES, Breschi L. Degree of conversion and interfacial nanoleakage expression of three one-step self-etch adhesives. Eur J Oral Sci 2009;117:463-469.

22. Odor TM, Pitt Ford TR, Mcdonald F. Adrenaline in local anaesthesia: the effect of concentration on dental pulpal circulation and anaesthesia. Endodontic and Dental Traumatology 1994;10:167-173.

23. Olgart L, Gazelius B. Effect of adrenalin and felypressine (octapressin) on blood flow and sensory nerve activity on the tooth. Acta Odontol Scand 1977;35:69-75.

24. Pereira PNR, Sano H, Ogata M, Zheng L, Nakajima M, Tagami J, Pashley DH. Effect of region and dentin perfusion on bond strengths of resin-modified glass ionomer cements. J Dent 2000;28:347-354.

25. Premdas CE, Pitt Ford TR. Effect of palatal injections on pulpal blood flow in premolars. Endod Dent Tramatol 1995;11:274-8.

26. Purk JH, Dusevich V, Atwood J, Spencer BD, Kruse D, Webb T Williams A, Tira D. Microtensile dentin adhesive bond strength under different positive pulpal pressures. Am J Dent 2009;22:357-360.

27. Reis A, AlbuquerquE M, Pegoraro M, Mattei G, Bauer JRO, Grande RHM, Klein-Junior CA, Baumhardt-Neto R, Loguercio AD. Can the durability of one-step self-etch adhesives be improved by double application or by an extra layer of hydrophobic resin? J Dent 2008;36:309-315.

28. Reis A, Klein-Junior CA, Coelho-De-Souza FH, Stanislawczuc R, Loguercio AD. The use of warm air-stream for solvent evaporation: effects on the durability of resin-dentin bonds. Oper Dent 2010;35:29-36.

29. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20:18-25.

30. Sauro S, Pashley DH, Mannocci F, Tay FR, Pilecki P, Sherriff M, Watson TF. Micropermeability of current self-etching and etch-and-rinse adhesives bonded to deep dentine: a comparison study using a double-staining/confocal microscopy technique. Eur J Oral Sci 2008;116:184-193.

31. Sauro S, Pashley DH, Montanari M, Chersoni S, Carvalho RM, Toledano M, Osorio R, Tay FR, Prati C. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater 2007;23:705-713.

32. Sengun A, Ozturk Bozer F. The effect of simulated intrapulpal pressure on bond strength to enamel and dentin. J Oral Rehabil 2003;30:550-555.

33. Silva SMA, Carrilho MRO, Marquezini Junior L, Garcia FCP, Manso AP, Alves MC, Carvalho RM. Effect of an additional hydrophilic versus hydrophobic coat on the quality of dentinal sealing provided by two-step etch-and-rinse adhesives. J Appl Oral Sci 2009;17:184-189.

34. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002; 30:371-382.

35. Tay FR, Pashley DH, Suh BI, Garcia-Godoy F, Yiu CK. Single-step, self-etch adhesives behaves as permeable membranes after polymerization. Part II. Silver tracer penetration evidence. Am J Dent 2004;17:315-322.

36. Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81(7):472-476.

37. Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? J Can Dent Assoc 2003; 69:726-731.

38. Vachiramon V, Vargas MA, Pashley DH, Tay FR, Geraldeli S, Qian F, Armstrong SR. Effects of oxalate on dentin bond after 3-month simulated pulpal pressure. J Dent 2008;36:178-185.

39. Van Landuyt KL, Snauwaert J, De Munck J, Coutinho E, Poitevin A, Yoshida Y, Suzuki K, Lambrechts P, Van Meerbeek B. Origin of interfacial droplets in one-step adhesives. J Dent Res 2007;86:739-744.

40. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin:current status and future challenges. Oper Dent 2003;28:215-35.

41. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond strength tests and clinical outcomes. Dent Mater 2010;26:100-121.

42. Ye Q, Wang Y, Spencer P. Nanophase separation of polymers exposed to simulated bonding conditions.J Biomed Mater Res Part B:Appl Biomater 2009;88B: 339–348.

43. Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. Biomaterials 2004;25:5789-5796.

APÊNDICE

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VICTOR PINHEIRO FEITOSA
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AUTOR

AMÉRICO BORTOLAZZO CORRER
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Piracicaba
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ANEXO 1

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CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Um método simples para simulação de pressão intrapulpar: Nanoinfiltração e resistência de união à microtração"**, protocolo nº 167/2009, dos pesquisadores Victor Pinheiro Feitosa e Mário Alexandre Coelho Sinhoreti, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 09/12/2009.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"A simple method to simulate pulp pressure: Nanoleakage and microtensile bond strength"**, register number 167/2009, of Victor Pinheiro Feitosa and Mário Alexandre Coelho Sinhoreti, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 12/09/2009.

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

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

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

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