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Efeito da pressão pulpar interna na resistência de união e nanoinfiltração das interfaces de união resina-dentina produzidas por cimentos auto-adesivos: um estudo *in vitro*

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Orientador: Prof. Dr. André Figueiredo Reis
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A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, intitulada "EFEITO DA PRESSÃO PULPAR INTERNA NA RESISTÊNCIA DE UNIÃO E NANOINFILTRAÇÃO DAS INTERFACES DE UNIÃO RESINA-DENTINA PRODUZIDAS POR CIMENTOS AUTO-ADESIVOS: UM ESTUDO *IN VITRO*" em sessão pública realizada em 27 de Janeiro de 2010 considerou o candidato Rodrigo Sversut de Alexandre aprovado com louvor.

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Guarulhos, 27 de Janeiro de 2010.

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Epígrafe

Como é que você reage às quedas que sofre na vida?

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Já começou vencer aquele que se levantou para recomeçar o caminho.

Pe. Fábio de Melo

Resumo:

Os objetivos deste estudo foram avaliar o efeito da pressão pulpar simulada (PPS) na resistência de união (RU) à dentina e padrão de nanoinfiltração produzido por diferentes cimentos auto-adesivos. Três cimentos auto-adesivos (RelyX Unicem - UN; RelyX U100 - UC; Clearfil SA Luting - SA) e dois cimentos resinosos convencionais, um que emprega técnica do condicionamento ácido (Rely X ARC - RX) e um que emprega um autocondicionante de um passo (Panavia F - PF) foram utilizado neste estudo. Um grupo adicional incluiu o uso de autocondicionante de dois passos (Clearfil SE Bond) previamente a aplicação do Panavia F(PS). Sessenta terceiros molares tiveram suas porções coronárias planificadas. Em metade dos espécimes foram submetidos à pressão de 15 cm de H_2O por 48 horas, começando 24 horas antes, durante e 24 horas depois dos procedimentos de cimentação. Em seguida, os espécimes restaurados foram seccionadas em palitos com área de secção de aproximadamente 1 mm^2 e submetidos ao teste de microtração com velocidade de 1 mm/min. Os padrões de fratura foram determinados com auxílio de Microscópio Eletrônico de Varredura (MEV). Os dados foram analisados estatisticamente com análise variância Anova 2- way e Tukey's studentized para múltiplas comparações (α=0,05). Dois dentes adicionais em cada grupo foram seccionados serialmente em fatias de 0,9mm de espessura e submetidos ao protocolo de nanoinfiltração com $AgNO_3$ e analisados em MEV. A resistência de união do grupo com condicionamento ácido prévio (RX) foi influenciada negativamente pela pressão pulpar. Os demais grupos não apresentaram alterações estatisticamente significantes, com exceção de grupo UC que apresentou aumento significativo. A PPS aumentou a deposição de prata em todos os grupos, exceto para UC e UN. O uso de autocondicionante de 2 passos com PF pode melhorar a resistência de união e reduzir nanoinfiltração, reduzindo o efeito da PPS. A pressão pulpar prejudica a resistência de união e nanoinfiltração de PF e RX, mas não interferiu negativamente o desempenho dos cimentos auto-adesivos testados

Palavras chave: cimentos auto-adesivos, dentina, cimentos resinosos, resistência de união, pressão pulpar, nanoinfiltração.

ABSTRACT

The aims of this study were to evaluate the effect of simulated hydrostatic pulpal pressure (SPP) on the microtensile bond strength (μTBS) to dentin and nanoleakage patterns produced by different self-adhesive luting agents. Three self-adhesive luting agents (RelyX Unicem – UN; RelyX U100 – UC; Clearfil SA Luting - SA) and 2 conventional luting agents, one that uses a 2-step etch-and-rinse adhesive (Rely X ARC - RX), and one that uses a 1-step self-etching adhesive (Panavia F - PF) were used in this study. An additional group included the use of a 2-step self-etching primer adhesive system (Clearfil SE Bond) prior to the application of Panavia F (PS). Sixty human molars were abraded to expose occlusal surfaces. Cylindrical composite blocks were luted with resin cements in the absence or presence of pulpal pressure. Half of the specimens were subjected to 15 cm H_2O of hydrostatic pressure for 24 hours before cementation procedures, and continued for 24 hours after luting procedures. Afterwards, restored teeth were serially sectioned into beams with a cross-sectional area of approximately 1 mm^2 at the bonded interface and were tested in tension with a crosshead speed of 1 mm/min. Failure mode was determined using scanning electron microscopy (SEM). Data were statistically analyzed by 2-way ANOVA and Tukey's studentized range HSD test (α=.05). Two additional teeth in each group were serially sectioned into 0.9 mm-thick slabs, which were submitted to a nanoleakage protocol with $AgNO_3$ and analyzed in the SEM. The μTBS values of the etch-and-rinse group (RX) were negatively influenced by simulated pulpal pressure. The other groups didn't present statistically significant differences, except for UC, that presented increased μTBS values. Except for UC and UN, SPP increased silver deposition in all groups.

Conclusions: The use of a two-step self-etching adhesive with PF may improve bond strength and reduce nanoleakage, reducing the effect of SPP. Hydrostatic pulpal pressure affected bond strengths and nanoleakage behavior of PF and RX, but did not influence negatively the performance of the self-adhesive cements tested.

Key Words: self-adhesive cements; dentin; resin cements; pulpal pressure; microtensile bond strength; nanoleakage.

SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

Em dentes vitais a dentina apresenta-se hidratada através da saída de fluído dentinário pelos túbulos, devido à pressão positiva da polpa, estimada em aproximadamente 15 à 20 centímetros de água (Ciucchi *et al.*, 1995; Sauro *et al.*, 2007; Hiraishi *et al.*, 2009). Muitas pesquisas sobre resistência de união têm salientado a importância da utilização da simulação da pressão pulpar durante o procedimento de aplicação do sistema adesivo (Mitchem *et al.*, 1988; Tao & Pashley, 1989; Tao *et al.*, 1991; Elhabashy *et al.*, 1993; Ozok *et al.*, 2004; Moll *et al.*, 2005; Hebling *et al.*, 2007). A utilização desta variável tem mostrado que a umidade do fluído tubular pode influenciar a resistência de união e habilidade de sistemas adesivos e cimentos resinosos promoverem o selamento da dentina (Ciucchi *et al.*, 1995; Sauro *et al.*, 2007; Hiraishi *et al.*, 2009). Muitos tratamentos restauradores diretos e indiretos, tais como facetas, inlays, onlays e coroas são realizados em dentes com vitalidade pulpar. Assim sendo, a simulação da pressão pulpar se faz importante para aproximar as condições dos estudos "*in vitro*" da realidade clínica.

A adesão ao substrato dentinário pode ser realizada através de duas técnicas: condicionamento ácido prévio ou autocondicionamento. A primeira técnica baseia-se na aplicação do ácido fosfórico para remoção da *smear layer* e desmineralização da dentina subjacente a uma profundidade de 3 a 6 µm (Perdigao *et al.*, 1996). No entanto, esta técnica tem sido considerada crítica (Spencer *et al.*, 2000), devido a necessidade de controle da umidade (Kanca, 1992; Tay *et al.*, 1996). Com o intuito de reduzir as dificuldades da técnica e simplificar os procedimentos de aplicação, uma segunda técnica foi desenvolvida, na qual primers ou adesivos autocondicionantes compostos de monômeros ácidos são aplicados sobre a dentina sem a necessidade de lavagem e controle da umidade. Nesta técnica há a manutenção da *smear layer e smear plug,* o que contribui para uma baixa permeabilidade e movimentação de fluidos provenientes da dentina durante o processo de adesão (Hashimoto *et al.*, 2004; Sauro *et al.*, 2007; Hiraishi *et al.*, 2009). Além disso, os adesivos autocondicionantes, teoricamente, não produzem uma discrepância entre a profundidade de condicionamento e infiltração do adesivo (Tay & Pashley, 2001; De Munck *et al.*, 2003a; Van Meerbeek *et al.*, 2003). No entanto,

adesivos autocondicionantes de passo único podem produzir uma contínua desmineralização, mesmo após a sua fotoativação, criando uma zona de fibrilas de colágeno desprotegida de mineral (Wang & Spencer, 2005). Este fenômeno é atribuído à presença de monômeros ácidos não polimerizados na região da interface, que em contato com a água proveniente da dentina continuam o processo de desmineralização (Wang & Spencer, 2005). O desempenho inferior dos adesivos autocondicionantes de passo único tem sido atribuído à maior concentração de monômeros hidrófilos, menor grau de conversão em virtude da alta acidez da solução e alta permeabilidade mesmo após sua polimerização (De Munck *et al.*, 2003a; Wang & Spencer, 2004; De Munck *et al.*, 2005; Van Meerbeek *et al.*, 2005). A técnica simplificada, tanto para adesivos convencionais de 2 passos como para os adesivos autocondicionantes de 1 passo, trouxeram prejuízo em termos de durabilidade da união (Tay *et al.*, 2002a; De Munck *et al.*, 2003b; Reis *et al.*, 2004; Tay *et al.*, 2004a; Tay *et al.*, 2004b; Reis *et al.*, 2007a). O emprego de uma última camada rica em monômeros hidrófobos favorece a impermeabilização, diminuindo a degradação da interface (Reis *et al.*, 2004; De Munck *et al.*, 2005).

Mesmo com os avanços nos componentes poliméricos e inorgânicos, trabalhos têm demonstrado a degradação da união de materiais resinosos aos tecidos dentais ao longo do tempo na presença de água (Sano *et al.*, 1999; Hashimoto *et al.*, 2000; De Munck *et al.*, 2003b; Giannini *et al.*, 2003; Reis *et al.*, 2004; Reis *et al.*, 2007a; Reis *et al.*, 2007b; Reis *et al.*, 2008). A redução da resistência de união de sistemas adesivos à dentina é atribuída à degradação hidrolítica das fibrilas colágenas e da resina adesiva (Gopferich, 1996; Tay *et al.*, 2003a; Itthagarun *et al.*, 2004). A busca por um agente de união que promova uma união duradoura ao substrato dentinário e seja de fácil aplicação tem sido um desafio, e alternativas têm sido buscadas para se aumentar a longevidade da união (Hebling *et al.*, 2005; Carrilho *et al.*, 2007; Sadek *et al.*, 2007). O fenômeno de degradação pode ser observado através do ensaio de nanoinfiltração. O termo “nanoinfiltração” foi introduzido para se descrever a ocorrência de espaços nanométricos entre 20-100 nm dentro da camada híbrida, mesmo na ausência de uma fenda na interface de união (Sano *et al.*, 1994; Sano *et al.*, 1995a). Esta técnica utiliza um traçador de baixo peso molecular como o nitrato de prata ($AgNO_3$) para evidenciar tais porosidades na interface quando a área de união é analisado em

microscopia eletrônica de varredura ou transmissão (Sano *et al.*, 1995b). A deposição de grãos de prata na interface de união é atribuída à existência de regiões onde as fibrilas colágenas não foram totalmente envolvidas pela resina adesiva, ou onde a resina não foi adequadamente polimerizada. Estas porosidades podem permitir a movimentação de fluidos pela interface de união, o que contribui para a degradação da união ao longo do tempo (Sano *et al.*, 1999; Hashimoto *et al.*, 2001). Além da deposição de prata na camada híbrida, isso pode ocorrer na camada de adesivo (Tay *et al.*, 2002b). Este fenômeno foi denominado de *water-trees* (ou árvores de água). As diferenças na hidrofilia e no conteúdo de água dos adesivos podem influenciar diretamente os padrões de nanoinfiltração das interfaces.

As principais causas de substituição de restaurações diretas em resina composta são a ocorrência de fratura ou desgaste do material restaurador causados por carga mastigatória excessiva, sensibilidade dental, cáries recorrentes, necrose pulpar ou ausência de pontos de contato (Van Meerbeek *et al.*, 1998). Assim, as restaurações indiretas são indicadas em casos de cavidades extensas com grande perda de estrutura dental. Dentre as vantagens das restaurações indiretas podemos citar que: a contração de polimerização ocorre fora da cavidade no caso de restaurações em resina composta, melhor restabelecimento do ponto de contato, maior estabilidade de cor ao longo do tempo e melhores propriedades mecânicas (Vishnu *et al.*, 2007). Por estas razões, procedimentos restauradores indiretos constituem uma substancial porção dos procedimentos restauradores estéticos atuais. Restaurações indiretas estéticas como *inlays, onlays*, laminados e coroas são cimentadas sobre o substrato dental vital através da utilização de cimentos resinosos (Peumans *et al.*, 2000; Hikita *et al.*, 2007). Os agentes de cimentação podem ser divididos em cinco classes principais: cimento de fosfato de zinco, de policarboxilato de zinco, de ionômero de vidro, ionômero de vidro modificado por resina e resinoso (Diaz-Arnold *et al.*, 1999). Os cimentos resinosos apresentam como vantagens: melhor retenção das restaurações cerâmicas principalmente em conseqüência do aumento de resistência à fratura devido à melhor transmissão e distribuição das tensões funcionais através da interface adesiva (Burke, 1999; Burke *et al.*, 2002); menor solubilidade; reforço da estrutura dental enfraquecida e melhor selamento marginal quando comparados com a cimentação tradicional com os cimentos de fosfato de zinco e ionoméricos (Van Meerbeek *et al.*, 1998; Senyilmaz *et al.*, 2007).

Tradicionalmente, a união dos cimentos resinosos ao substrato dental se dá através da associação com a aplicação prévia de um sistema adesivo. Assim sendo, a técnica de aplicação do material é considerada crítica, sujeita a fatores relativos ao material e ao operador (Frankenberger *et al.*, 2000), que podem levar a ocorrência de sensibilidade pós-operatória e ao insucesso do tratamento restaurador (Mak *et al.*, 2002). Dentre os fatores relacionados ao material, temos descrito na literatura a incompatibilidade entre alguns cimentos resinosos e alguns sistemas adesivos convencionais de 2 passos e autocondicionantes de passo único (Tay et al., 2003b). A incompatibilidade entre sistemas de cimentação e adesivos está na reação de monômeros ácido presentes na formulação destes adesivos(Cheong *et al.*, 2003). Mesmo após a polimerização estes monômeros estão presentes na camada mais superficial não polimerizada, pela ação do oxigênio(Cheong *et al.*, 2003). Em condições onde a polimerização do cimento se dá, exclusivamente, através da reação de polimerização química, os monômeros ácidos reagem com a amina terciária dos cimentos, impossibilitando a reação de polimerização na interface. Para evitar este fenômeno os fabricantes laçaram adesivos de polimerização dual e adicionaram sais sulfinatos aromáticos de sódio, como componentes diferentes da reação peróxido-amina, o que evitaria a incompatibilidade entre os sistemas (de Menezes *et al.*, 2006; Arrais *et al.*, 2008).

Seguindo a evolução dos materiais adesivos e com o propósito de simplificar a técnica de cimentação, foi introduzido no mercado um cimento auto-adesivo a base de resina, que dispensa qualquer pré-tratamento da dentina (Hecht *et al.*, 2002; Reich *et al.*, 2005). O cimento auto-adesivo RelyX UNICEM foi o primeiro material auto-adesivo introduzido no mercado, e logo alcançou a aprovação dos clínicos, devido a facilidade de aplicação. Acompanhando esta tendência, diversos fabricantes lançaram seus cimentos auto-adesivos. No entanto, pouca informação a respeito destes materiais existe na literatura.

Todos os materiais que apresentam capacidade de auto-condicionar a estrutura dentária combinam três função em um ou dois frascos: condicionar, estabilização da rede de colágeno (primer) e impermeabilizar (bond). Estes materiais devem conter monômeros resinosos ionizáveis (grupos funcionais fosfato ou carboxílico), monômero hidrófobo, água e um solvente orgânico (Pashley *et al.*,

2002; De Munck *et al.*, 2005). Em virtude desta composição, os sistemas mais simplificados apresentam uma forte característica hidrófila. Alguns pesquisadores têm mostrado que sistemas adesivos de passo único comportam-se como membranas semi-permeáveis, as quais permitem a difusão de água mesmo após sua polimerização (Tay *et al.*, 2002a; Tay *et al.*, 2004a), mostrando ser ineficientes na redução da permeabilidade quando aplicados diante da simulação da pressão pulpar (Ciucchi *et al.*, 1995; Sauro *et al.*, 2007; Hiraishi *et al.*, 2009).

Assim, a avaliação dos efeitos da pressão pulpar e da umidade promovida por este fenômeno na união produzida por materiais auto-adesivos através do ensaio de resistência de união e os padrões de nanoinfiltração são um tópico relevante dentro da Odontologia Restauradora, visto que a utilização destes materiais tende a se difundir rapidamente entre os clínicos.

2. PROPOSIÇÃO

O objetivo deste estudo foi avaliar o efeito da pressão pulpar na resistência de união e nanoinfiltração nas interfaces resina-dentina produzida por diferentes cimentos resinosos auto-adesivos e cimentos que empregam sistemas adesivos autocondicionantes ou com condicionamento ácido prévio, verificando se a presença ou ausência desta pode apresentar algum efeito sobre a união ao substrato dentinário.

3. METODOLOGIA E RESULTADOS

A presente tese está baseada no artigo “The effect of simulated pulpal pressure on the bond strength and nanoleakege of self-adhesive luting agents to human dentin”, que será submetido para avaliação pelo periódico Journal of Prosthetic Dentistry.

The effect of simulated pulpal pressure on the bond strength and nanoleakage of self-adhesive luting agents to human dentin

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ABSTRACT

Objectives: The aims of this study were to evaluate the effect of simulated hydrostatic pulpal pressure (SPP) on the microtensile bond strength (μTBS) to dentin and nanoleakage patterns produced by different luting agents.

Materials and methods: Three self-adhesive luting agents (RelyX Unicem (UN), RelyX U100 (UC), and Clearfil SA Luting (SA)) and 2 conventional luting agents, one that uses a 2-step etch-and-rinse adhesive (Rely X ARC - RX), and one that uses a 1-step self-etching adhesive (Panavia F - PF) were used in this study. An additional group included the use of a 2-step self-etching primer adhesive system (Clearfil SE Bond) prior to the application of Panavia F (PS). Sixty human molars were abraded to expose occlusal surfaces. Cylindrical composite blocks were luted with resin cements in the absence or presence of pulpal pressure. Half of the specimens were subjected to 15 cm H_2O of hydrostatic pressure for 24 hours before cementation procedures, and continued for 24 hours after luting procedures. Afterwards, restored teeth were serially sectioned into beams with a cross-sectional area of approximately 1 mm^2 at the bonded interface and were tested in tension with a crosshead speed of 1 mm/min. Failure mode was determined using scanning electron microscopy (SEM). Data were statistically analyzed by 2-way ANOVA and Tukey's studentized range HSD test (α=.05). Two additional teeth in each group were serially sectioned into 0.9 mm-thick slabs, which were submitted to a nanoleakage protocol with $AgNO_3$ and analyzed in the SEM.

Results: The μTBS values of the etch-and-rinse group (RX) were negatively influenced by simulated pulpal pressure. The other groups didn't present statistically significant differences, except for UC, that presented increased μTBS values. Except for UC and UN, SPP increased silver deposition in all groups.

Conclusions: Hydrostatic pulpal pressure affected bond strengths and nanoleakage behavior of RX, but did not influence negatively the performance of the cements tested. The use of a two-step self-etching adhesive with PF may improve bond strength and reduce nanoleakage, reducing the effect of SPP.

Key Words: self-adhesvie cements; dentin; resin cements; pulpal pressure; microtensile bond strength; nanoleakage.

INTRODUCTION

Resin-based luting agents were introduced in an attempt to overcome the inherent problems of zinc phosphate cements and provide better handling and esthetic properties (Sigemori et al., 2005). In order to promote adhesion to tooth structures, conventional resin cements use etch-and-rinse or self-etching adhesive systems (Carvalho et al., 2004). Dentin consists on the main substrate available for adhesion in prosthetic procedures, especially in vital teeth. Dentin is a hydrated hard tissue in the vital state, when there is an outward flow of dentinal fluid through the dentinal tubules with a positive pulpal pressure, estimated to be approximately 15 cm H2O (Ciucchi et al., 1995). Water presents deleterious effects for adhesive procedures, such as the plasticization of the polymer chains, which can result in reduction of mechanical properties and hydrolytic degradation of resin and collagen fibrils (Hashimoto et al., 2003; Ito et al., 2005; Reis et al., 2004a; Reis et al., 2007a; Reis et al., 2007b).

The etch-and-rinse multi-step adhesive technique has been considered complex and sensitive (Frankenberger et al., 2000). This technique produce complete smear layer and smear plug removal, increasing outward flow of dentinal fluid (Nakabayashi & Pashley, 1998). In addition, an incompletely infiltrated hybrid layer has been reported (Spencer et al., 2000; Wang & Spencer, 2002). Some adhesive systems behave as semi permeable membranes (Tay et al., 2002a) and can allow outward fluid flow through dentin adhesive interface, even after polymerization (Hashimoto et al., 2004; Hiraishi et al., 2009; Sauro et al., 2007). These disadvantages probably account for higher incidence of postoperative sensitivity after bonding procedures (Christensen, 2002), pulpal damage (de Souza Costa et al., 2005) and premature degradation of the resin-dentin interface (Reis et al., 2004b; Reis et al., 2007a; Reis et al., 2007b).

Self-etching adhesive systems were developed in an attempt to reduce the technique sensitivity of etch-and-rinse systems. Self-etching monomers simultaneously etch and infiltrate dentin, providing a micromechanical retention after polymerization (Tay & Pashley, 2001). The maintenance of smear plugs minimize moisture contamination by dentinal fluid transudation when compared with the use of etch-and-rinse adhesives (Pereira et al., 1999). Simplified single-step all-in-one self-

etching adhesives have been reported to allow water diffusion even after polymerization (Hashimoto et al., 2004; Sauro et al., 2007; Tay et al., 2002a; Tay et al., 2004).

A new type of luting material that does not require any pretreatment of the tooth surface with adhesive systems has been developed, the so-called self-adhesive cements (Abo-Hamar et al., 2005; Behr et al., 2004; Radovic et al., 2008). These materials aim to combine the favorable properties of conventional (zinc phosphate, glass ionomer and polycarboxylate cements) and resin luting agents (Radovic et al., 2008). After the first self-adhesive cement was developed (RelyX Unicem; 3M ESPE, St. Paul, Minn), it rapidly gained popularity among clinicians due to its simplified "mistake-free" application technique. Their application on smear layer-covered substrates maintain dentin permeability in very low levels (Hiraishi et al., 2009), contributing with reduced post-operative sensitivity and lower susceptibility to moisture degradation (Mazzitelli et al., 2008). However, limited information is available with regard to the bonding mechanism, longevity and nanoleakage of self-adhesive cements (De Munck et al., 2004; Gerth et al., 2006; Piwowarczyk et al., 2007; Radovic et al., 2008).

The aim of this study was to evaluate the effects of simulated pulpal pressure (SPP) on bond strength and nanoleakage in resin-dentin interfaces produced by different cementation strategies.

MATERIALS AND METHODS

Tooth preparation

Seventy two recently extracted caries-free third molars stored in 0.1% thymol (Symrise GmbH, Holzminden, Germany) solution at 4°C were used in this study. Teeth were obtained by protocols that were approved by the review board of the Guarulhos University (Guarulhos, São Paulo, Brazil). After disinfection and removal of soft tissues, flat coronal dentin surfaces were exposed with 600-grit SiC paper (3M of Brazil Ltd, Sumaré, Brazil) under running water to create a standardized smear layer and avoiding exposition of pulp chamber.

Teeth were assigned to 2 experimental groups (with or without SPP), which were distributed into 6 experimental subgroups (n=7) according to the luting technique. Three self-adhesive cements: RelyX Unicem (UN), RelyX U100 (UC), Clearfil SA Luting (SA); and 2 conventional luting agents, one that uses a 2-step etch-and-rinse adhesive (Single-bond 2 + RelyX ARC - RX), and one that uses a 1-step self-etching adhesive (ED Primer + Panavia F - PF), were used in this study. An additional group included the use of a 2-step self-etching primer adhesive system (Clearfil SE Bond) prior to the application of Panavia F (PS). Luting agents were mixed and placed according to manufacturers' instructions (Table 1).

The teeth submitted to SPP had their roots removed using a diamond saw (Isomet, Buehler, Lake Bluff, IL) 2 mm below the cement-enamel junction. Pulpal tissue was gently removed so as not to damage the predentin region. In order to simulate pulpal pressure on dentin surface, each tooth was bonded to a Plexiglass platform (3 cm x 3 cm x 0.3 cm) penetrated by an 18 gauge stainless steel tube and fixed with cyanoacrylate adhesive (Loctite Super Bonder Gel; Henkel, Düsseldorf, Germany). The pulp chamber was filled with distilled water via polyethylene tubing connected to a syringe barrel with 10 ml of distilled water and suspended 15 cm in relation to the tooth crown. Thus, each specimen was connected to a hydraulic pressure device that delivered 15 cm water pressure (Tao & Pashley, 1989).The teeth were kept under this hydrostatic pressure for 48 hours, starting 24 hours before luting procedures.

Luting procedures for microtensile bond strength

Five teeth of each group were used for the microtensile bond strength evaluation. Four-mm-thick composite resin discs, 12 mm in diameter, were prepared by layering 2-mm-thick increments of a microhybrid composite resin (Filtek Z250, shade A1; 3M ESPE) into a silicone mold. Each increment was light activated (700 mW/mm^2) for 40 seconds with a halogen light (Optilux 501; Kerr Corp, Orange, Calif, USA). One side of the composite resin discs was abraded with 600-grit SiC paper under water cooling to create a flat surface with standardized roughness. The composite surface was airborne-particle abraded with 50-µm aluminum oxide particles (Asfer Ind. Quím Ltda, São Caetano do Sul, SP, Brazil) for 10 seconds. Before luting procedures were performed, the composite resin discs were

ultrasonically cleaned in distilled water for 10 minutes, rinsed with running water, air dried, and silanated (RelyX Ceramic Primer; 3M ESPE).

After application of the luting agent according to the manufacturer's instructions, the composite resin disc was pressed on the cement using proper digital pressure, after which excess cement was removed. Specimens were light activated for 40 seconds with the same halogen light from the buccal, lingual, and occlusal directions. Bonded specimens were stored in distilled water for 24 hours and the specimens submitted to SPP were under constant pulpal pressure.

Luting procedures for nanoleackage analysis

Two specimens were prepared for each group. After luting agents were mixed and applied onto flat dentin surfaces, a polyester strip was placed over the luting agent and a glass plate was used to apply proper digital pressure while the luting agent was light-activated for 40s with a halogen light (Optilux 501; Demetron/Kerr, Danbury, Conn, USA). Next, a thin layer of a low-viscosity resin composite (Clearfil Majesty Flow, Kuraray Med. Inc, Okayama, Kurashiki, Japan) was applied and light-activated for 40 seconds. After similar storage conditions described above, teeth were sectioned perpendicular to the adhesive-tooth interface into 0.9 mm thick slabs using a diamond saw (Isomet 1000; Buehler Ltda, Lake Bluff, Ill, USA).

Bonded slabs were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. In order to rehydrate specimens and avoid desiccation artefacts (Agee et al., 2003), they were immersed in distilled water for 20 min prior to immersion in the tracer solution for 24 h. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al. (2002b). Tooth slabs were placed in the tracer solution in total darkness for 24 h, rinsed thoroughly in distilled water and immersed in a photodeveloping solution for 8h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the interface.

Scanning Electron Microscopy

Specimens were fixed in Karnovsky's solution and embedded in epoxy resin (Epoxycure, Buehler Ltd, Lake Bluff, IL, USA). Afterwards, they were polished with

400, 600, 1200 and 2400-grit SiC paper and 6, 3, 1 and 0.25 μm diamond paste (Arotec, Cotia, SP, Brazil). Then, specimens were dehydrated in ascending ethanol series, and coated with carbon (MED 010, Balzers Union, Balzers, Liechtenstein). Resin-dentin interfaces were observed with a scanning electron microscope (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, United Kingdom) operated in the backscattered electron mode. After SEM analysis, representative leakage patterns at the cement-dentin interfaces for each system were photographed at 500x magnification.

Microtensile bond strength evaluation

Teeth were serially sectioned perpendicular to the adhesive-tooth interface into slabs, and the slabs into beams with a cross-sectional bonded area of approximately 1 mm2 using a diamond saw (ISOMET 1000; Buehler Ltd, Lake Bluff, Ill)(Pashley et al., 1999). Beams were fixed to the grips of a universal testing machine (EZ Test; Shimadzu Corp, Kyoto, Japan) using a cyanoacrylate adhesive (Loctite Super Bonder Gel; Henkel, Düsseldorf, Germany) and tested in tension at a crosshead speed of 1 mm/min until fracture. Maximum tensile load was divided by specimen cross-sectional area to express results in units of stress (MPa). Five beams were selected from each restored tooth, and the average value for each tooth was used in the calculations. Bond strength values were statistically evaluated using a 2-way ANOVA and the Tukey's studentized range HSD test (α=.05). Pretest failures were not included in the statistical analysis (Pashley et al., 1999; Reis et al., 2003). Statistical analyses were performed using a statistical software program (SAS for Windows V8; SAS Institute, Inc, Cary, NC). Failure modes were determined by examination of fractured specimens with a scanning electron microscope (SEM) (LEO 435 VP; LEO Electron Microscopy Ltd, Cambridge, UK). Specimens were mounted on aluminum stubs and gold-sputter coated (MED 010; BAL-TEC AG, Balzers, Liechtenstein) prior to viewing at different magnifications. Failure mode at the fractured interface was classified into 1 of 4 types: CD (cohesive failure in Dentin), AD (adhesive failure between hybrid layer and dentin), CC (cohesive failure in the cement), or ADR (adhesive failure between the luting agent and composite

resin). Instead of classifying failures as mixed, the area percentage of each type of failure in each specimen was recorded.

RESULTS

Microtensile bond strength

Mean (SD) μTBS values are presented in Table 2. The ANOVA revealed a significant difference among groups (df=50; F=25; P<.01) and interaction between resin cements and pulpal pressure condition. Without SPP, RX and PS presented the highest bond strength values (Table 2). PF, UN, UC and SA did not present significant differences. With SPP the highest μTBS values were obtain by RX, PS and UC, which did not differ among them. However, there were no differences between UC, UN and SA. PF obtained the lowest bond strength values, but it was not significantly different from UN and SA.

For RX, the SPP significantly reduced bond strength. UN, SA and PS were not influenced by SPP. Even though no significant difference was detected for PF when SPP was applied, a considerable reduction in bond strength was recorded. On the other hand, the self-adhesive cement UC was positively influenced by SPP, resulting in significantly higher bond strength values.

Distribution of failure modes is presented in Figure 1. The predominant type of failure for RX and PS was cohesive failure in resin cement, independent of the application of pulpal pressure (Figs. 2 and 3). On the other hand, the predominant failure mode for all other groups was adhesive between dentin and the luting agent. A similar adhesive failure pattern was observed for UC and UN groups. On the resin side of the fractured beams an irregular globular structures were observed (Fig. 4).

Nanoleakage

SPP increased nanoleakage in all groups, except for the self-adhesive cements UN and UC. When RX was applied without SPP silver deposition was observed in some regions within the hybrid layer (Fig. 7A). When SPP was applied, increased silver deposition was observed. RX with SPP revealed silver deposition within the entire thickness of the hybrid layer and tags (Fig 7B). PS with or without SPP presented small silver deposition at the base of the hybrid layer (Fig 7C and D).

Some regions of the interface produced by PF without SPP presented adhesive layer, hybrid layer and tags completely impregnated by silver (Fig.7E). PF with SPP presented gaps between cement layer and dentin (Fig.7F). In this group isolated areas of silver impregnation were observed between cement and resin (Fig 7F and G).

The self-adhesive cements presented lower silver impregnation than the other cement systems (Fig. 8). UN and UC presented reduction on silver deposition when submitted to SPP. In UN and UC without SPP, little silver deposition was observed at the cement-dentin interface (Fig. 8A and C). When they were submitted to SPP, silver deposition was observed in a few regions (Fig. 8B and D). SA presented lower silver impregnation in the absence of SPP (Fig. 8E). However, the nanoleakage pattern with SPP was similar to UN and UC without SPP (Fig. 8F).

DISCUSSION

In this study, diverse current types of resin cement systems were tested. RX uses an etch-and-rinse adhesive as pre-treatment of dentin. Thus, this material removes the smear layer together with mineral of underlying dentin using a phosphoric acidic solution. After this procedure, dentin permeability is drastically increased and an outward fluid flow is constant (Hashimoto et al., 2004; Ozok et al., 2004). In the present study, the etch-and-rinse group presented decreased bond strength when it was submitted to SPP (Table 2). This fact can be related to the diffusion of water into the adhesive layer, reducing the mechanical properties of the polymer matrix by swelling and reducing the frictional force between the polymer chains, a process known as ‘plasticization’ (Ito et al., 2005; Reis et al., 2004b; Yiu et al., 2004). Potential water-binding domains within hybrid layers and adhesive layers in resin-dentin interfaces are traced by ammoniacal silver nitrate (Reis et al., 2007a; Tay et al., 2002b). Backscattered SEM micrographs of the interface of conventional cements revealed silver deposition within the hybrid layer (Fig. 7). The differences among adhesives nanoleakage patterns is due to differences in hydrophilicity and water content (Reis et al., 2007a). Even in the absence of SPP, all conventional cements systems presented a certain degree of nanoleakage, mainly within the

hybrid layer (Fig. 7A, C and E). It depends on the adhesive used, their mode of application and composition. The presence of water within dentin adhesives composition plays an important role in both total- and self-etching techniques. Water-based adhesives used in total-etch systems have been shown to solvate dried matrices, being able to re-expand dentin collagen (Pashley et al., 2002). Probably, the water is not eliminated during adhesive procedures, producing the nanoleakage pattern observed on Figure 7A. Besides the presence of water in their composition, the two-step etch-and-rinse adhesive produce a semi permeable membrane due its high concentration of hydrophilic monomers and solvents (Hashimoto et al., 2004; Tay et al., 2002a). Hydrophilic resin monomers attract water molecules permit movement of water molecules from dentin across adhesive layer through formation of water channels (Hashimoto et al., 2006; Sauro et al., 2007). Figure 7B showed evident increase in nanoleakage, probably due to fluid flow produced by SPP. This water-filled channel has been considered a site of hydrolytic degradation and crack propagation points during bond strength testing (De Munck et al., 2003; De Munck et al., 2005).

Another phenomenon that contributes to reduction of bond strength is water diffusion during the slow setting process of these resin cements from de underlying hydrated dentin structure across the polymerized hydrophilic adhesive layer via an osmotic gradient (Tay et al., 2002a). According to Hiraishi et al. (2009), continuous water uptake via adhesive layer could result in an unstable porous region, increasing the degradation along the interface between the adhesive and resin cement, turning this bonding interface into a weak link when pulpal pressure is simulated (Carvalho et al., 2004; Tay et al., 2003). Porous regions were observed in fractured specimens of RX with SPP, suggesting the presence of water channels (Fig 2). In higher magnification non-uniform globular structures were identified, which might suggest a poor polymerization (Sigemori et al., 2005). Besides, backscattered SEM micrographs of PF submitted to SPP revealed silver deposition between adhesive cement and resin (Fig 7F and G), suggesting permeability within this system.

Even though no significant difference was detected for PF when SPP was applied, a considerable reduction in bond strength was recorded. Previous studies have reported a negative influence of pulpal pressure for Panavia F (Carvalho et al.,

2004; Hiraishi et al., 2009). When pulpal pressure was simulated, PF presented the lowest bond strengths. This performance is probably associated with the higher concentration of hydrophilic and ionic resin monomers in ED Primer, resulting in the formation of a highly permeable layer after polymerization (Mak et al., 2002; Tay et al., 2002a). According to Hiraishi,et al. (2009), the primed dentin is highly permeable, allowing water to diffuse from dentin across the hybrid layer, and form water droplets in the interface of dentin-resin cement (Fig. 7F), resulting in low bond strengths. Hydrophilic monomers, such as HEMA, produce more water attraction and can result in a reduction in the degree of polymerization of the resin cement, reducing mechanical properties (Mak et al., 2002). Besides, water is an essential component in self-etching systems, in order to enable ionization of acidic monomers and demineralization of underlying enamel and/or dentin (Tay & Pashley, 2001). This statement can be confirmed by Figure 7E, showing high amounts of silver deposition, even without SPP. According to Hiraishi et al (2005), for ideal bonding performance, the water concentration must be sufficient to provide adequate ionization of the acidic monomers, but without lowering the resin concentration too much to optimize their bonding efficacy to dentin. The high water concentration at the interface during polymerization setting, probably contributed with the reduction in monomer concentration when PF was submitted to SPP. This fact can explain lower bond strength associated with gap formation at the interface (Fig. 7F). The failure modes for PF were exclusively adhesive between resin cement and dentin. However, the fracture of SPP occurred frequently on hybrid layer, where it was not possible to visualize the tubular lumen (Fig. 7), suggesting a poorly cured adhesive layer. On the other hand, the fracture of non SPP occurred at the base of the hybrid layer and exposed collagen fibrils can be observed (Fig. 6).

The lower bond strengths observed by Panavia F, in comparison with Panavia F + Clearfil SE Bond, with or without SPP, probably occurred because PF uses a 1-step self-etching dual-polymerizing primer (ED Primer)(Mak et al., 2002). It has been reported that inhibition of the polymerization of the luting agent (Panavia F) could occur due to the presence of acidic monomers within the ED Primer composition (Mak et al., 2002). However, as light activation was performed immediately upon luting, this effect is probably negligible (De Munck et al., 2004). When Clearfil SE Bond was used prior to the application of the Panavia F system, increased bond

strengths were observed. No significant difference was observed between PS and RX. However, differently from RX, the bond strength of PS group was not influenced by SPP. Only a very small layer of silver deposition was observed, even with SPP. (Fig. 7C and D). These observations can be attributed to the hydrophobic, filled adhesive layer that is applied over the self-etching primer and polymerized before application of ED Primer and the luting agent itself. This hydrophobic layer can reduce permeability of dentinal fluids between dentin and the luting agent (Brackett et al., 2005; Carvalho et al., 2004; King et al., 2005). In addition, the presence of smear plugs are very important to reduce significantly the rate of fluid flow through the interface even in the presence of intrapulpal pressure, “in vivo” and “in vitro” (Hashimoto et al., 2004; Hebling et al., 2007; Sauro et al., 2007). Direct light activation of the adhesive system probably resulted in a better monomer conversion within the hybrid and adhesive layers, resulting in higher bond strengths. In the PS group, ED Primer was applied over Clearfil SE Bond to assist in the chemical polymerization of the luting agent. It might be expected that the acidity of ED Primer would not be buffered, as it did not contact the mineralized dentin surface; however, the presence of aromatic sodium sulphinate salts probably reduced the concentration of acidic monomers (De Munck et al., 2004). The presence of silver deposition on base of PS hybrid layer (Fig. 7C and 7D) can be explained by intrinsic water permanence, high hydrophilic primer composition or a continuous demineralization, even after polymerization (Reis et al., 2007b)

Three different self-adhesive materials were used in this study. The self-adhesive cements RelyX U100 (UC) and RelyX Unicem (UN) were developed by the same manufacturer and are marketed under the same name in some countries. According to the manufacturer, the only difference between these products is the delivery system. While UN requires an activator, triturator, and applicator, UC can be hand mixed. Another self-adhesive cement used was Clearfil SA Luting, that needs to be hand mixed. Without SPP, self-adhesive cements presented significantly lower values than the multistep systems, except for PF, which was significantly similar (Table1). When submitted to SPP self-adhesives maintained their bond strength, except for UC that presented increased bond strengths. UC bond strength values were significantly higher than PF, and it was not significantly different from the multi-

step systems PS and RX. In addition, it was not significantly different from the self-adhesive cements UN and SA.

The low initial pH of UN and UC (pH<2 in the first minute, according to the manufacturer), and SA (pH 2-3 in the first minute, according to the manufacturer), produces almost no demineralization and hybrid layer formation on dentin surface (De Munck et al., 2004; de Souza Costa et al., 2005; Goracci et al., 2006; Monticelli et al., 2008; Yang et al., 2006). This finding might be attributed to the high cement viscosity, which hinders the wetting and infiltrating of the dentin surface by the luting agent (De Munck et al., 2004). The favorable bond strength and very low silver impregnation (Fig. 8) observed for self-adhesive has been attributed to the micromechanical retention and chemical interaction between monomer acidic phosphate groups and dentin/enamel hydroxyapatite (Hiraishi et al., 2009; Mazzitelli et al., 2008; Radovic et al., 2008).

The primary polymerization can be initiated by either light exposure or an autopolymerizing reaction. The polymerization mechanism results in a highly crosslinked polymer with high molecular weight (Radovic et al., 2008). Furthermore, an increase in pH from 1 to 7 is observed as a consequence of the reaction between phosphate groups and both alkaline filler particles and hydroxyapatite from enamel and dentin, to neutralize resin acidity (Han et al., 2007; Reich et al., 2005). The pH neutralization results in water formation and a more hydrophilic cement, which enhances the cement's wetting ability on the dentin surface and the cement tolerance to water. Water is crucial for self-adhesive luting agents to release hydrogen ions required for smear layer demineralization (Moszner et al., 2005), and is also reused in the reaction between multifunctional acidic phosphate monomers and alkaline filler particles. Such a phenomenon is speculated to be responsible for a change in the nature of the cement from hydrophilic to hydrophobic, which is thought to improve adhesive stability. When SPP is applied, water transudation increases acidic monomers aggressiveness, improving smear layer dissolution and dentin demineralization (Hiraishi et al., 2005). It also optimizes these acid-basic reactions allowing better setting (Mazzitelli et al., 2008). This possible improvement produced by water was evidenced by the bond strength values and nanoleakage patterns observed when UC was submitted to SPP (Fig. 8D). The bond strength of SA was

not statistically different when submitted to SPP and its silver deposition was more evident. This can be justified by difference in chemical composition of the materials (Table 1). However, SA presented an increase of cohesive fractures in the resin cement, which means that its bond strength was superior than the cement cohesive strength (Figure 1). During failure mode analysis of UC and UN, the presence of a globular structure was observed on the resin side of fractured beans. These structures were frequent in adhesive fractures (Fig.4 and Fig. 8A, B, C and D). These are probably a result of incorporation of voids during mixture of the cements.

Within the limits of this study, it may be concluded that the influence of SPP was material dependent. The SPP negatively bond strength and nanoleakage for RX, that uses a two step etch-and-rinse adhesive system and PF that uses a single-step self-etching primer. The use of a two-step self-etching adhesive reduced the effect of SPP for PF, improving bond strength and reducing silver deposition. The immediate bond strength and nanoleakage of self-adhesive luting agents was not influenced by SPP.

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TABLES AND FIGURES

Table 1. Cements, lot number, manufacturers, delivery system, composition, and application technique

Type	Manufacturers (lot number)	Delivery system (cement)	Composition	Application technique
Dual-polymerizing resin cement + 2-step etch-and-rinse adhesive system	RelyX ARC (GEHG) + Adper Single Bond 2 (7MY) 3M ESPE, St. Paul, Minn	Automatic dispenser, 2 pastes, hand mixed for 10 s	Cement: bis-GMA, TEGDMA polymer, zirconia/silica filler Etchant: 35% H_3PO_4 Adhesive: bis-GMA, HEMA, UDMA, dimethacrylates,ethanol, water, camphorquinone , photoinitiators, polyalkenoic acid copolymer, 5-nm silica particles	a (15 s); b (15 s); c; d; e; i (10 s); mix cement; apply mixture
Dual-polymerizing resin cement + 1-step self-etching adhesive	Panavia F (paste A, 00249D; paste B, 0027A) + ED Primer (primer A, 00262A; primer B, 00137A) Kuraray Medical, Inc, Tokyo, Japan	One-step self-etching adhesive + resin cement, dual polymerizing 2 pastes, hand mixed	Primer A: HEMA, 10-MDP, 5-NMSA, water, accelerator Primer B: 5-NMSA, accelerator, water, sodium benzene sulphinate Paste A: 10-MDP, silanated silica, hydrophobic aromatic and aliphatic dimethacrylate, hydrophilic dimethacrylate photoinitiator, dibenzoyl peroxide Paste B: silanated barium glass, sodium fluoride, sodium aromatic sulfinate, dimethacrylate monomer, BPO	h (A+B) (leave undisturbed for 60 s); mix cement; apply mixture; i (40s)
Dual-polymerizing resin cement + 2-step self-etching adhesive system	Panavia F (paste A, 00249D; paste B, 0027A) Clearfil SE Bond (00788A) Kuraray Medical, Inc	Two-step self-etching adhesive + ED Primer + resin cement, dual polymerizing 2 pastes, hand mixed	Primer: MDP, HEMA, hydrophilic dimethacrylate, dl-camphorquinone, N,N-diethanol p-toluidine, water Bond: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, N, N-diethanol p-toluidine, silanated colloidal silica Paste A and Paste B: as described above	f (20 s); e; g; i (10 s); h (ED Primer); e; mix cement; apply mixture; i (40 s)
Dual-polymerizing self-adhesive resin cement	RelyX U100 (366321) 3M ESPE	Clicker dispenser 2 pastes, hand mixed	Base: glass fiber, methacrylated phosphoric acid esters, dimethacrylates, silanated sílica, sodium persulfate Catalyst: glass fiber, dimethacrylates, silanated sílica, p-toluene sodium sulfate, calcium hydroxide	Mix cement; apply mixture; i (40 s)
Dual-polymerizing self-adhesive resin cement	RelyX Unicem (365979) 3M ESPE	Capsules, mechanically mixed for 10 s	*Powder:* glass powder, silica, calcium hydroxide, self-curing initiators, pigments, light-curing initiators, substituted pyrimidine, peroxy compound. *Liquid:* methacrylated phosphoric esters, dimethacrylates, acetate, stabilizers, self-curing initiators, light-curing initiators	Automix cement; apply mixture i (40 s)
Dual-polymerizing self-adhesive resin cement	Clearfil SA luting (008AA) Kuraray Medical, Inc	Dual polymerizing 2 pastes, hand mixed	Paste A - MDP, Bis-GMA, TEGDMA, Hydrophobic aromatic dimethacrylate dl-Camphorquinone, Benzoyl peroxide, Initiator, Silanated barium glass filler, Silanated colloidal silica. Paste B - Bis-GMA, Hydrophobic aromatic dimethacrylate, Hydrophobic aliphatic dimethacrylate, Accelerators, Pigments, Surface treated sodium fluoride, Silanated barium glass filler, Silanated colloidal silica.	Automix cement; apply mixture i (40 s)

Application technique = a: acid etch; b: rinse surface; c: dry with cotton pellet; d: apply 1-bottle adhesive; e: gently air dry; f: apply primer; g: apply adhesive; h: apply mixture; i: light polymerize; j: autopolymerize. Bis-GMA: bisphenol A diglycidyl ether methacrylate; 4-META: 4-methacryloyloxyethyl trimellitate anhydride; UDMA: urethane dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; 10-MDP: 10-methacryloxydecyl dihydrogen phosphate; 5-NMSA: N-methacryloxyl-5-aminosalicylic acid; TEGDMA: triethylene glycol dimethacrylate.

Table 2 – Dentin bond strength values (MPa) for different resin cements systems

Materials	Product type	No pulpal presure (NP)		Pulpal pressure(P)	
		Mean (SD)	Tukey	Mean (SD)	Tukey
RELYX ARC (RX)	Two-step etch-and-rinse adhesive/resin cement	53.0 (8.6)	Aa	34.8 (11.3)	Ab
PANAVIA F + CLEARFIL SE BOND (PS)	Two-step self-etching adhesive/resin cement	45.5 (6.9)	Aa	38.0 (10.2)	Aa
PANAVIA F + ED PRIMER (PF)	One-step self-etching adhesive/resin cement	14.1 (4.6)	Ba	7.8 (1.4)	Ca
RELYX U100 (UC)	Self-adhesive cement	14.2 (5.6)	Ba	24.2 (2.3)	ABb
RELYX UNICEM (UN)	Self-adhesive cement	17.5 (7.4)	Ba	20.4 (7.6)	BCa
CLEARFIL SA LUTING (SA)	Self-adhesive cement	13.1 (11.1)	Ba	14.3 (5.3)	BCa

Values are means (SD) in MPa. Different letters (upper case – within column, lower case – within row) are significantly different by Tukey test.

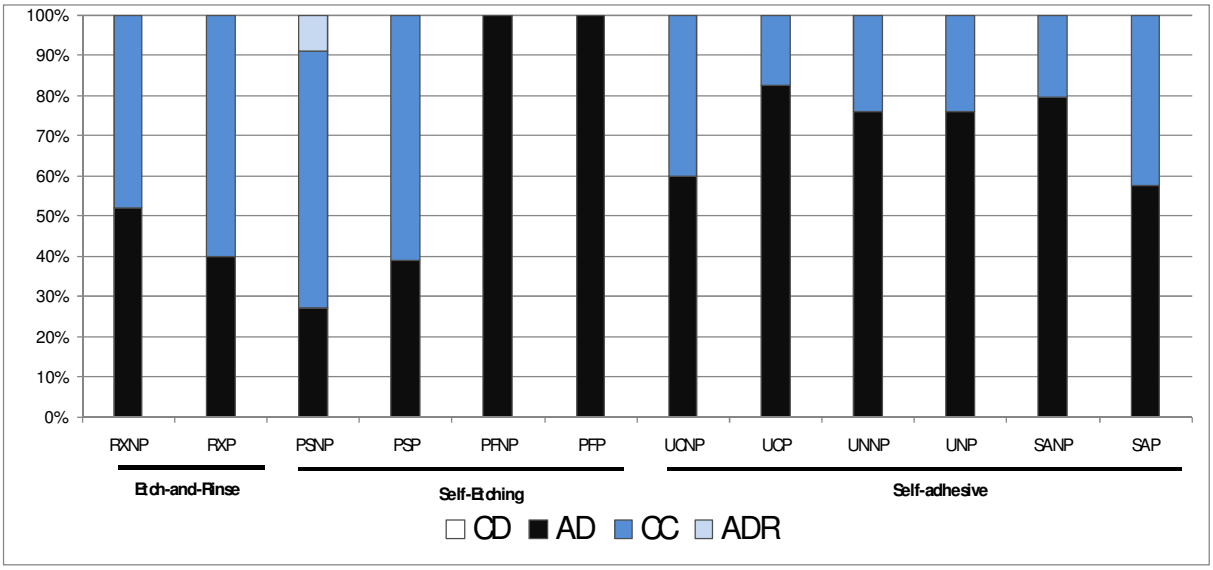

Figure 1 - Distribution of failure modes within groups. CD, cohesive failure in dentin; AD, adhesive failure between dentin and luting agent; CC, cohesive failure in resin cement; ADR, adhesive failure between luting agent and composite resin.

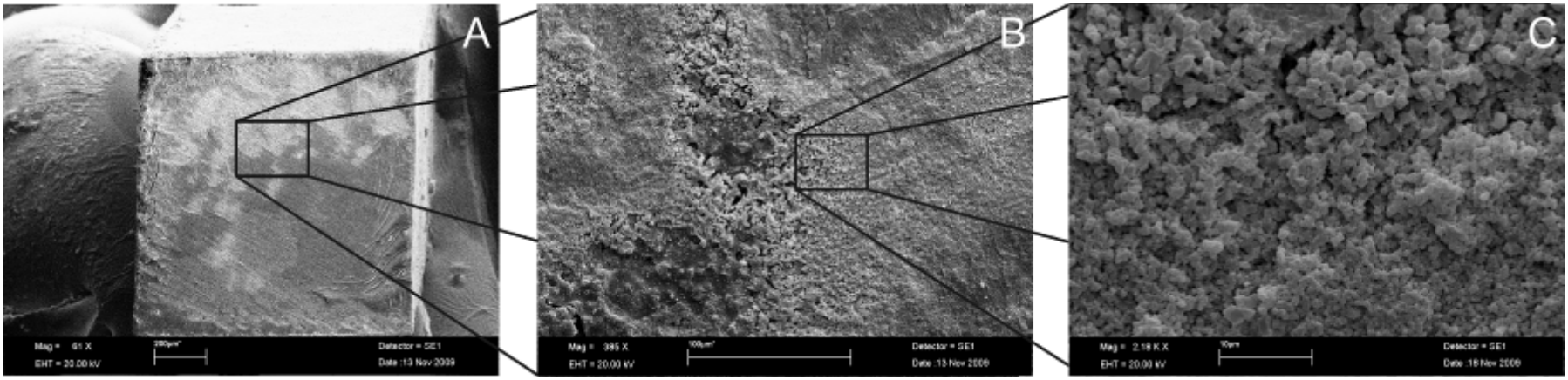

Figure 2 - Representative SEMs of the most predominant failure mode of RX submitted to SPP. (A) Cohesive failure in the cement is observed for RX. (B) At higher magnification, non-uniform globular structures were identified.

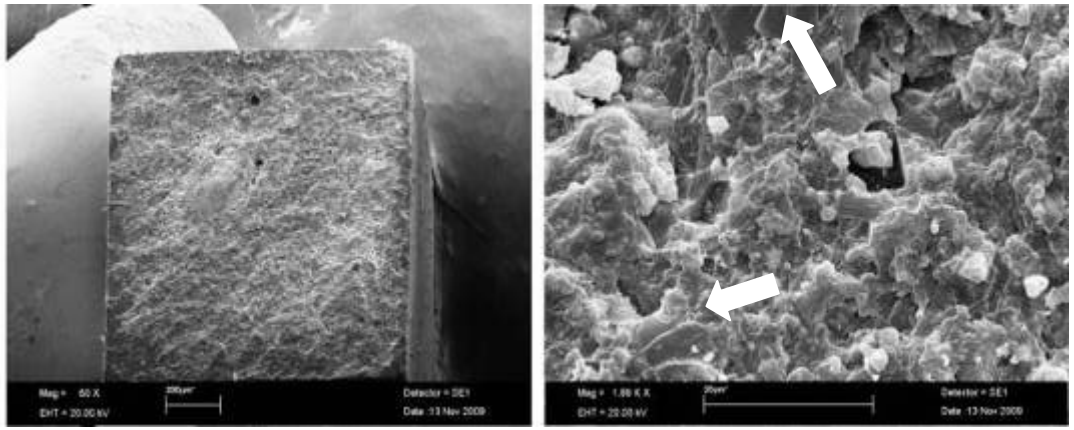

Figure 3 - Representative SEMs of the most predominant failure mode of PS non SPP. Cohesive failure in the cement is observed for PS. (B) At higher magnification, filler particles of the resin cement can be observed (arrows).

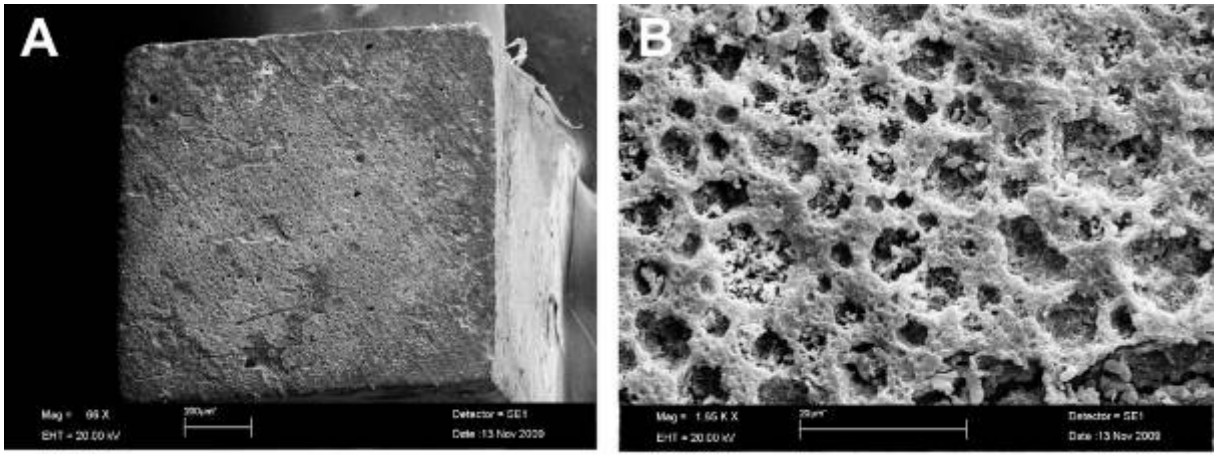

Figure 4 – Representative SEMs of the most predominant failure mode of UN non SPP. (A and B) On the resin side of the fractured beams an irregular globular structure was observed.

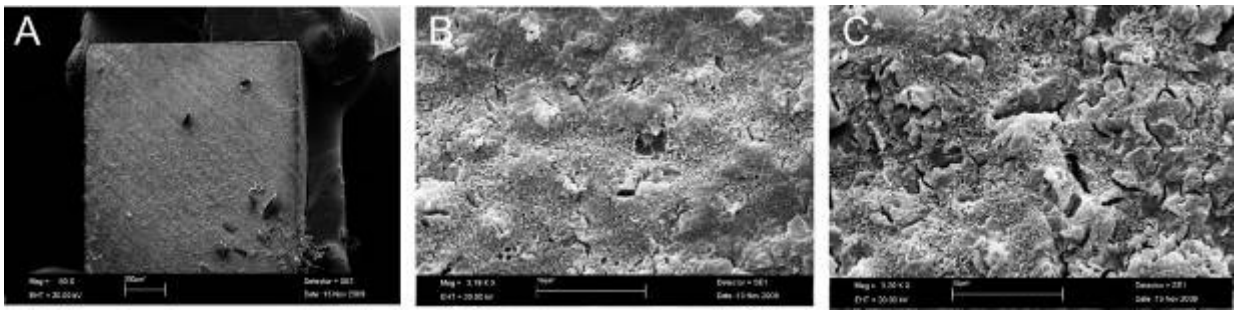

Figure 5 – Representative SEMs of the most predominant failure mode for PF on SPP group. (B and C) At higher magnification, porosity was observed at the surface .

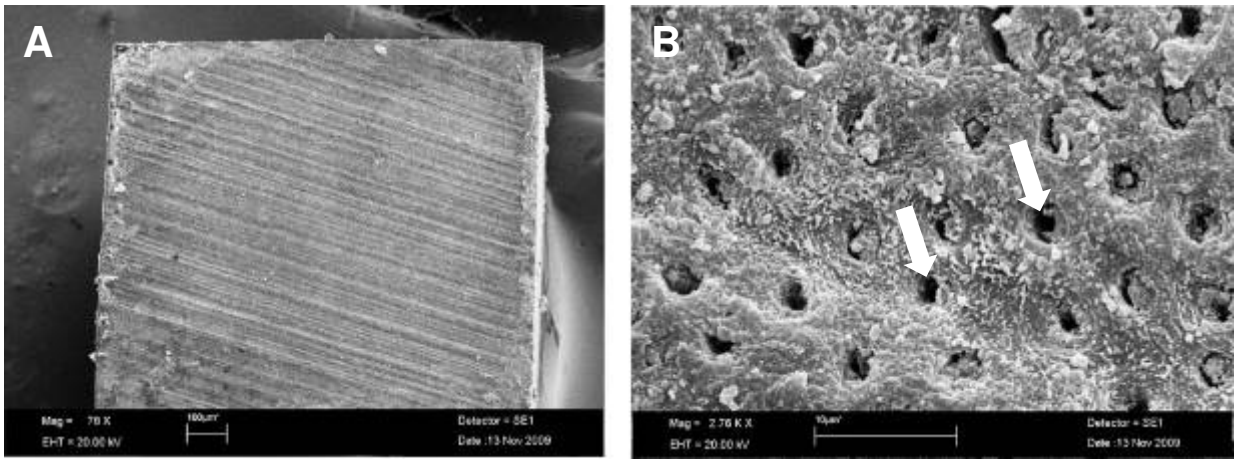

Figure 6 – Representative SEMs of the most predominant failure mode for PF without SPP group. At higher magnification, the fracture occurred at the base of the hybrid layer. Exposed collagen fibrils and lumen of dentin tubules can be observed (arrows).

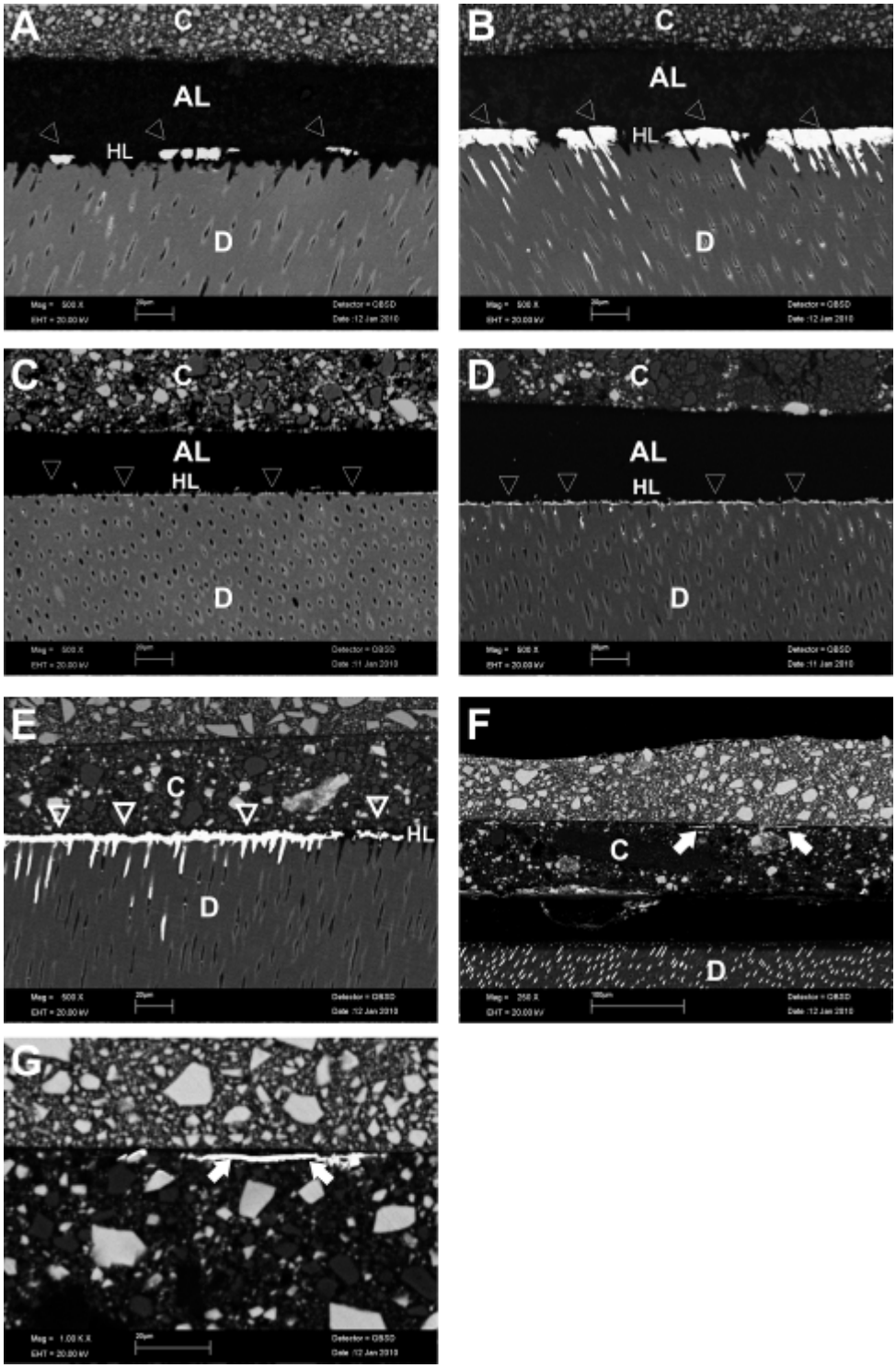

Figure 7 – Backscattered SEM micrographs of resin-dentin interfaces produced by conventional resin cements. A - RX nanoleakage pattern without SPP. B - RX nanoleakage pattern with SPP. C and D - PS without and with SPP silver deposition observed at the base of the hybrid layer, respectively. E - The adhesive layer of PF without SPP was highly silver impregnated. F - Gap formed on the cement-dentin interface when PF was submitted to SPP and silver deposition between cement and resin (white arrows). G - Higher magnification micrographs of PF with SPP. C – cement; AL – adhesive layer; D – dentin.

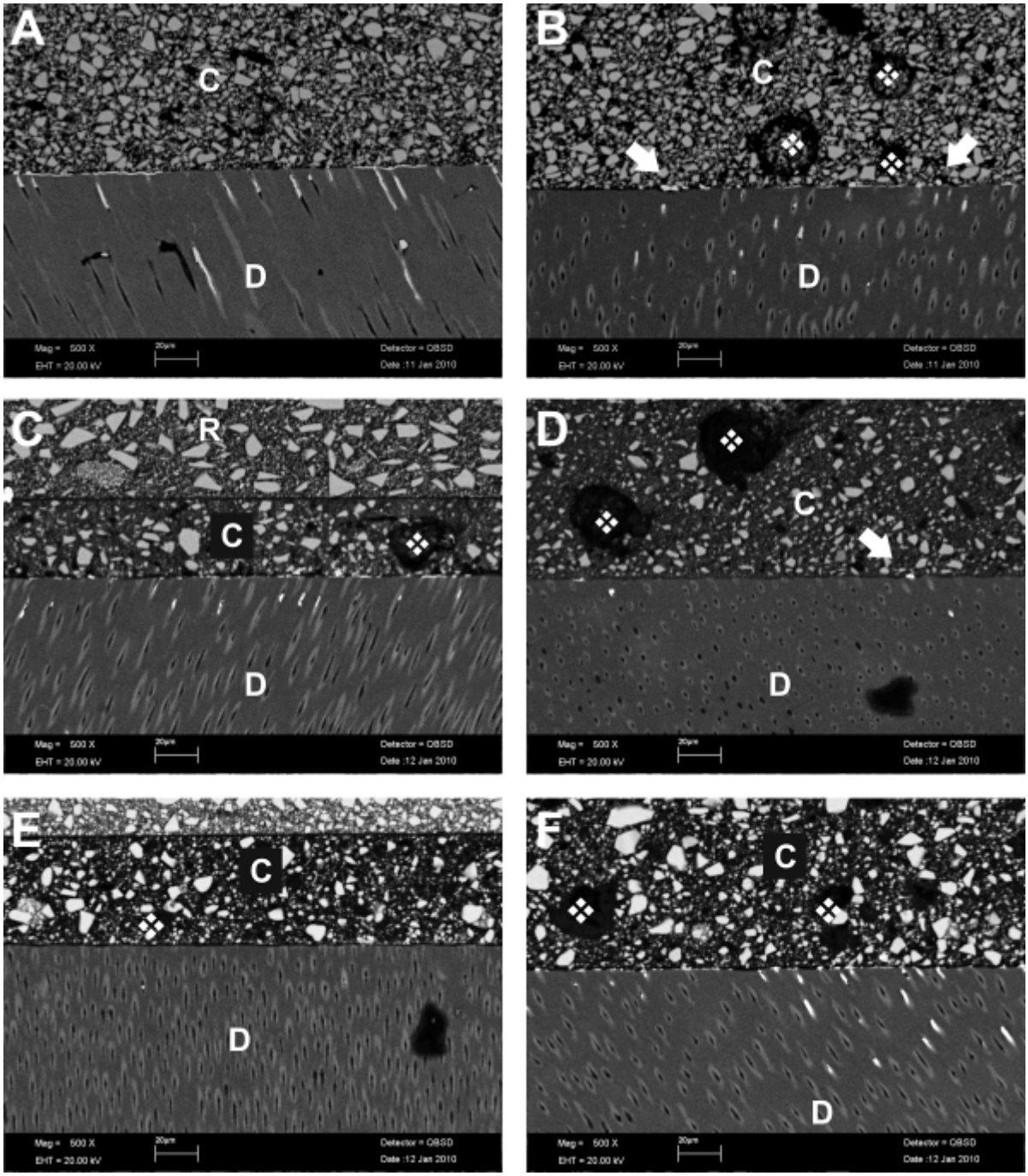

Figure 8 - Backscattered SEM micrographs of the interface produced by self-adhesive cements. A and B - Nanoleakage pattern produced by UN without and with SPP, respectively. C and D - Silver deposits observed at the cement-dentin interface of UC without and with SPP, respectively. E and F - SA interface without and with SPP. (White arrows – silver deposition); (❖ - avoids without silver impregnation)

4. CONCLUSÃO

Com base nos resultados obtidos neste estudo pode ser concluído que:

- a influência da pressão pulpar foi material dependente.

- a simulação da pressão pulpar apresentou efeito deletério à resistência de união e nanoinfiltração de cimentos convencionais que empregam adesivos com condicionamento ácido total prévio à sua aplicação e adesivos autocondicionantes de um passo;

- a resistência de união dos cimentos auto-adesivos testados não sofreu influência negativa da simulação da pressão pulpar;

- a utilização de um adesivo autocondicionante de dois passos aumenta a resistência de união e pode reduzir os efeitos da pressão pulpar para o PF;

- os cimentos auto-adesivos testados são menos susceptíveis à nanoinfiltração do que os cimentos resinosos convencionais.

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6-ANEXO

UnG
CEPPE
Centro de Pós-Graduação, Pesquisa e Extensão

Guarulhos, 07 de outubro de 2008.

Exmo. Sr.
Prof. André F. Reis

PARECER Nº 94/2008

Referência: **Aprovação de Projeto**
SISNEP/384 - "Efeito 'in vitro' da pressão pulpar na resistência de união, nanoinfiltração e características ultramorfológicas das interfaces de união resina-dentina produzidas por cimentos auto-adesivos ao longo do tempo"

O Comitê de Ética em Pesquisa da Universidade Guarulhos analisou o Projeto de Pesquisa de sua autoria "Efeito 'in vitro' da pressão pulpar na resistência de união, nanoinfiltração e características ultramorfológicas das interfaces de união resina-dentina produzidas por cimentos auto-adesivos ao longo do tempo" - SISNEP/384, na reunião de 07.10.2008, e no uso das competências definidas na Res. CNS 196/96, considerou o Projeto acima **aprovado.**

As orientações abaixo devem ser consideradas pelo Pesquisador Responsável durante a realização da pesquisa, visando que a mesma se desenvolva respeitando os padrões éticos:

- O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado.
- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou, aguardando seu parecer, exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade de regime oferecido a um dos grupos da pesquisa que requeiram ação imediata.
- Eventuais modificações ou emendas e eventos adversos ao protocolo, devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas.
- Esclarecemos a necessidade da apresentação de relatório de andamento até **15.02.10** e relatório final até **15.02.11.**

Luciene Cristina de Figueiredo
Coordenadora do Comitê de Ética em Pesquisa