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CARLOS EDUARDO PENA

AVALIAÇÃO ULTRAMORFOLÓGICA EM MICROSCOPIA ELETRÔNICA DE TRANSMISSÃO E VARREDURA DA INTERFACE DE UNIÃO COMPÓSITO-DENTINA PRODUZIDA POR DIFERENTES CIMENTOS RESINOSOS CONVENCIONAIS E AUTO-ADESIVOS

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Co-orientador: Prof. Dr. Marcelo Giannini

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3. Prof. Dr. Cesar Augusto Galvão Arrais (UnG)_____

Guarulhos, 05 de Março de 2009.

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“...para pedir à Deus deve-se unir as mãos, mas não cruzar os braços.”

Padre Gervázio

RESUMO

Objetivo: O propósito deste estudo foi avaliar ultramorfologicamente em Microscopia Eletrônica de Varredura (MEV) e Transmissão (MET) as interfaces resina-dentina produzidas por diferentes cimentos resinosos auto-adesivos submetidos a um protocolo de nanoinfiltração, e compará-los à cimentos resinosos convencionais. **Materiais e métodos:** Foram utilizados 6 cimentos auto-adesivos: RelyX Unicem (UN), RelyX U100 (UC), SmartCem 2 (SC), G-Cem (GC), Maxcem (MC), Set (SET); e dois cimentos resinosos convencionais, sendo que um utiliza um adesivo de dois passos com condicionamento ácido prévio (Rely X ARC - RX) e um que utiliza um adesivo auto-condicionante de passo único (Panavia F - PF) . Um grupo adicional foi realizado utilizando um adesivo auto-condicionante de dois passos (Clearfil SE Bond) previamente à aplicação do cimento Panavia F (PS). Foram utilizados 36 terceiros molares distribuídos em 9 grupos de acordo com o material de cimentação. Após 24 horas de armazenamento em água, os dentes restaurados foram seccionados em fatias de 0,9 mm, as quais foram submetidas ao protocolo de nanoinfiltração em $AgNO_3$ e observadas em MET e MEV. **Resultados:** Diferentes padrões de nanoinfiltração foram observados para os diferentes grupos. Os cimentos resinosos auto-adesivos UC e UN apresentaram pequena ou nenhuma deposição de prata, comparáveis aos cimentos resinosos convencionais, enquanto MC, SC e SET não resistiram aos procedimentos de preparo dos espécimes. GC apresentou considerável deposição de prata ao longo da interface. O grupo adicional PS não apresentou diferenças do grupo original PF. **Conclusões:** As interfaces resina-dentina produzidas pelos cimentos auto-adesivos apresentaram diferentes características entre si e também diferiram dos cimentos resinosos convencionais.

Palavras chave: cimentos auto-adesivos, cimentos resinosos, microscopia eletrônica de transmissão, microscopia eletrônica de varredura, nanoinfiltração.

ABSTRACT

Objectives: The aim of this study was to evaluate the ultramorphology of the resin-dentin interfaces produced by different self-adhesive cements submitted to a nanoleakage protocol by means of Transmission (TEM) and Scanning Electron Microscopy (SEM), and to compare them with conventional resin cements. **Materials and Methods:** Six self-adhesive cements were used in the present study: RelyX Unicem (UN), RelyX U100 (UC), SmartCem 2 (SC), G-Cem (GC), Maxcem (MC), Set (SET); and two conventional resin cements: 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). An additional group included the use of a 2-step self-etching adhesive system (Clearfil SE Bond) prior to the application of Panavia F (PS). Thirty-six human molars were assigned to 9 groups according to the luting material. After 24h of water-storage, restored teeth were serially sectioned into 0.9 mm-thick slabs, which were submitted to a nanoleakage protocol with $AgNO_3$ and observed in the TEM or SEM. **Results:** Different nanoleakage patterns were observed for the different groups. UC and UN self-adhesive resin cements presented little or no silver deposition, comparable to the conventional resin cements, while MC, SC and SET did not resist specimen preparation procedures. GC presented remarkable silver deposition along the interface. The additional group PS did not present differences from the original group PF. **Conclusions:** The characteristics of the resin-dentin interfaces were rather product-dependent than related to the type of resin cement.

Keywords: self-adhesive cements, resin cements, TEM, SEM, nanoleakage

SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

Atualmente, os clínicos e pacientes vêm apresentando um interesse crescente pela Odontologia estética, pois dentes atraentes são considerados um fator importante na aparência pessoal. As restaurações estéticas podem ser empregadas para a restauração ou recontorno estético dos dentes, necessitando pouco ou nenhum desgaste de estrutura dental sadia. Avanços na Odontologia Adesiva permitem ao dentista melhorar a estética dental com relativa simplicidade e de forma conservadora (VAN MEERBEEK et al., 1998; MJÖR et al., 2000).

As restaurações adesivas apresentam inúmeras vantagens sobre as restaurações não-adesivas. Tradicionalmente, a retenção e estabilização das restaurações requeriam remoção de grande quantidade de tecido dental sadio. Além de permitir um preparo cavitário conservador a adesão também reduz a micro-infiltração na interface dente-restauração, prevenindo a entrada de fluídos orais e bactérias para o interior das paredes cavitárias, que são a principal causa de sensibilidade pós-operatória, pigmentação marginal e cáries recorrentes, os quais podem reduzir a longevidade clínica das restaurações (PHILLIPS, 1982; DUKE, 1993; VAN MEERBEEK et al., 1998).

Historicamente, restaurações diretas em resina composta falham devido à 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, em casos de restaurações extensas com grande perda de estrutura dental, restaurações indiretas são indicadas tendo como vantagens: a contração de polimerização ocorre fora da cavidade (no caso das resinas compostas), melhor restabelecimento do ponto de contato e anatomia (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 atualmente sobre o substrato dental através da utilização de cimentos resinosos (MANHART et al., 2000; PEUMANS et al., 2000; HIKITA et al., 2007).

Existem diferentes agentes de cimentação para retenção de restaurações indiretas disponíveis para os clínicos, e que podem ser divididos em cinco classes principais: cimento de fosfato de zinco (CFZ), de policarboxilato de zinco (CPZ), de ionômero de vidro (CIV), ionômero de vidro modificado por resina (CIVMR) e resinoso (CR) (DIAZ-ARNOLD et al., 1999). Estudos demonstram que os cimentos resinosos melhoram a retenção e a eficácia 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; SENYILMAZ et al., 2007). Além de apresentarem menor solubilidade, a cimentação adesiva tem o potencial de reforçar a estrutura dental enfraquecida e melhorar o selamento marginal quando comparados com a cimentação tradicional com os cimentos de fosfato de zinco e ionoméricos (VAN MEERBEEK et al., 1998; KRAMER et al., 2000; SENYILMAZ et al., 2007).

Os cimentos resinosos são tradicionalmente utilizados em associação com sistemas adesivos (DE MUNCK et al., 2004; REICH et al., 2005; HIKITA et al., 2007). Até meados da década de 90, os sistemas adesivos mais utilizados apresentavam 3 passos de aplicação, posteriormente surgiram os de dois passos, os de passo único, e mais recentemente, surgiram no mercado os materiais de cimentação auto-adesivos que não necessitam da aplicação prévia de nenhum agente de união para se aderirem à estrutura dental. Estes materiais são utilizados na cimentação de restaurações indiretas e pinos pré-fabricados (BEHR et al., 2004; ABO-HAMAR et al., 2005; GORACCI et al., 2005). No entanto, pouco se sabe a respeito do mecanismo de união e da longevidade de tais procedimentos na prática clínica (DE MUNCK et al., 2004; GERTH et al., 2006; PIWOWARCZYK et al., 2007).

A adesão ao substrato dentinário por meio de adesivos pode ser realizada através de duas técnicas: condicionamento ácido prévio ou auto-condicionamento. A técnica do condicionamento ácido prévio, ou técnica úmida de união, 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 (PERDIGÃO et al., 1996). Subseqüentemente ao condicionamento e irrigação abundante com água, é necessário que a dentina permaneça úmida para que a rede de fibrilas de colágeno se mantenham expandidas, permitindo assim a infiltração da resina adesiva. No entanto, esta técnica tem sido considerada crítica (SPENCER et al., 2000), devido à

dificuldade e subjetividade do procedimento de controle da umidade, que pode influenciar negativamente a qualidade da camada híbrida (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 auto-condicionantes compostos de monômeros ácidos são aplicados sobre a dentina sem a necessidade de lavagem e controle da umidade. Acredita-se que os sistemas auto-condicionantes desmineralizam a dentina e infiltram seus monômeros simultaneamente, evitando a ocorrência de fibrilas desprotegidas (TAY & PASHLEY., 2001; CARVALHO et al., 2005).

Devido à sua utilização em associação com sistemas adesivos, a técnica de aplicação dos cimentos resinosos convencionais é considerada crítica, sujeita a fatores relativos ao material e ao nível de conhecimento do operador (FRANKENBERGER et al., 2000), que podem levar a ocorrência de sensibilidade pós-operatória e ao insucesso do tratamento restaurador (MAK et al., 2002). Seguindo a evolução dos materiais adesivos e com o propósito de simplificar a técnica de cimentação, foi introduzido no mercado um cimento à base de resina denominado de auto-adesivo, que dispensa qualquer pré-tratamento do substrato dental (HECTH 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 à facilidade de aplicação (DE MUNCK et al., 2004). Este cimento auto-adesivo é baseado em um novo monômero, carga e tecnologia de polimerização. O fabricante afirma que a matriz orgânica consiste de um novo monômero multifuncional (éster do ácido fosfórico metacrilato). Este pode reagir com as partículas de carga do cimento resinoso e a hidroxiapatita da dentina e esmalte (HIKITA et al., 2007). Acompanhando esta tendência, diversos fabricantes lançaram suas versões para os cimentos auto-adesivos. No entanto, pouca informação a respeito destes materiais existe na literatura. A grande maioria das informações geradas até a presente data está relacionada ao primeiro material auto-adesivo (RelyX UNICEM).

Todavia, apesar dos avanços alcançados pelos polímeros adesivos, trabalhos apontam para uma possível degradação da interface de 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., 2003; GIANNINI et al., 2003; REIS et al., 2004a; 2007b; 2007c; 2007d). A redução da resistência de união à

dentina é atribuída à degradação das fibrilas colágenas e/ou da resina adesiva (TAY et al., 2003; GÖPFERICH, 1996). A busca por um material 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 (CARRILHO et al., 2007; HEBLING et al., 2005; SADEK et al., 2007).

Evidências do fenômeno de degradação podem ser observadas 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. Posteriormente, esta área de união é observada em microscocopia eletrônica de varredura e/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. A degradação da união tem sido atribuída à penetração de fluidos nestas porosidades (SANO et al., 1999; HASHIMOTO et al., 2001).

Assim, a determinação das características ultramorfológicas e padrões de nanoinfiltração das interfaces de união produzida por materiais auto-adesivos através do protocolo de nanoinflitração é 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 através de Microscopia Eletrônica de Varredura (MEV) e de Transmissão (MET) a ultramorfologia das interfaces resina/dentina produzidas por cimentos resinosos convencionais e auto-adesivos, submetidos ao ensaio de nanoinfiltração após armazenamento em água por 24 horas.

3. METODOLOGIA E RESULTADOS

A presente dissertação está baseada no artigo “Transmission and Scanning Electron Microscopy Analysis of the Composite-Dentin Interfaces Produced by Self-Adhesive and Conventional Resin Cements”.

Transmission and Scanning Electron Microscopy Analysis of the Composite-Dentin Interfaces Produced by Self-Adhesive and Conventional Resin Cements

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ABSTRACT

Objectives: The aim of this study was to evaluate the ultramorphology of the resin-dentin interfaces produced by different self-adhesive cements submitted to a nanoleakage protocol by means of Transmission (TEM) and Scanning Electron Microscopy (SEM), and to compare them with conventional resin cements. **Materials and Methods:** Six self-adhesive cements were used in the present study: RelyX Unicem (UN), RelyX U100 (UC), SmartCem 2 (SC), G-Cem (GC), Maxcem (MC), Set (SET); and two conventional resin cements: 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). An additional group included the use of a 2-step self-etching adhesive system (Clearfil SE Bond) prior to the application of Panavia F (PS). Thirty-six human molars were assigned to 9 groups according to the luting material. After 24h of water-storage, restored teeth were serially sectioned into 0.9 mm-thick slabs, which were submitted to a nanoleakage protocol with $AgNO_3$ and observed in the TEM and SEM. **Results:** Different nanoleakage patterns were observed for the different groups. UC and UN self-adhesive resin cements presented little or no silver deposition, comparable to the conventional resin cements, while MC, SC and SET did not resist specimen preparation procedures. GC presented remarkable silver deposition along the interface. The additional group PS did not present differences from the original group PF. **Conclusions:** The characteristics of the resin-dentin interfaces were rather product-dependent than related to the type of resin cement.

Keywords: self-adhesive cements, resin cements, TEM, SEM, nanoleakage

INTRODUCTION

Bonding of resin-based composite materials to tooth hard tissues has been simplified in the latest years. A few years ago, most adhesives were available in three application steps, which were combined into two steps (etch-and-rinse or self-etching) and later, in one single self-etching application step. Indirect adhesive procedures constitute a substantial portion of esthetic restorative procedures. Until recently, all resin cements required the application of one of these adhesive systems, either self-etching or etch-and-rinse, to prepare the tooth prior to cementation.[1-3] However, the multi-step application technique has been reported to be complex and sensitive, and can compromise bonding effectiveness.[4]

A new concept of luting materials has been recently developed, which does not require any pretreatment of the tooth surface, originating the so-called self-adhesive cements.[5-7] These materials aim to combine the favorable properties of traditional (zinc phosphate, glass ionomer and polycarboxilate cements) and conventional resin cements, eliminating their shortcomings.[8] After the first self-adhesive cement was introduced into the market (RelyX UNICEM) it rapidly gained popularity among clinicians due to its simplified “mistake-free” application technique. Thus, several manufacturers developed their own self-adhesive cements.

Limited information, however, is currently available with regard to the bonding mechanism, longevity and clinical performance of self-adhesive cements.[1,8-10] The ability of some self-adhesive cements to demineralize and infiltrate smear-layer covered dentin has been questioned.[7,11] Despite the favorable microleakage behavior reported for UNICEM,[12-14] the sealing ability can vary between materials.[15] The interfaces ultrastructural characteristics have only been described for the first developed self-adhesive cement.[1,7]

The aim of this study was to evaluate the ultramorphology of the resin-dentin interfaces produced by six self-adhesive cements submitted to a nanoleakage protocol by means of Transmission (TEM) and Scanning Electron Microscopy (SEM), and to compare them with conventional etch-and-rinse and self-etching resin cements. The null hypothesis to be tested is that there is no difference in the silver deposition patterns and ultramorphological characteristics of the cement-dentin interface produced by the different luting materials.

MATERIALS AND METHODS

Thirty-six caries-free recently extracted third molars stored in 0.1% Thimol solution at 4ºC were used in this study. Teeth were obtained by a protocol approved by the review board of the Guarulhos University (#152/2007). After disinfection and removal of soft tissues, flat occlusal dentin surfaces were exposed with 600-grit SiC papers under running water to create a standardized smear layer.

Teeth were randomly assigned to 9 experimental groups, which were restored with one of the 9 luting techniques. Six self-adhesive cements were used in the present study: RelyX Unicem (UN – 3M ESPE), RelyX U100 (UC – 3M ESPE), SmartCem 2 (SC – Dentsply Caulk), G-Cem (GC – GC Corp.), Maxcem (MC – Kerr Corp.), Set (SET - SDI); and two conventional resin cements: one that uses a 2-step etch-and-rinse adhesive (Adper Single Bond Plus / Rely X ARC - RX), and one that uses a 1-step self-etching adhesive (ED Primer / Panavia F - PF). An additional group was made by using a 2-step self-etching adhesive system (Clearfil SE Bond) prior to the application of ED Primer and Panavia F (PS). Resin cements were mixed and inserted according to manufacturers' instructions (Table 1).

Luting procedures for TEM analysis

Half of the specimens (n=2) were prepared for TEM analysis. After resin cements were mixed and applied onto the flat dentin surfaces, a polyester strip was placed over the resin cement and a glass plate was used to apply proper digital pressure while the resin cement was light-cured for 40s with a halogen light (OPTILUX 501, Kerr Corp., Danbury, CT, USA). Light output was monitored at 650mW/cm^2. Next, in order to facilitate ultrathin sectioning, a thin layer of a low-viscosity resin composite (Protect Liner F, Kuraray Med. Inc, Kurashiki, Okayama,

Japan) was applied and light-cured for 40s. After 24h of water storage, teeth were sectioned perpendicular to the adhesive-tooth interface into 0.9 mm thick slabs using a diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA).

Luting procedures for SEM analysis

For SEM analysis, composite resin blocks were prepared by layering one 2 mm-thick increment of a micro-hybrid composite resin (Z250 3M ESPE, St. Paul, MN, USA) into a silicon mold, which was light-cured for 40s with a halogen light. One side of the composite resin blocks was abraded with #600 SiC-paper under water-cooling to create a flat surface with standardized roughness. The composite surface was air-abraded with 50 µm aluminum-oxide particles for 10s. Before luting procedure the composite resin blocks were ultrasonically cleaned in distilled water for 10 min, rinsed with running-water, air-dried and silanated with Rely X Ceramic Primer (3M ESPE, St Paul, MN, USA).

After application of the resin cement according to manufacturer's instructions (Table 1), the indirect composite block was pressed on the cement using proper digital pressure, after which excess cement was removed. Specimens were light-cured for 40 s with a halogen light from the occlusal, buccal and lingual surfaces. Restored teeth were stored in distilled water for 24 h.

Nanoleakage Evaluation

The silver impregnation protocol was carried out in two different ways. For the TEM evaluation, bonded slabs were subjected to the silver impregnation protocol, while for the SEM evaluation, the teeth were sectioned after silver impregnation. This approach permitted us to evaluate the ultrastructural properties and silver uptake at

the resin-dentin interfaces under the TEM and the ability of the cements to prevent nanoleakage through the enamel margins when observed under the SEM.

Bonded slabs/teeth 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 artifacts,[16] 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. (2002).[17] 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 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the interface. Specimens that were prepared for the SEM were then sectioned into 0.9 mm-thick slabs and additionally photodeveloped for 8 h.

Transmission Electron Microscopy

Specimens were examined with the TEM to compare silver uptake patterns along resin-dentin interfaces. Undemineralized specimens were fixed in Karnovsky's solution, post-fixed in osmium tetroxide, dehydrated in ascending ethanol series (30 to100%) and embedded in epoxy resin (Dr. Spurr, Electron Microscopy Sciences, Hatfield, PA, USA). Propylene oxide was used as a transitional fluid. Representative 90 nm-thick ultrathin sections were prepared with an ultramicrotome (Leica UC6, Leica Microsystems GmbH, Wetzlar, Germany) and collected on 100-mesh carbon/formvar-coated copper grids. Without additional staining, they were observed in a TEM (Zeiss EM 900, Zeiss, Munich, Germany) operated at 80KV. Silver deposition patterns were compared among the different luting products. As the etch-and-rinse adhesives produce a hybrid layer that is approximately ten times thicker

than those produced by the self-etching primer and the self-adhesive systems, different magnifications were used for observation of the bonded interfaces.

Scanning Electron Microscopy

Specimens were fixed in Karnovsky's solution, post-fixed in osmium tetroxide 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 operated at 20KV (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, UK).

RESULTS

Figures 1 through 9 present the interfacial characteristics of the resin dentin interfaces produced by the different luting materials. The characteristics of the resin-dentin interfaces produced by the multi-step systems and the silver deposition pattern were different between materials. The multi-step systems presented a distinct hybrid layer. The hybrid layer presented by the etch-and-rinse system RX was approximately 5 μm-thick (Fig.1), whereas the hybrid layer produced by PF and PS was approximately 10 times thinner, presenting 0.5 μm (Figs. 2 and 3). RX presented the typical characteristics of etch-and-rinse systems: thick hybrid layer, funnel-shaped dentin tubules entrance, and long resin tags (Fig. 1). Almost no silver deposition was observed for RX (Fig. 1A). PF and PS presented the typical characteristics of mild self-etching systems: a thin, partially demineralized hybrid layer, measuring approximately 0.5 μm; and partially demineralized smear plugs, which result in small resin tags (Figs. 2 and 3). Silver deposition was only detected under high magnification TEM. Tiny silver deposits were mainly observed at the top of the hybrid layer. The only difference between the interfaces presented by PF and PS was the approximately 6 μm-thick adhesive layer that was present due to the application of Clearfil SE Bond. Even though ED primer was applied over the polymerized adhesive system, it was not distinguishable under the TEM.

The resin-dentin interfaces produced by the self-adhesive cements presented characteristics and silver deposition patterns that were distinct from the multi-step systems, and differed remarkably among the different self-adhesive materials. TEM observation revealed that UN presented an intimate contact with dentin surface. It appears to incorporate the smear layer, slightly interacting with dentin. No silver deposition was observed for this system (Fig. 4). On the other hand, UC, which is the

clicker version of the same cement, presented silver deposition at the interface (Fig. 5).

The self-adhesive cement GC appeared to infiltrate dentin deeper than the other self-adhesive resin cements. An interaction zone of approximately 0.5 μm thick was observed. A basal zone of partially etched but uninfiltrated dentin was observed in some regions of this self-adhesive cement. Such a zone was characterised by the occurrence of silver deposits within the interfibrillar spaces of the mineralized dentin, and was located at a region beneath the hybridized complex (Fig. 6).

Figures 7, 8 and 9 show representative images of the self-adhesive cements MC, SC and SET, which did not resist specimens preparation procedures and the vacuum at the SEM and disrupted the resin-dentin interface. Massive silver deposition was observed for the MC specimens (Fig. 7). Interestingly, SEM observation revealed that despite the fragile dentin bond, enamel margins of all self-adhesive cements were still bonded (Figs. 4D, 5B, 8B and 9B).

Apparently, for all resin cements tested, the resin-enamel margins surrounding the restorations were able to protect interfaces against silver penetration, since silver deposition was not easily distinguishable under the SEM.

DISCUSSION

Dental luting agents provide the link between a fixed prosthesis and the supporting prepared tooth structure. They should be able to provide a strong and durable bond of indirect materials to tooth structure, as well as an effective dentin sealing.[18] Self-adhesive cements were developed with the intent to combine the favorable properties of conventional (zinc phosphate, glass ionomer and polycarboxilate cements) and resin cements, eliminating their shortcomings.[8]

The present study demonstrated that there are remarkable differences in the silver deposition and ultramorphological characteristics of the interfaces produced by conventional multi-step systems and self-adhesive cements. The resin-dentin interfaces produced by the resin cements that require previous application of an adhesive system presented a well-defined hybrid layer.[19] The hybrid layer produced by the etch-and-rinse adhesive is well documented in the literature.[20,21] It presents a thick hybrid layer of approximately 5 μm, with long resin tags. Our null hypothesis was rejected, because there were remarkable differences in the silver deposition patterns and interfacial ultramorphology of the resin cements tested.

The resin-dentin interfaces produced by Panavia F presented typical characteristics of those reported for mild self-etching primers.[20,22] A thin 0.5 μm thick, partially demineralized hydroxyapatite-containing hybrid layer was observed. Very little silver deposition was recorded for this system. TEM revealed tiny silver grains at the top of the hybrid layer. Except for the thicker adhesive layer, produced by the application of the hydrophobic resin of Clearfil SE Bond, a very similar interface was observed for PF and PS. It has been reported that the monomer 10-methacryloxydodecyl dihydrogen phosphate (10-MDP), present in Clearfil SE Bond and in ED primer, chemically interacts with hydroxyapatite, forming a very stable

bond. Yoshida et al. (2004)[23] reported that the calcium salt formed by 10-MDP presents a low dissolution rate in water when compared to other functional monomers present in commercially available adhesives. This interaction seems to be important for bonding efficacy and stability over time.[23,24]

The resin-dentin interfaces produced by the self-adhesive cements presented characteristics and silver deposition patterns that were distinct from the multi-step systems and differed remarkably among the different self-adhesive materials. The self-adhesive cements UNICEM (UN) and U100 (UC) are both developed by the same manufacturer, and marketed under the same name in some countries. According to manufacturer, the only difference between them is the delivery system. UN requires an activator, triturator and applier, while UC is the clicker version of the same material, and can be hand mixed. However, UN presented no signs of silver deposition along the interface, and UC presented some regions of silver deposition along the interface (Figs. 3 and 4).

Despite the low initial cement pH (<2 in the first minute according to the manufacturer), almost no demineralization/infiltration was observed on the dentin surface for UNICEM. This finding is in agreement with the study performed by De Munck et al (2004).[1] This might be attributed to the higher cement viscosity if compared to self-etching primers, which hinders the resin cement to wet and infiltrate the dentin surface.[1] Limited ability to demineralize and infiltrate dentin substrate has been reported for UN, which can explain why no true hybrid layer is formed when applied to dentin.[1,7,11] In order to promote a micromechanical interlocking with dentin collagen fibrils, these cements should be able to etch the substrate in a relatively short time, requiring optimal wetting properties to ensure a fast interaction with dentin.[25] Chemical interaction with dentin hydroxyapatite has been reported for

UNICEM,[9] which can help to explain the favorable bond strength results reported for this self-adhesive material.[8]

TEM observation of G-Cem revealed a deeper demineralization if compared to UN and UC, of approximately 1 μm depth. However, high amounts of silver deposition were detected for GC (Fig. 6). A basal zone of partially etched but uninfiltrated dentin was observed in some regions of this self-adhesive cement. Such a zone was characterized by the occurrence of silver deposits within the interfibrillar spaces of the mineralized dentin, and was located at a region beneath the hybridized complex. A similar pattern of silver deposition has been reported for some self-etching adhesives by Carvalho et al (2005).[26] The bonding mechanism of GC has been reported by the manufacturer to be based on the glass-ionomer technology modified by exchanging polyacrylic acid with the acidic functional monomers 4-MET and phosphoric-acid esters.[15] Water in the cement composition of GC is expected to aid the conditioning reaction, reducing the time needed for interacting with de substrate. However, the relatively weak chemical bonding potential of 4-MET and the high molecular weight of the functional monomer are expected to contribute poorly to the supposed chemical reaction, within a clinically reasonable time.[23]

The self-adhesive cements MC, SC and SET did not resist specimen preparation procedures and the resin-dentin interfaces disrupted before TEM observation. SEM analysis demonstrated that gaps were formed along the resin-dentin interface. Conversely, resin bonding to peripheral enamel remained intact (Figs 8B and 9B). Han et al (2007)[27] reported low pH values for GC, MC, SC and UN a few seconds after manipulation. However, after 48 hours, only UN presented a neutral pH (pH 7.0). The pH reported 48h after polymerization was 2.4 for MC, 3.6 for GC and 4.0 for SC.[27] Even though an initial low pH is important for etching of enamel

and dentin, if a low pH is maintained for a long time, it might adversely influence the adhesion of the mixed cement to tooth structures.[27,28] This observation can explain why no silver deposition was observed for UN. It's not clear if the chemistry of UC is exactly the same of UN. The dominant setting reaction of UN is the radical polymerization, and it additionally presents a glass-ionomer concept in order to assure neutralization of the initial acidic system. A neutralization effect may occur during setting, since these chemical reactions involve water release and alkaline filler that may raise the pH level.[5,29] This neutralization effect may also be exerted by dentin-buffering components contained in the smear layer.[30,31]

Interestingly, when specimens were observed under the SEM, silver deposition was not easily observed. The electron-lucent areas observed in some of the SEMs can be attributed to charging, rather than to the presence of silver deposits. Apparently, the peripheral resin-enamel margins around the restorations were able to protect interfaces against silver penetration. It has been reported that the presence of a resin-enamel bond is able to reduce the degradation rate of resin-dentin interfaces.[32,33]

In summary, among the self-adhesive cements, UN and UC presented the lowest silver deposition. GC presented high amounts of silver deposits, and MC, SC and SET did not resist specimen preparation procedures. UN and UC seem to be more indicated for luting indirect materials to tooth substrate. Further studies are necessary to investigate the durability of the interfaces produced by these materials.

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

Type	Product (lot#) Manufacturer	Delivery system (Cement)	Composition	Application Technique
Dual cure Resin Cement + 2-step etch&rinse adhesive system	RelyX ARC (GEHG) + Adper Single Bond 2 (8RW) 3M ESPE, St Paul, MN, USA	Clicker dispenser, 2 paste hand mixed for 10 s	Etchant: 35% H_3PO_4 Adhesive: Bis-GMA, HEMA, UDMA, dimethacrylates, ethanol, water, canphorquinone , photoinitiators, polyalkenoic acid copolymer, 5-nm silica particles Cement: Bis-GMA, TEGDMA polymer, zirconia/silica filler	a (15s); b (15s); c; d; e; i.(10s); mix cement; apply mixture
Dual cure Resin Cement + 1-step self-etching adhesive	Panavia F (paste A 00248C; paste B 0026B) + ED Primer (primer A 00255A; primer B 00131A) Kuraray Medical Inc, Kurashiki, Japan	One step self-etching adhesive + Resin cement Dual cure 2 paste/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 photo-iinitiator, 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 cure Resin Cement + 2-step self-etching adhesive system	Panavia F + (paste A 00248C; paste B 0026B) + Clearfil SE Bond (00788A) Kuraray medical Inc., Kurashiki, Japan	Two step self-etching adhesive + ED primer + Resin cement Dual cure 2 paste/hand-mixed	Primer: MDP, HEMA, hydrophilic dimethacrylate, dl-canphorquinone, N,N-diethanol p-toluidine, water Bond: MDP, bis-GMA, HEMA, hydrophobic dimethacrylate, dl-canphorquinone, N,N-diethanol p-toluidine, silanated colloidal silica Paste A and Paste B: As described above	f (20 s); e; g; i (10s); h (ED primer); e; mix cement; apply mixture; i (40 s)
Dual cure Self-adhesive Resin Cement	G-CEM (0702191) GC Corp, Tokio, Japan	Capsules Mechanically mixed 10s	Powder: fluoroaluminosilicate glass, initiator, pigment Liquid: 4-META , phosphoric acid ester monomer, water, UDMA, dimethacrylate, silica powder,initiator, stabilizer	Auto-mix cement; Apply mixture; i (40s) or j (5min)
Dual cure Self-adhesive Resin Cement	U100 (287269) 3M ESPE, Seefeld, Germany	Clicker dispenser 2 paste hand mixed	Base: glass fiber, methacrylated phosphoric acid esters, dimethacrylates, silaneted sílica, sodium persulfate Catalyst: glass fiber, dimethacrylates, silanated sílica, P-Toluene sodium sulfate, calcium hydroxide	mix cement; apply mixture; i (40s) or j (5min)
Dual cure Self-adhesive Resin Cement	Unicem (293599) 3M ESPE, Seefeld, Germany	Capsules, Mechanically mixed, 10s	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.	Auto-mix cement, Apply mixture, i (40s) or j (5min)
Dual cure Self-adhesive Resin Cement	Maxcem (2954635) Kerr Corp, Orange, CA, USA	Paste/paste dual syringe, direct dispensing through a mixing tip	Resin: multifunctional DMAs, GPDM, proprietary Redox initiators and photoinitiators Filler: barium, fluoroaluminosilicate, fumed silica (66 wt.%)	Auto-mix cement; Apply mixture; i (20s) or j (3min)
Dual cure Self-adhesive Resin Cement	SmartCem 2 (0807311) Dentsply Caulk, Milford, DE, USA	Paste/paste dual syringe. direct dispensing through a mixing tip	UDMA; Di- and Tri-Methacrylate resins; Phosphoric acid modified acrylate resin; Barium Boron FluoroAluminoSilicate Glass; Organic Peroxide Initiator; Camphorquinone Photoinitiator; Phosphene Oxide Photoinitiator; Accelerators; Butylated Hydroxytoluene; UV Stabilizer; Titanium Dioxide; Iron Oxide; Hydrophobic Amorphous Silicon Dioxide	Auto-mix cement; Apply mixture; i (40s) or j (6min)
Dual cure Self-adhesive Resin Cement	SeT (50711292) SDI, Bayswater Victoria, AUS	Capsules, mechanically mixed, 10s	Methacrylated phosphoric esters, UDMA, photoinitiator 67 wt% (45 vol%) Fluoroaluminosilicate glass, pyrogenic silica	Auto-mix cement; Apply mixture i (20s) or j (5min)

Application technique – a: acid etch; b: rinse surface; c: dry with cotton-pellet; d: apply one-bottle adhesive; e: gently air dry; f: apply primer; g: apply adhesive; h: apply mixture; i: light cure; j: self-cure

FIGURES AND LEGENDS

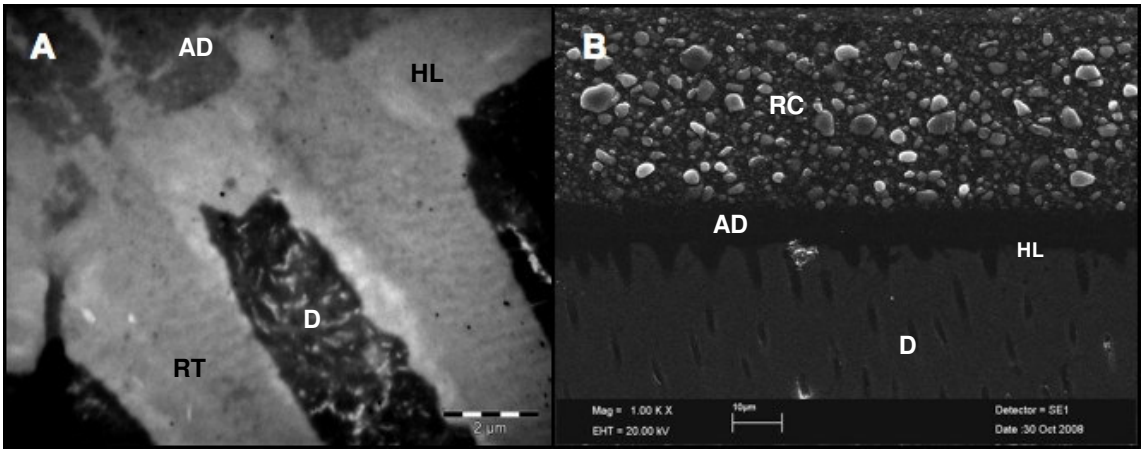

Figure 1. Representative transmission (A) and scanning (B) electron micrographs of the resin-dentin interfaces produced by RX. The hybrid layer presented by the etch-and-rinse system was approximately 5 µm thick (HL). RX presented the typical characteristics of etch-and-rinse systems: thick hybrid layer, funnel-shaped dentin tubules entrance, and long resin tags (RT). Very little silver deposition was observed. D – dentin; RC – resin cement; AD – adhesive. Original Magnification: A – 3000X, B – 1000X.

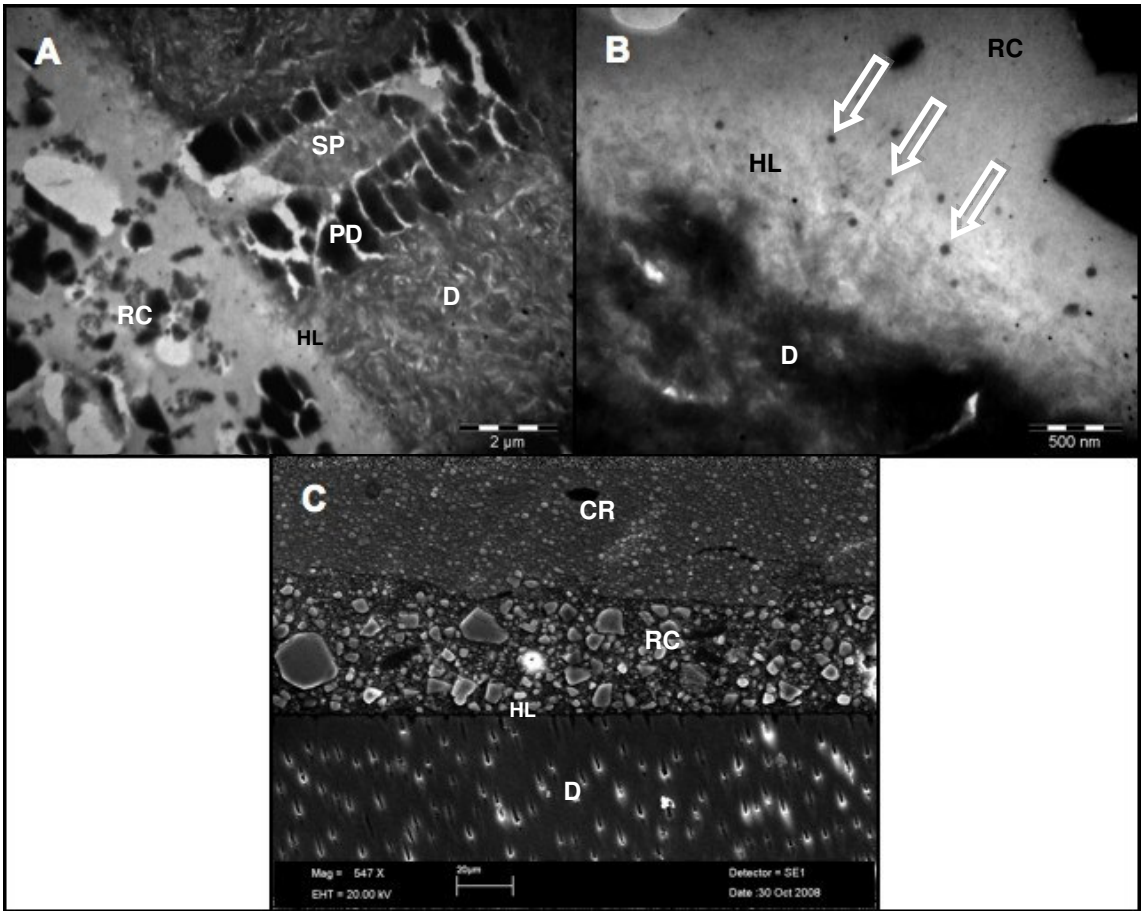

Figure 2. Representative TEMs of undemineralized, unstained sections (A and B) and SEM (C) of the composite-dentin interface produced by the self-etching resin cement. PF presented a thin, partially demineralized hybrid layer (HL), measuring approximately 0.5 µm and partially demineralized smear plugs (SP), which result in small and thin resin tags. Silver deposition (arrows) was only detected under high magnification TEM (B). Tiny silver deposits were mainly observed at the top of the hybrid layer. D – dentin; RC – resin cement; PD – peritubular dentin; CR – composite resin. Original Magnification: A – 3000X, B – 12000X, C – 547X.

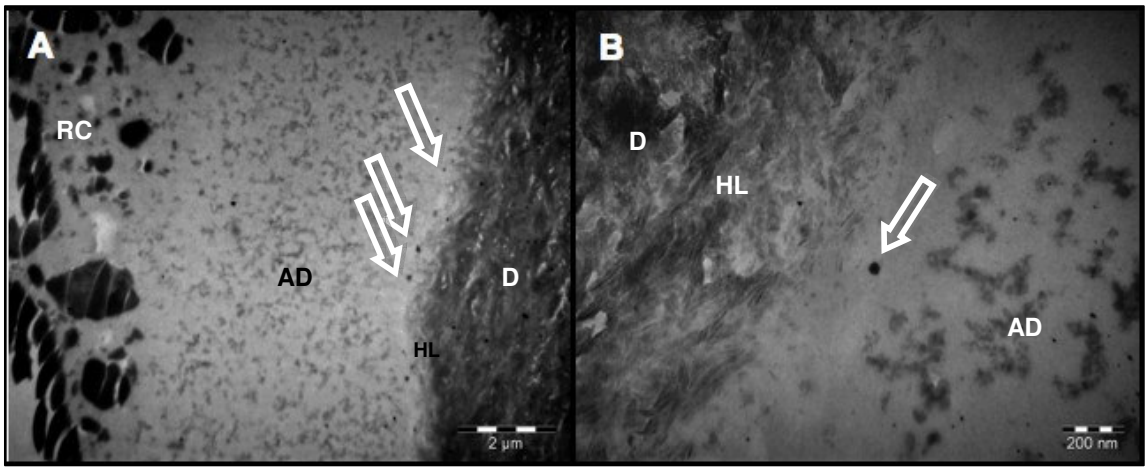

Figure 3. Representative TEMs of undemineralized, unstained sections of the composite-dentin interface produced by the application of Clearfil SE Bond and Panvia F. PS presented a thin, partially demineralized hybrid layer (HL), measuring approximately 0.5 µm. Silver deposition (arrows) was only detected under high magnification TEM. Tiny silver deposits were mainly observed at the top of the hybrid layer (arrows). The only difference between the interfaces presented by PF and PS was the approximately 6 µm thick adhesive layer (AD) that was present due to the application of Clearfil SE Bond. D – dentin; RC – resin cement. Original Magnification: A – 3000X, B – 20000X.

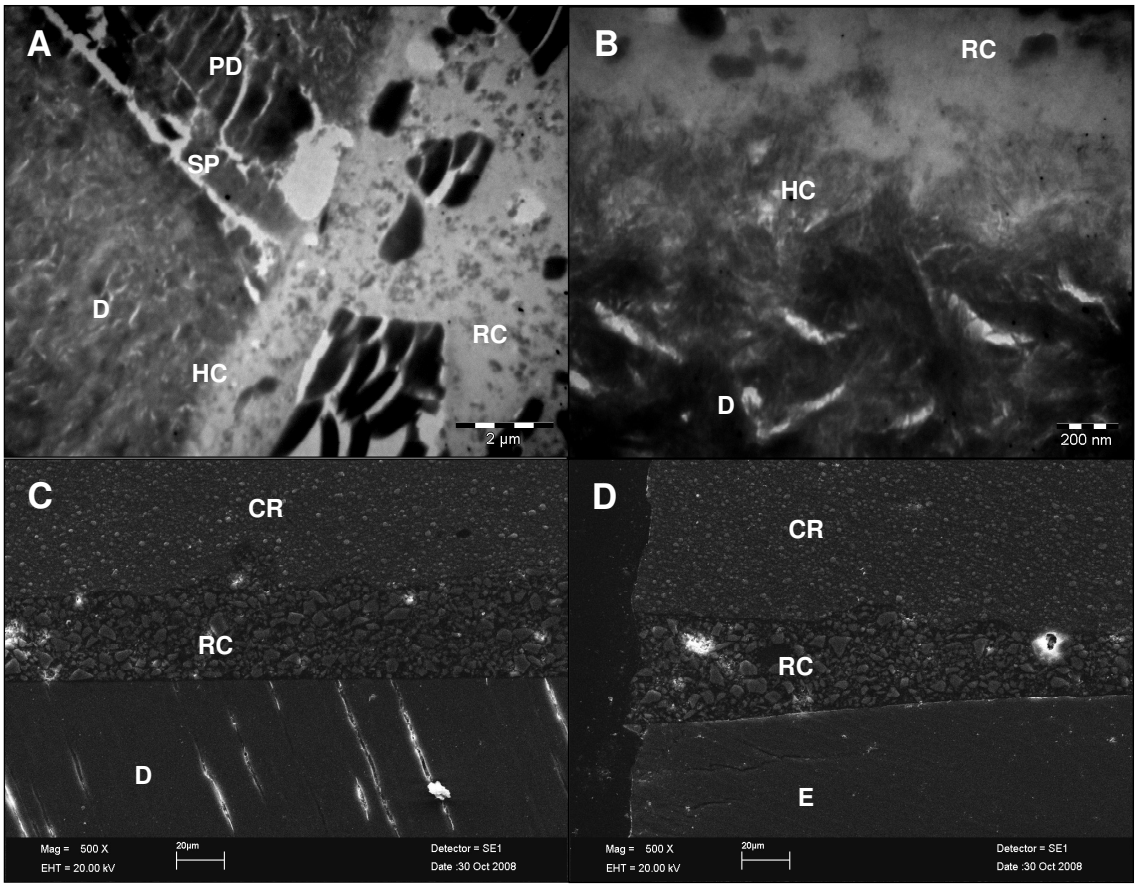

Figure 4. Representative TEMs (A and B) and SEMs (C and D) of the resin-dentin interface produced by the self-adhesive cement UNICEM. TEM observation (A and B) revealed that UN presented an intimate contact with dentin surface, appears to incorporate the smear layer, and slightly interact with dentin, forming a hybridized complex (HC). No silver deposition was observed for this system. D – dentin; PD – peritubular dentin; SP – smear plug; HC – hybridized complex; RC – resin cement; CR – composite resin; E - enamel. Original Magnification: A – 3000X, B – 2000X, C – 500X, D – 500X.

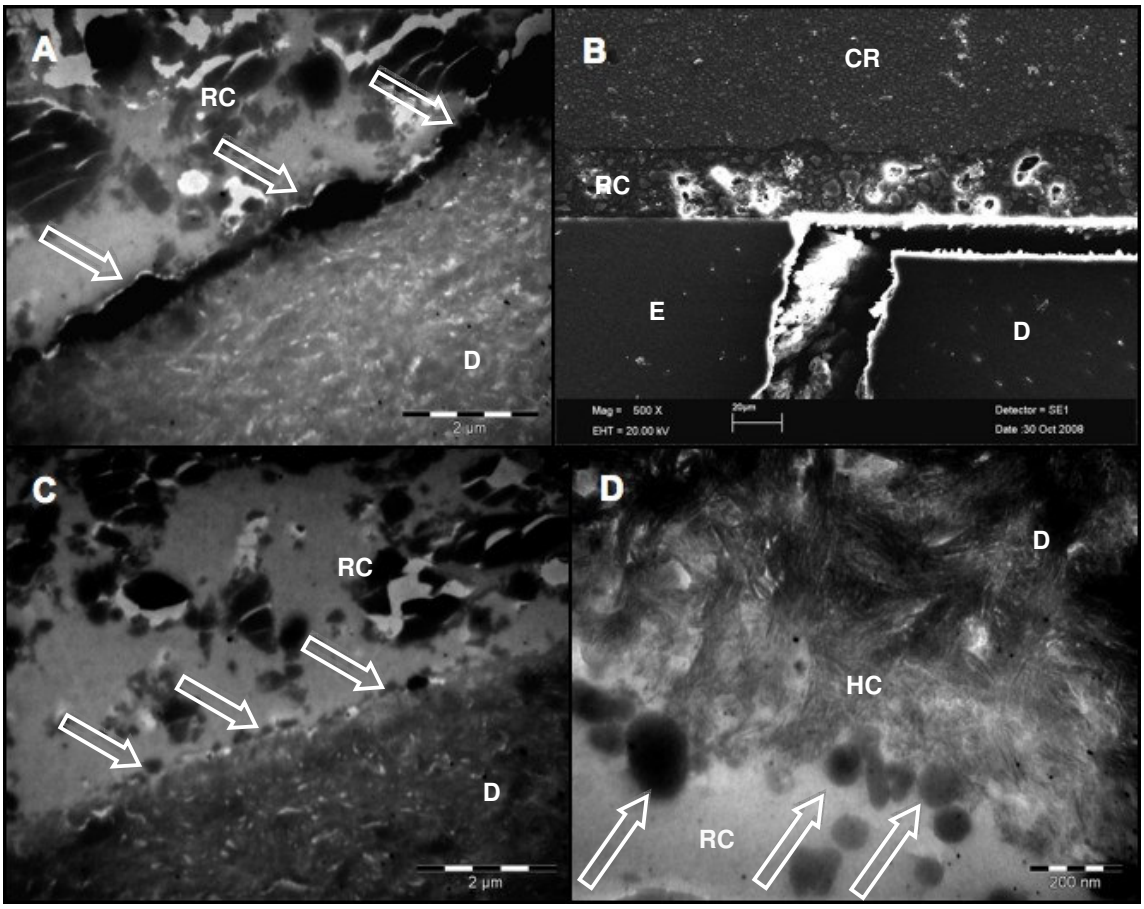

Figure 5. Representative TEMs (A, C and D) and SEM (B) of the resin-dentin interface produced by the self-adhesive cement U100. TEM observation revealed the presence of silver deposits in some regions of the interface (arrows). UC also presented an intimate contact with dentin surface, incorporating the smear layer, and forming a hybridized complex (HC). (B) The vacuum produced by the SEM disrupted the resin-dentin bond, however the resin enamel bond remained intact. D – dentin; HC – hybridized complex; RC – resin cement; CR – composite resin; E - enamel. Original Magnification: A – 4400X, B – 500X, C – 4400X , D – 30000X.

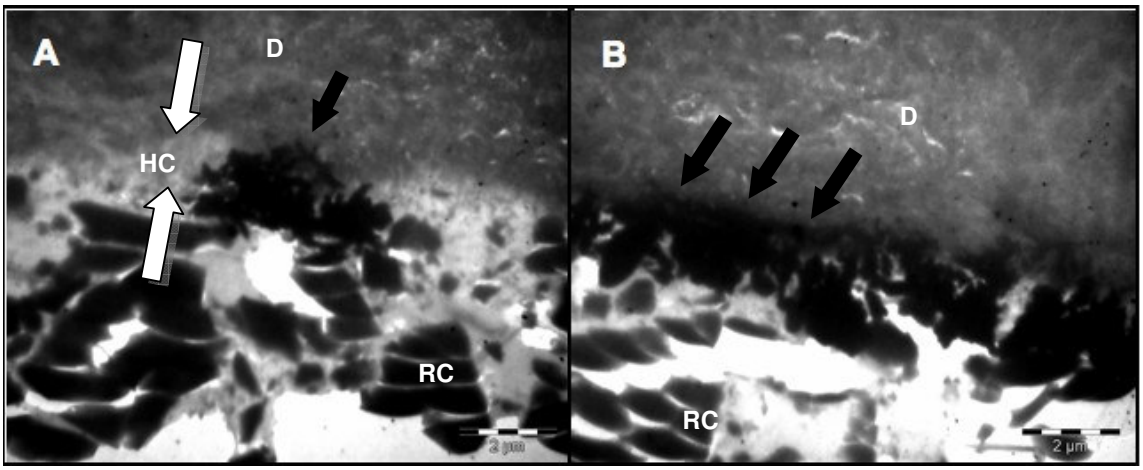

Figure 6. Representative TEMs of undemineralized, unstained sections of the composite-dentin interface produced by the self-adhesive cement G-Cem. GC appeared to infiltrate dentin deeper than the other self-adhesive resin cements. An interaction zone of approximately 0.5 µm was observed (white arrows). Silver deposition was observed within the hybridized complex. Additionally, a basal zone of partially etched but uninfiltrated dentin was observed in some regions of this self-adhesive cement. Such a zone was characterized by the occurrence of silver deposits within the interfibrillar spaces of mineralized dentine, and was located at a region beneath the hybridized dentin (black arrows). D – dentin; RC – resin cement. Original Magnification: A – 3000X, B – 3000X.

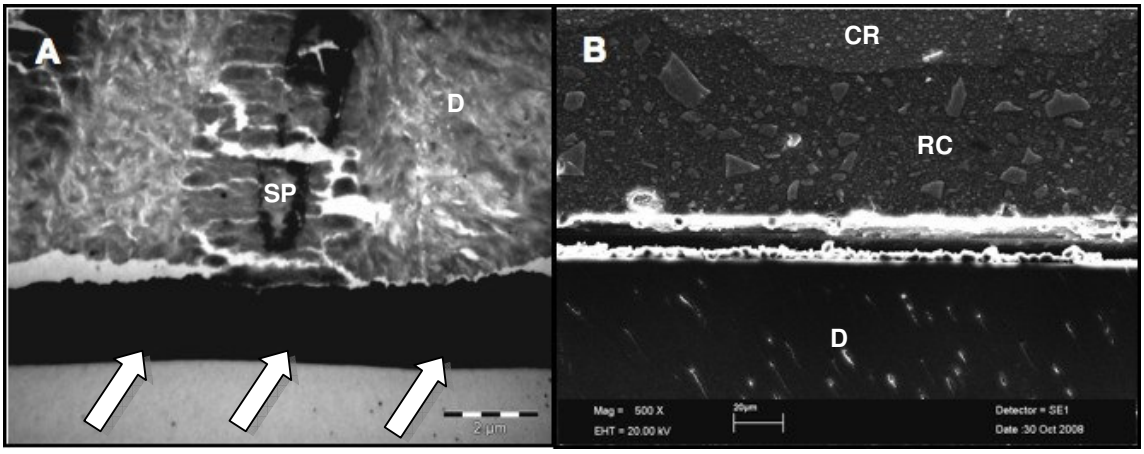

Figure 7. Representative images of the self-adhesive cement MC. Massive silver deposition was observed for the MC specimens, which did not resist specimens preparation procedures and the vacuum at the SEM and disrupted the resin-dentin interface. D – dentin; RC – resin cement; CR – composite resin. Original Magnification: A – 3000X, B – 500X.

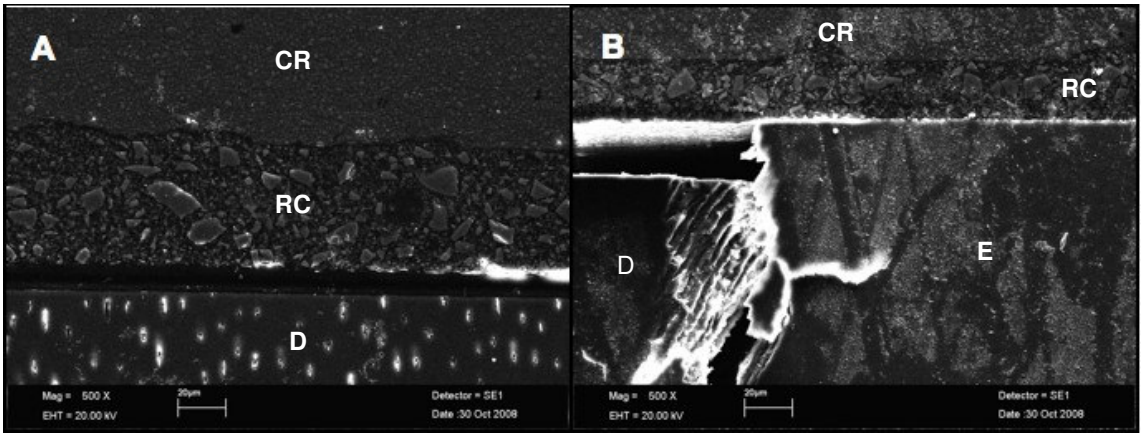

Figure 8. Representative SEMs of the self-adhesive cement Smartcem, which did not resist specimen preparation procedures and the vacuum at the SEM and disrupted the resin-dentin interface. Interestingly, SEM observation revealed that despite the fragile dentin bond, enamel margins were still bonded. D – dentin; RC – resin cement; E – enamel; CR – composite resin. Original Magnification: A – 500X, B – 500X.

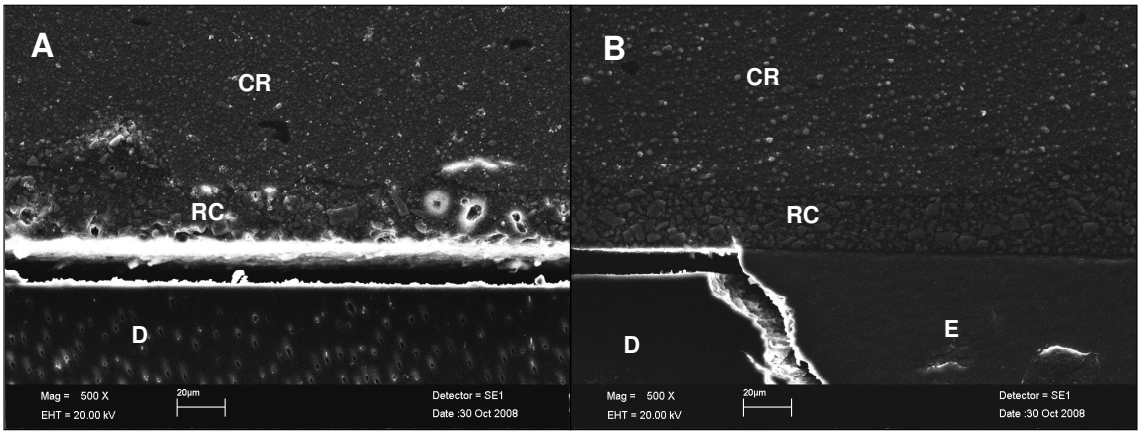

Figure 9. Representative SEMs of the resin-dentin interface produced by the self-adhesive cement SET. This self-adhesive cement did not resist specimen preparation procedures and the vacuum at the SEM and disrupted the resin-dentin interface. Interestingly, SEM observation revealed that despite the fragile dentin bond, enamel margins remained bonded. D – dentin; RC – resin cement; E – enamel; CR – composite resin. Original Magnification: A – 500X, B – 500X

4. CONCLUSÃO

Dentro das limitações deste estudo podemos concluir que:

Quando observados através de Microscopia Eletrônica de Transmissão, os cimentos resinosos auto-adesivos U100 e UNICEM apresentaram pouca ou nenhuma deposição de prata (nanoinfiltração). O cimento G-Cem apresentou grandes quantidades de deposição de prata enquanto as interfaces de união produzidas pelos cimentos Maxcem, Smartcem e SET não resistiram aos procedimentos de preparo para MET.

Através da observação em Microscopia Eletrônica de Varredura, não foi observada a deposição de prata. A margem de esmalte possivelmente protegeu a interface compósito dentina contra a infiltração do traçador.

As interfaces de união produzidas pelos cimentos resinosos convencionais RelyX ARC, Panavia F e Panavia F + SE Bond apresentaram características compatíveis com aquelas apresentadas pelos respectivos sistemas de união.

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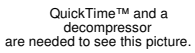
7. ANEXOS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TERMO DE CONSENTIMENTO
DOAÇÃO DE MATERIAL BIOLÓGICO

Por esse instrumento particular declaro, para os efeitos éticos e legais, que eu (nome) _____, (nacionalidade) _____, (profissão)_____, portador do R.G. _____, C.I.C._____, residente e domiciliado à Rua _____ , telefone _____, na cidade de _____ , Estado de _____ , permito a utilização do(s) terceiro(s) molar(es) extraído(s) devido à indicações cirúrgicas ou ortodônticas na pesquisa ANÁLISE DA RESISTÊNCIA DE UNIÃO, NANOINFILTRAÇÃO E CARACTERÍSTICAS ULTRAMORFOLÓGICAS DAS INTERFACES DE UNIÃO RESINA-DENTINA PRODUZIDAS POR CIMENTOS AUTO-ADESIVOS AO LONGO DO TEMPO, coordenada pelo Prof. Dr. André Figueiredo Reis (Pesquisador Responsável).

Estou ciente que estas amostras serão armazenadas no Laboratório de Biomateriais I – UnG para serem utilizados em pesquisas futuras também com o objetivo único de estudar a adesão de materiais restauradores às estruturas dentais. A guarda e autorização de uso deste material é responsabilidade do Pesquisador Responsável (Prof^{o}. André Figueiredo Reis), comprometendo-se a submeter para aprovação do Comitê de Ética toda nova pesquisa que utilizará este material, e quando for o caso, da Comissão Nacional de Ética em Pesquisa – CONEP.

Em nenhuma hipótese a identidade dos participantes do estudo será declarada. As amostras serão armazenadas em tubos plásticos codificados por números, ou seja, sem a colocação dos nomes dos indivíduos participantes da pesquisa.

Os participantes do estudo, poderão ser contatados para o fornecimento de informações a respeito do resultado do estudo ou para obtenção de consentimento específico para uso em novo projeto de pesquisa. Da mesma forma, poderão buscar novos esclarecimentos em qualquer momento.

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_____ Assinatura do Doador

_____ Dr. André Figueiredo Reis – CRO-SP 68.253 Pesquisador Responsável

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AUTORIZAÇÃO PARA USO DA INSTITUIÇÃO

Autorizo o pesquisador André Figueiredo Reis (responsável pelo projeto) a utilizar o Laboratório de Pesquisa em Odontologia I - Biomateriais e os equipamentos necessários para a realização do projeto “Análise da resistência de união, nanoinfiltração e características ultramorfológicas das interfaces de união resina-dentina produzidas por cimentos auto-adesivos ao longo do tempo”.

Guarulhos, 17 de Outubro de 2007.

_____

Profa. Dra. Magda Feres

Coordenadora de Pós-Graduação e Pesquisa
em Odontologia
RG 37957403-2