



ARIENE ARCAS TOPAL PAES LEME

PROCEDURES FOR REDUCING THE INCOMPATIBILITY BETWEEN
SIMPLIFIED-STEP ADHESIVE SYSTEMS AND
DUAL-CURED RESIN CEMENTS

PROCEDIMENTOS PARA REDUÇÃO DA INCOMPATIBILIDADE
ENTRE SISTEMAS ADESIVOS SIMPLIFICADOS E CIMENTOS
RESINOSOS DE DUPLA ATIVAÇÃO

Piracicaba
2013



UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

ARIENE ARCAS TOPAL PAES LEME

PROCEDURES FOR REDUCING THE INCOMPATIBILITY BETWEEN
SIMPLIFIED-STEP ADHESIVE SYSTEMS AND DUAL-CURED
RESIN CEMENTS

Orientador: Prof. Dr. Mário Alexandre Coelho Sinhoreti

*PROCEDIMENTOS PARA REDUÇÃO DA INCOMPATIBILIDADE ENTRE
SISTEMAS ADESIVOS SIMPLIFICADOS E CIMENTOS RESINOSOS
DE DUPLA ATIVAÇÃO*

Tese apresentada Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, como parte dos requisitos exigidos para obtenção do título de Doutora em Materiais Dentários.

Thesis presented to the Piracicaba School of Dentistry of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Dental Materials.

Este exemplar corresponde à versão final da tese defendida pela aluna Ariene Arcas Topal Paes Leme e orientada pelo Prof. Dr. Mario Alexandre Coelho Sinhoreti.

Assinatura do orientador

Piracicaba

2013

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Odontologia de Piracicaba
Marilene Girello - CRB 8/6159

L542p Leme, Ariene Arcas Topal Paes, 1986-
Procedimentos para redução da incompatibilidade entre sistemas adesivos simplificados e cimentos resinosos de dupla ativação / Ariene Arcas Topal Paes Leme. – Piracicaba, SP : [s.n.], 2013.

Orientador: Mário Alexandre Coelho Sinhoreti.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Cimentos de resina. 2. Nanoindentação. 3. Sistemas adesivos. I. Sinhoreti, Mário Alexandre Coelho, 1969-. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Procedures for reducing the incompatibility between simplified-step adhesive systems and dual-cured resin cements

Palavras-chave em inglês:

Resin cements

Nanoindentation

Adhesive systems

Área de concentração: Materiais Dentários

Titulação: Doutor em Materiais Dentários

Banca examinadora:

Mário Alexandre Coelho Sinhoreti [Orientador]

Sandrine Bittencourt Berger

William Cunha Brandt

Simonides Consani

Marcelo Giannini

Data de defesa: 17-12-2013

Programa de Pós-Graduação: Materiais Dentários

FOLHA DE APROVAÇÃO



UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba



A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 17 de Dezembro de 2013, considerou a candidata ARIENE ARCAS TOPAL PAES LEME aprovada.

Handwritten signature in blue ink.

Prof. Dr. MARIO ALEXANDRE COELHO SINHORETI

Handwritten signature in blue ink.

Profa. Dra. SANDRINE BITTENCOURT BERGER

Handwritten signature in blue ink.

Prof. Dr. WILLIAM CUNHA BRANDT

Handwritten signature in blue ink.

Prof. Dr. SIMONIDES CONSANI

Handwritten signature in blue ink.

Prof. Dr. MARCELO GIANNINI

RESUMO

Neste estudo avaliou-se o efeito: (1) da adição de resina de troca aniônica (AER) a um cimento experimental, para reduzir a incompatibilidade entre sistemas adesivos simplificados e cimento resinoso de polimerização dupla, quando ativado quimicamente; (2) da aplicação de uma camada extra de adesivo entre o sistema adesivo simplificado e o cimento resinoso, na resistência da união, propriedades nanomecânicas da camada de cimento e micropermeabilidade na interface adesiva. Discos de resina composta foram cimentados com os sistemas adesivos ACE ALL Bond TE ([ACE] Bisco Inc.) e Scotchbond Universal ([SCU] 3M ESPE). No capítulo um, foram utilizados os cimentos experimentais controle (sem AER, mesma composição do DuoLink), experimental-1 (AER na forma OH) e experimental-2 (AER forma OH+F). Após a cimentação, foram obtidos palitos, os quais foram avaliados com os testes de microtração e nanoindentação da camada de cimento, após 24 h e seis meses de envelhecimento. No capítulo dois, foi utilizado o cimento DuoLink (Bisco Inc.) e os grupos foram separados considerando-se a aplicação ou não de uma camada extra de resina hidrófoba. Após 24 h de armazenagem, os dentes foram cortados e os palitos submetidos ao teste de microtração, nanoindentação da camada de cimento e micropermeabilidade através da observação da presença de rodamina B na interface adesiva. Os dados foram analisados com Anova 3 fatores para o primeiro capítulo e Anova 2 fatores para o segundo capítulo, seguidos pelo teste de Tukey com $\alpha=0,05$, para a comparação entre as médias. Não houve diferença na resistência da união entre os grupos controle e os de cimento com AER, bem como entre o controle e os grupos com aplicação da camada extra de adesivo. A resistência da união permaneceu estável após seis meses de armazenamento. Em 24 h, maior módulo de elasticidade reduzido (E_r) e nanodureza (H) foram observados para os cimentos com AER em relação aos dos grupos controle ($p<0,001$), assim como após aplicação da camada extra de adesivo maiores E_r e H foram observados em relação aos grupos em que o cimento foi aplicado diretamente em contato com o adesivo simplificado ($p<0,001$). Maior resistência da união foi observada nos grupos cimentados com SCU ($p<0,001$). Não foi observada diferença na quantidade de infiltração por rodamina entre os grupos. Pode-se concluir que os métodos avaliados para reduzir os efeitos negativos da incompatibilidade entre os sistemas adesivos simplificados e os cimentos resinosos avaliados sob ativação química resultaram em maiores módulo de elasticidade reduzido e nanodureza da camada de cimento; entretanto, não influenciaram na resistência adesiva.

Palavras-chave: cimentos de resina, nanoindentação, resistência da união, sistemas adesivos.

ABSTRACT

This study evaluated the effect of: (1) adding anion exchange resin (AER) to an experimental resin cement aiming to reduce incompatibility between simplified-step adhesive systems and dual-cured resin cement when chemically activated; (2) the application of an extra adhesive resin layer between the simplified adhesive system and resin cement, on the microtensile bond strength, nanomechanical properties of the cement layer and micropermeability at the adhesive layer. Resin composite discs were luted with the adhesive systems ACE ALL Bond TE ([ACE] Bisco Inc.) and Scotchbond Universal ([SCU] 3M ESPE). In the chapter one, experimental resin cements were used, being control (no AER added, same composition as of DuoLink), experimental-1 (OH form of AER) and experimental-2 (OH+F form of AER). After cementation procedures, beams were obtained and evaluated for microtensile bond strength and nanoindentation of the cement layer, after 24 h and six months aging. In chapter two, the cement DuoLink (Bisco Inc.) was used and groups were divided after application or not of an extra adhesive resin layer. After 24 h, teeth were cut and beams underwent evaluation of the microtensile bond strength, nanoindentation of the cement layer and micropermeability by means of observing rhodamine B infiltration at the adhesive interface. Data were analyzed using 3-way Anova for the first chapter and 2-way Anova for the second chapter, followed by Tukey's post-hoc test with $\alpha=0.05$. There were no differences on the bond strength among control and the AER added groups, as among control and the groups with application of the extra adhesive resin layer. The bond strength remained stable after six months aging. At 24 h, higher reduced modulus of elasticity (E_r) and nanohardness (H) were observed for the AER added cements compared to the controls ($p<0.001$), as well as after the application of the extra adhesive resin layer, higher E_r and H were observed when compared to the groups which the cement was applied directly above the simplified-step adhesive system ($p<0.001$). Higher bond strength was observed for the groups luted with SCU ($p<0.001$). No difference was observed on the rhodamine infiltration among the groups. It can be concluded that the evaluated methods aiming to reduce the negative effects of the incompatibility between simplified-step adhesive systems and resin cements evaluated under self-cure activation resulted in higher reduced modulus of elasticity and nanohardness of the cement layer; however, no influence was observed on the microtensile bond strength.

Key Words: resin cements, nanoindentation, bond strength, adhesive systems.

SUMÁRIO

DEDICATORIA	XIII
AGRADECIMENTOS	XV
INTRODUÇÃO	1
CAPÍTULO 1: Effect of anion exchange resin-added resin cements and simplified-step adhesive systems on bond strength and nanomechanical properties of the cement layer	4
CAPÍTULO 2: Extra hydrophobic resin layer effect on bond strength, nanomechanical properties and micropermeability of adhesively luted indirect resin restoration	24
CONSIDERAÇÕES GERAIS	45
CONCLUSÃO	49
REFERÊNCIAS	50
ANEXOS	51
APÊNDICE	53

*Dedico este trabalho aos meus pais,
Luiz Antonio e Clery,
exemplos de caráter e dedicação.
Jamais mediram esforços para as minhas
formações profissional e pessoal.
Agradeço por todo incentivo, orientação,
compreensão e amor.*

*A minha irmã **Ariadne**, presente
em todos os momentos da minha vida,
pela amizade, companheirismo e amor.*

AGRADECIMENTOS

A **Deus**, pelo apoio em todos os momentos, por iluminar e guiar meus passos, colocando sempre em meu caminho pessoas corretas, levando a mais uma conquista.

Ao **Prof. Dr. Mário Alexandre Coelho Sinhoreti**, Titular da Área Materiais Dentários, do Departamento de Odontologia Restauradora, da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, meu orientador, pelo inestimável apoio, dedicação e paciência durante os cinco anos de trabalho juntos, na orientação deste estudo e principalmente pela amizade. Obrigada pela confiança e por ajudar no meu crescimento, tanto pessoal como científico.

A **Prof. Dra. Ana Karina Bedran-Russo**, Professora do Departamento de Odontologia Restauradora da Universidade de Illinois, em Chicago, EUA, pela amizade, acolhida e atenção a mim dispensadas durante todo o período de doutorado sanduíche. Acima de tudo, por todos os ensinamentos, tanto relacionados valores que agreguei a minha vida pessoal após esta experiência, como a inestimável contribuição científica para a realização deste estudo. Muito obrigada.

À direção da Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, na pessoa de seu Diretor **Prof. Dr. Jacks Jorge Júnior** e do Diretor Associado **Prof. Dr. Alexandre Augusto Zaia**.

À coordenação geral de Pós-Graduação da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas na pessoa da sua coordenadora **Profa. Dra. Renata Cunha Matheus Rodrigues Garcia**, e do coordenador do Programa de Pós-Graduação em Materiais Dentários **Prof. Dr. Marcelo Giannini**.

Ao **Prof. Dr. Simonides Consani**, Titular da Área Materiais Dentários, do Departamento de Odontologia Restauradora, da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, grande educador, pela sua dedicação durante o curso, sempre incentivando a busca pelo conhecimento.

Ao **Prof. Dr. Lourenço Correr-Sobrinho**, Titular da Área Materiais Dentários, do Departamento de Odontologia Restauradora, da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, pela amizade, incentivo e contribuição para a minha formação.

Ao **Prof. Dr. Mario Fernando de Goes**, Titular da Área Materiais Dentários, do Departamento de Odontologia Restauradora, da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, pelo convívio e contribuição em minha formação por meio de todo conhecimento transmitido e dedicação.

A **Profa. Dra. Regina Maria Puppim-Rontani**, Titular da Área Odontopediatria, do Departamento de Odontologia Infantil, da Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, por todos os ensinamentos dedicados ao longo desses anos essenciais para a minha formação.

Ao **Prof. Dr. Marcelo Giannini**, Professor da Área Dentística, do Departamento de Odontologia Restauradora, da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, Coordenador do Programa de Pós-graduação em Materiais Dentários, pela amizade e pela contribuição em meu crescimento científico e pessoal.

Ao técnico especializado do laboratório da Área Materiais Dentários, da Faculdade de Odontologia de Piracicaba, engenheiro e mestre em Materiais Dentários **Marcos Blanco Cangiani (Marcão)**, pela amizade, paciência, convívio e

grande colaboração na execução de todos os trabalhos. Sua ajuda e carisma foram fundamentais.

A **Selma Aparecida Barbosa de Souza Segalla**, técnica da Área Materiais Dentários da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, pela amizade, paciência, convívio e ajuda em todos os momentos solicitados.

Ao meu namorado, **Patrick Kraus**, pela compreensão e companheirismo. Obrigada também por abrir as portas da sua casa, me permitindo participar junto a você e sua família de momentos tão especiais.

Aos amigos, **Cristina Vidal, Breno S. Rodrigues, Thaiane Aguiar e Fabio Barreto**, por toda ajuda e companheirismo durante minha estadia em Chicago. A amizade de vocês foi de importância inestimável para amenizar a saudade da família e dos amigos aqui no Brasil.

As minhas grandes amigas de Pós-graduação, **Polliana Scaffa, Giovana Araújo, Ana Paula Piovezan Fugolin, Andreia Bolzan de Paula, Gislaine Padovani, Adriana Carvalho, Fabiana Scarparo, Livia Galvão, Cristina Caldas, Ailla Lancelotti** pela amizade e companheirismo ao longo desses anos. Sempre presentes e dispostas a ajudar. Obrigado pelos agradáveis momentos de convívio.

As grandes amigas **Margareth Coutinho e Sonia Maria Fernandes**, pela amizade e torcida durante todo esse tempo.

Aos amigos(as) e colegas da FOP, **Victor Feitosa, Américo Bortolazzo, Guilherme Guarda, Karla Mychellyne, Marcos Bonfim, Rafael Pinno Vitti, Larissa Sgarbosa, Renata Bacelar, Luciano Gonçalves e William Brandt**, pelos bons momentos de amizade.

A todos os **colegas** de Pós-graduação deste e de outros programas.

À **Fundação e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)**, pela bolsa concedida.

À empresa de materiais odontológicos Bisco Inc., na pessoa do **Dr. Liang Chen**, pelo auxílio no preparo dos materiais experimentais.

A minha avó **Ivanir** e todos os meus **familiares**, pelo apoio e incentivo dispensados durante toda a minha vida.

A **todos** que indiretamente contribuíram ou torceram para a realização deste trabalho.

Meus sinceros agradecimentos.

“O êxito da vida não se mede pelo caminho que você conquistou,
mas sim pelas dificuldades que superou no caminho.”

Abraham Lincoln

INTRODUÇÃO

Para a cimentação de restaurações indiretas, sejam elas de resina composta ou de cerâmica, a escolha recai no cimento resinoso associado a um sistema adesivo capaz de polimerizar com pouca intensidade de luz ou mesmo na ausência dela (Darr e Jacobsen, 1995). Ainda, devido à existência de um anteparo entre a ponta da unidade fotoativadora e o material de cimentação, pode reduzir a quantidade de fótons disponíveis para ativar o fotoiniciador (Meng *et al.*, 2008; Piva *et al.*, 2008). Assim, grande parte da reação de polimerização se inicia pela formação de radicais livres decorrentes da reação entre o peróxido de benzoíla e a amina terciária.

Devido à complexidade e possibilidade de erros durante a aplicação de sistemas adesivos, como por exemplo, a correta dosagem de solução primer e catalisador e aplicação na superfície dentinária com a umidade adequada, há uma tendência na simplificação dos procedimentos de aplicação dos adesivos, reduzindo assim o tempo de atendimento clínico e a possibilidade de erro. Entretanto, foi observado que os sistemas adesivos simplificados, nos quais as porções hidrófilas e hidrófobas estão contidas no mesmo frasco, mesmo após a polimerização permitem a passagem de água (Chersoni *et al.*, 2005), agindo então como membranas permeáveis. Aliado a isso, principalmente para adesivos auto-condicionantes de passo único, a presença de monômeros acídicos na superfície da camada de adesivo, a qual tem a polimerização inibida (Rueggeberg e Margeson, 1990), pode levar a uma reação indesejada com compósitos de ativação química, sejam eles para restauração ou para cimentação. Esta reação foi reportada como incompatibilidade entre sistemas adesivos simplificados e compósitos resinosos de ativação química ou dupla (Sanares *et al.*, 2001; Franco *et al.*, 2002; Tay *et al.*, 2003; Schittly *et al.*, 2010). Os monômeros acídicos remanescentes na superfície da camada de adesivo podem reagir e assim, inativar a amina terciária no compósito de ativação química, (Sanares *et al.*, 2001; Cheong *et al.*, 2003) que por sua vez, deveria reagir com o peróxido de benzoíla, garantindo a formação de

radicais livres e, conseqüentemente, iniciando a reação de polimerização com ativação química. Assim, a polimerização do compósito quimicamente ativado ficaria prejudicada.

Para tentar eliminar esse efeito adverso na polimerização de resinas à base de metacrilatos, foi proposto incluir agentes redutores como sais de ácido sulfínico e propanal nos sistemas adesivos. Estes sais agiriam como ativadores da reação de polimerização e não seriam sensíveis ao pH ácido (Tay *et al.*, 2003). Foram encontrados resultados positivos com este método (Suh *et al.*, 2003); entretanto, em presença de umidade, a inclusão destes agentes redutores não foi capaz de evitar a incompatibilidade (Tay *et al.*, 2003). Também foi sugerido o uso de uma camada de resina adesiva hidrófoba após a aplicação do adesivo, a qual serviria como barreira, impedindo que a água atravessasse a camada de adesivo e evitasse o contato direto entre sistema adesivo simplificado e o cimento resinoso de ativação química e/ou dupla. Contudo, este procedimento poderia resultar em aumento na espessura da camada adesiva, e assim, afetar a adaptação da peça em um procedimento restaurador indireto (Tay *et al.*, 2003).

Outro possível meio de evitar a incompatibilidade entre cimentos de ativação química e adesivos simplificados relatado na literatura (Finger *et al.*, 2005, Kanehira *et al.*, 2006) seria a incorporação de resinas de troca aniônica (AER). Este material foi aplicado inicialmente entre a camada de sistema adesivo simplificado e cimento resinoso de ativação química, resultando em melhor resistência da união, em comparação aos grupos em que o cimento de ativação química foi aplicado diretamente sobre o adesivo. As AERs, são encontradas em íons na forma de cloreto (Cl⁻) e por meio de trocas iônicas podem ser convertidas em outras formas, como por exemplo, em hidroxila (OH⁻). Após serem convertidas em OH⁻, possuem pH básico e podem reagir com os monômeros acídicos presentes na camada de adesivo, evitando que estes inativem a amina terciária (Kanehira *et al.*, 2006; Endo *et al.*, 2007).

Assim, seria interessante avaliar tanto o efeito da aplicação de uma camada adesiva hidrófoba após o uso de sistemas adesivos simplificados, como o efeito da

adição de AERs a um cimento resinoso quimicamente ativado, na redução da incompatibilidade com sistemas adesivos simplificados. Para isso, na primeira parte deste estudo (Capítulo 1) foi avaliada a efetividade da adição de resinas de troca aniônica (AER) a um cimento resinoso experimental, como meio de evitar a incompatibilidade com os sistemas adesivos simplificados. Na segunda parte (Capítulo 2) foi avaliada a utilização de uma camada extra de adesivo hidrófobo, entre o sistema adesivo e o cimento resinoso quimicamente ativado.

O presente trabalho está apresentado no formato alternativo de tese de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas. O artigo referente ao Capítulo 1 está formatado e submetido ao periódico *Journal of Dentistry*. O artigo referente ao Capítulo 2 está formatado e será submetido ao periódico *Operative Dentistry*.

CAPÍTULO 1

Effect of anion exchange resin-added resin cements and simplified-step adhesive systems on bond strength and nanomechanical properties of the cement layer

Ariene Arcas Leme*¹, Americo Bortolazo Correr¹, Lourenço Correr-Sobrinho¹, Ana Karina Bedran-Russo², Mario Alexandre Coelho Sinhoreti¹

¹Department of Restorative Dentistry, Dental Materials, Piracicaba Dental School, University of Campinas; 901 Limeira Av; CEP: 13414-903; PO BOX: 52; Piracicaba SP; Brazil.

²Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, Chicago, IL, USA. 801 South Paulina Street, room 551 Chicago, IL 60612.

*Corresponding author:

Ariene Arcas Leme

Dental Materials, Department of Restorative Dentistry

Piracicaba Dental School, State University of Campinas-UNICAMP

901 Limeira Av.

Piracicaba, SP, Brazil

13414-903; PO BOX: 52;

Phone number: 55-67-99081986

Email: arienearcasleme@gmail.com

Short title: Bond strength and nanomechanical properties of resin cements

Keywords : resin cements, adhesive systems, bond strength, nanoindentation, incompatibility

ABSTRACT

Objectives: The effect of an anion exchange resin (AER) added to resin cement in reducing incompatibility between simplified-step adhesive systems and dual-cure resin cements was assessed by microtensile bond strength (μ TBS), reduced modulus of elasticity (E_r) and nanoindentation hardness (H) evaluations of the dentin-cement interface.

Methods: Experimental resin cements were prepared with hydroxyl (OH^-) and fluoride (F^-) forms of AER. Resin blocks were luted on flat occlusal dentin surfaces of 42 molars teeth using either ACE All Bond TE (ACE) or Scotchbond Universal (SCU) adhesive systems. The adhesive systems and resin cements [Control (no AER); Experimental-1 (AER in OH^- form) and Experimental-2 (AER in OH^+F form)] were used in self-cure mode. After 24 h, resin/dentin beams were obtained and prepared for μ TBS, E_r and H evaluations at two periods: immediate and after 6 months of water storage. The data were statistically analyzed using a 3-way Anova and Tukey's test ($\alpha=5\%$).

Results: There was no difference in μ TBS among the resin cements ($p>0.05$). SCU restored groups showed highest μ TBS ($p=0.000$). Significantly higher E_r were observed for experimental-1 and experimental-2 ($p=0.000$) at 24h, and higher H ($p=0.000$) at both periods. Significant decrease in H was observed after 6 months ($p=0.000$).

Conclusions: Adding AER improved resin cement nanomechanical properties, however no difference was observed on bond strength. The bond strength of adhesively luted indirect composite restorations was dependent of the adhesive system. Water aging affected H of the cement layer.

Clinical Significance: Incompatibility reaction did not affect bond strength of tested simplified-step adhesive systems and dual-cured resin cements. Adding anion exchange resins to dual-cured resin cements may improve their mechanical properties, when not light-cured. The exposed resin cement layer is susceptible to long-term water degradation.

1. Introduction

Self and dual-cure resin cements are currently used to lute indirect restorations and pre-fabricated posts. Besides the presence of the intermediate restoration between the light source and resin cement, the increased distance from the light source may result in reduced number of photons to initiate the polymerization(1). Thus, the polymerization will rely mostly on the self-cure activation (2, 3). The adhesive system is commonly used under self-curing activation to avoid large marginal discrepancies. Apart from ensuring a better adaptation of the indirect restoration, manufacturers have added an additional bottle of activator to the adhesive systems that when in contact with the resin cement, it is claimed to help with the resin cement polymerization (4).

Simplified-step adhesive systems may result in an excessively hydrophilic blend (5), more prone to water sorption and degradation of the adhesive interface (6). The incompatibility problem observed in certain simplified-step adhesives are due to 1. the adverse chemical interaction (7-11) between the nucleophilic amine from the resin cement and the unreacted acidic monomers at the adhesive system surface (12), and 2. the presence of water at the adhesive interface (13) likely to avoid polymerization (14), resulting in a higher concentration of remaining acidic monomers on the top layer of the adhesive system. Aiming to reduce the concentration of unreacted acidic monomers at the adhesive system surface, a reducing agent known as benzene sulphinic acid sodium salt was added to the adhesive systems formulation (15). No incompatibility problem was observed when the adhesive systems with added reducing agent were applied to dry dentin. However, having a completely dry tooth is not clinically possible (15). Thus, the water is still likely to impair the polymerization, leaving unreacted acidic monomers at the adhesive system surface.

Adding anion exchange resins (AER) to an unfilled resin has proven efficient in avoiding incompatibility (16). For this, the originally chloride (Cl⁻) form of the AER

was converted to an alkaline hydroxyl (OH⁻) resin form. The AER was then mixed to hydrophobic methacrylate monomers and applied as an additional layer. This prevented the acidic monomers to neutralize the nucleophilic tertiary amine (17, 18). Because dental resins initiators are claimed to work better when in a less acidic environment, it should be of interest to evaluate the effect of introducing the alkaline form of AER to the resin cement base. The AER are found in Cl⁻ form and can be converted to any ionic form.

Maintaining the bond between the tooth and the indirect restoration is imperative for the clinical success of composite or ceramic indirect restorations (19). All-ceramic restorations cannot resist to plastic deformation, thus the underneath cement layer is important help dissipating the stress resulting from chewing and other forces. The nanoindentation technique, using a mechanical properties microprobe, allowed assessment of the mechanical properties of materials in a submicron scale (20). Using this technique, the reduced modulus of elasticity (E_r) and nanoindentation hardness (H) can be determined on very thin and small material layers (21).

Therefore, the aim of this study was to evaluate the effect of adding anion exchange resin to a resin cement used with two simplified-step adhesive systems on the microtensile bond strength and the nanomechanical properties of the resin cement layer when luting an indirect composite restoration under self-cure polymerization. The hypotheses evaluated were: 1. adding ionic charged anion exchange resin to a dual cure resin cement can improve the E_r and H of the resin cement when used to lute an indirect composite restoration; 2. the microtensile bond strength of the luted restoration is affected by the type of adhesive system and resin cement used; and 3. water aging can decrease the microtensile bond strength and nanomechanical properties of the resin cement layer.

2. Material and Methods

2.1 Experimental resin cements preparation

Experimental resin cements were prepared using the base composition of Duolink cement (Bisco, Schaumburg, IL, USA): bisphenol A diglycidyl methacrylate (bis-GMA), Triethyleneglycol Dimethacrylate (TEGDMA), Urethane dimethacrylate (UDMA), glass filler; and catalyst: bis-GMA, TEGDMA, glass filler. The amount of initiators was kept constant for the three resin cements; however, the exact concentration is confidential (Bisco Inc.) and could not be provided. For the control group, there were no changes from the original composition. The anion exchange resin (Sigma-Aldrich Co., St. Louis, MO, USA), originally in chloride (Cl^-) form, was manually pulverized and converted to a hydroxyl (OH^-) and fluoride (F^-) form and added to the two experimental resin cements. Preliminary tests (flexural strength, Knoop hardness and degree of conversion tests) were carried out to determine the adequate AER concentration that would not have any significant impact on the mechanical properties of the experimental resin cements. The corresponding amount (4%) of OH^- -form and OH^-/F^- -form AER was incorporated to the base component of the resin cement, in weight. The amount of fillers was constant for all experimental resin cements (60/38% in weight/volume). The AER powder was added to the resin base and mixed using a centrifuge mixer SpeedMixer DAC 150FV (Flacktek, Landrum, SC, EUA), at 2500 rpm for 6 min. For the resin cement Experimental-1, 4% of anion exchange resin on the OH^- form was added to the base; and for the Experimental-2, 4% of anion exchange resin, consisting of 2% on the chloride OH^- form and other 2% on the F^- form was added to the base.

2.2 Specimen preparation

Composite resin blocks were prepared using the core build-up resin Bis-core (Bisco, Schaumburg, IL, USA). The round metallic matrix (10 mm diameter and 5 mm thick) was positioned over a glass slide and the core resin was incrementally

inserted to fill the mold with a little excess. Then, a mylar strip (Moyco Technologies, York, PA, USA) was held above the matrix with a 50 g weight and light-cured for 60 s with the halogen light-curing device Optilux 501 (Demetron/Kerr, Danbury, CT, USA).

For this study, 42 recently extracted human molars were used after approval from the local board committee (protocol #112/2011). Teeth were cleaned and occlusal enamel was removed with a #180-grit SiC paper (Buehler, Lake Bluff, IL, USA) to expose a flat surface of coronal dentin. Teeth were randomly assigned into 3 main groups (Control, Experimental-1 and Experimental-2) each with two subgroups (ACE ALL Bond TE [ACE/Bisco, Schaumburg, IL, USA] and Scotchbond Universal with Scotchbond Universal Dual Cure Activator [SCU/3M ESPE, St. Paul, MN, USA]). The smear layer was standardized using a #600-grit SiC paper (Buehler), for 30 s. The composition of the adhesive systems employed in this study is shown in Table 1.

For the three groups, luting procedures were performed following the respective adhesive manufacturers' instructions (Table 1). The adhesive system in contact with dentin and resin cement was allowed to self-cure.

Table 1 – Adhesive system composition and bonding procedures

Adhesive system	Composition	Dentin treatment	Resin disc treatment
ACE (Bisco)	Ethanol, NTG-GMA Salt, bis-GMA, HEMA, BPDM	Acid etched 15 s and water rinsed. One drop from each component of the adhesive system was dispensed in a case and mixed for 5 s. The adhesive system was applied to the moist dentin surface under agitation for 10 s. Excess solvent was volatilized for 15 s.	Phosphoric acid cleansing for 60 s, water rinsed and air dried. One layer of adhesive system was applied at the surface for 15 s. The excess solvent was volatilized for 15 s and light-cured for 20 s.
SCU (3M ESPE)	MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water initiators and silane	Phosphoric acid etching for 15 s and water rinsed. One drop of each bottle was dispensed in a case and the adhesive system was applied to the moist dentin surface under agitation for 20 s. Excess solvent was volatilized for 15 s.	Phosphoric acid etching for 60 s, rinsed and air dried. One layer of the adhesive system was applied at the surface for 20 s. The excess solvent was volatilized for 15 s and light-cured for 20 s.
Abbreviations: N-tolyglycine glycidyl methacrylate (NTG-GMA), 2-hydroxyethyl methacrylate (HEMA), bisphenol A diglycidyl methacrylate (bis-GMA), 10-methacryloyloxydecyl dihydrogen phosphate (MDP), biphenyl dimethacrylate (BPDM).			

For the luting procedures, the corresponding resin cement (Control, Experimental-1 and Experimental-2) was dispensed on the resin disc surface using an automix tip. After adhesive system application (Table 1), the resin disc was positioned on the dentin surface and hold in position for 7 min, with a 50 g weigh positioned above the resin disc, to standardize the seating pressure.

After luting procedures, the restored teeth were stored for 24 h in a lightproof container at 37°C and 100% humidity. After, the teeth were cut parallel to the long axis using a low speed precision saw (Isomet 1000, Buehler) to result in 1 mm² resin/dentin beams. Half of the resin/dentin beams were used for the immediate microtensile bond strength (μ TBS) and nanomechanical properties evaluation. The

remaining beams were stored and evaluated after 6 months aging in distilled water at 37 °C. The water was changed every 15 days.

2.3 Microtensile bond strength assay

After 24 h and 6 months of storage, the resin/dentin beams were fixed with a cyanoacrylate based adhesive (Loctite, Henkel Corp., Rocky Hill, CT, USA) to the device attached to the microtensile tester machine (Bisco Inc.). A tensile load at 1mm/min cross-head speed, applied until the rupture of the specimen. Bond strength (MPa) was calculated dividing the maximum load at failure by the specimen cross-sectional area. The corresponding data were statistically analyzed using three-way Anova and Tukey's post hoc test with significance level $\alpha=0.05$.

The fractured beams were examined to determine the failure mode, using a stereoscope loupe (Zeiss, Göttingen, Germany) under 60 x magnification. The failures were classified as adhesive failure at the adhesive/resin or adhesive/dentin interface (A); cohesive failure at the resin disc or the dentin (C) and mixed failure as a mixture of the two failures described above (M). Representative specimens were mounted in an aluminum stub, sputter-coated with gold and observed by scanning electron microscopy (SEM) (JEOL, JSM-5600LV, Tokyo, Japan), under 15 Kv.

2.4 Nanomechanical properties evaluation of the resin cement layer

After the respective storage time, two resin/dentin beams, from the same tooth as for the μ TBS, were embedded in epoxy resin (Buehler). Immediately before the nanoindentation test, the specimens were polished using #400, 600, 800 and 1200-grit SiC paper (Buehler Inc.) followed by the diamond suspensions 9, 6, 3, 1 and 0.05 μ m (Buehler Inc.). Samples were then positioned inside the nanoindenter chamber and a wax barrier was used to keep the specimens immerse in distilled water at room temperature, while performing the indentations.

The reduced modulus of elasticity (E_r) and nanoindentation hardness (H) of the resin cement layer was obtained from the resulting force-displacement curve, using a triboindenter (Hysitron Inc, Minneapolis, MN, USA) equipped with a

Berkovich fluid cell tip and calculated following the method of Oliver and Pharr (20). The initial calibration procedure was carried out using a fused silica sample with known elastic modulus ($E_r = 69.6$ GPa) and hardness ($H = 9.25$ GPa), in order to establish the correct relationship between depth of indent and tip contact area. A standardized trapezoidal load function set as 20-10-20s was applied with a 1,000 μ N peak load to the luting cement layer. The statistical analysis was performed for each E_r and H parameters using a three-way Anova and Tukey's post-hoc test with the significance level as 5%.

3. Results

No significant interaction was observed between the factors evaluated ($p > 0.05$) for the μ TBS data. No statistically significant difference was observed between the two evaluated times ($p = 0.194$) and among the different resin cements ($p = 0.779$). The adhesive system Scotchbond Universal attained the higher mean bond strength ($p = 0.000$), regardless of the resin cement and time evaluated (Table 2). The most common type of failure occurred at the adhesive interface (Table 2). Scanning electron micrographs of representative specimens show voids at the interface bonded with ACE and porosities at the resin cement layer in contact with SCU (Fig. 1).

Table 2 – Microtensile bond strength (MPa) and failure mode of all groups at the two periods evaluated.

Group		Period		Failure mode
		24 h	6 months	
Control	ACE	26.0 (13.8) B	25.0 (12.1) B	45.8% A; 34.7% C; 19.5% M
	SCU	34.6 (13.2) A	34.1 (7.1) A	66.7% A; 13% C; 20.3% M
Experimental-1	ACE	27.5 (8.6) B	18.3 (6.2) B	71.4% A; 14.4% C; 14.2% M
	SCU	34.1 (9.5) A	30.4 (6.2) A	60.8% A; 23.5% C; 15.7% M
Experimental-2	ACE	23.4 (9.6) B	19.7 (6.1) B	79.0% A; 12.7% C; 8.3% M
	SCU	33.8 (8.0) A	35.1 (13.3) A	56.9% A; 22.4% C; 20.7% M

Values are mean (standard deviation). Different letters indicate statistically significant difference between the adhesive systems ($p < 0.05$). Failure modes: (A) adhesive failure at the adhesive/resin or adhesive/dentin interface, (C) cohesive failure at the resin disc or the dentin and (M) mixed failure.

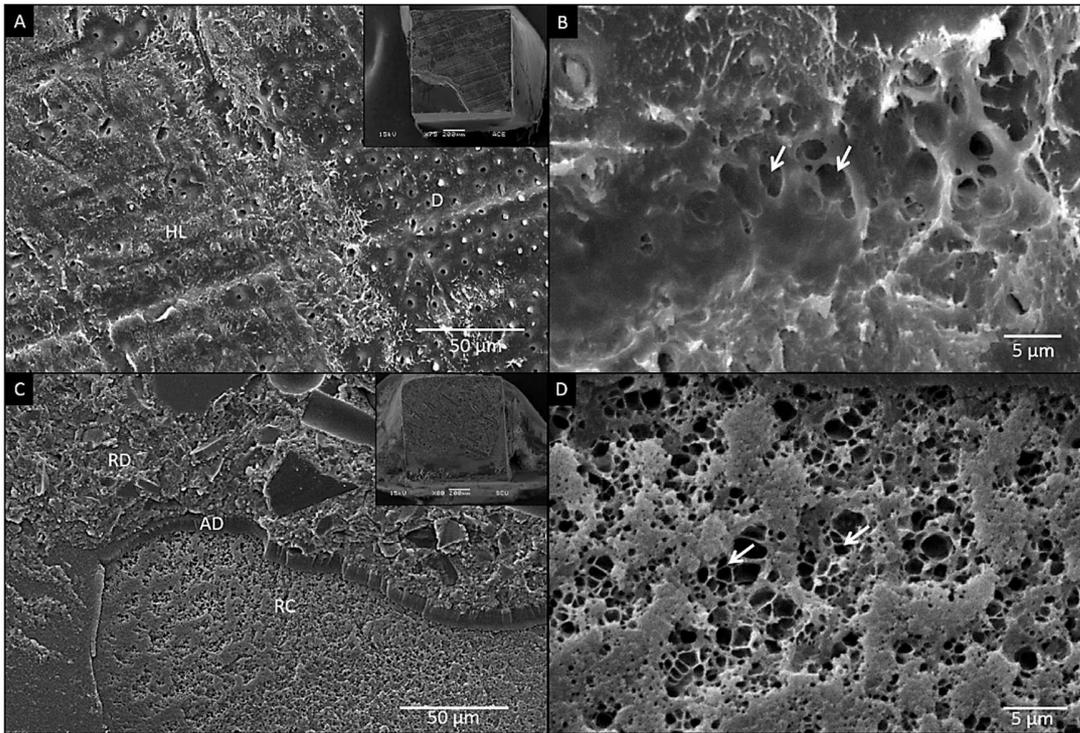


Fig. 1 – SEM micrographs of beams fractured at the adhesive and resin cement interface, in lower x500 and higher x3,000 magnification (HL: hybrid layer; D: dentin; RD: composite resin disc; AD: adhesive layer; RC: resin cement). Images A and B correspond to a fractured beam luted with ACE and the control resin cement. The white arrows in B indicate areas of porosity at the adhesive system. Images C and D were observed from a fractured beam luted with SCU and the control resin cement. In D, the porosities were observed at the resin cement layer in contact with the adhesive.

Graphs depicting the modulus of elasticity data are shown in Fig. 2, there was significant interaction between the factors time and resin cement ($p=0.010$). Regarding the adhesive system, no difference was observed on the modulus of elasticity at the resin cement layer ($p>0.05$). After 24h, the modulus of elasticity of the Experimental-1 and Experimental-2 groups were statistically higher than the control groups, regardless of the adhesive system ($p=0.000$). However, after 6

months of storage, no difference was observed on the modulus of elasticity among the three groups ($p>0.05$).

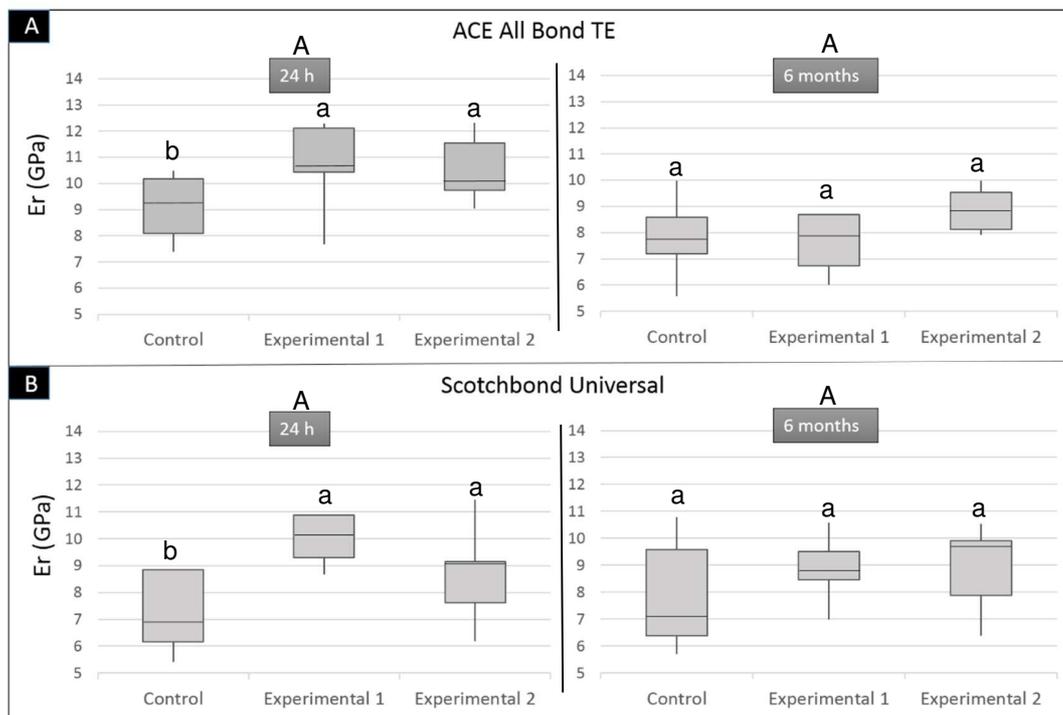


Fig. 2 – Graphs representing the reduced modulus of elasticity (E_r) of the Control, Experimental-1 and Experimental-2 groups, for the two adhesive systems evaluated after 24 h and 6 months of storage. Different upper-case letters indicate significant difference between the two periods and lower-case letters among the resin cements ($p=0.05$).

The graphs showing the nanohardness data are in Fig. 3, no interactions were observed among the factors ($p>0.05$). Regardless of the adhesive system, lower nanohardness was observed at the resin cement layer after 6 months of water aging ($p=0.000$). The experimental groups 1 and 2 attained statistically higher hardness than the control group ($p=0.000$).

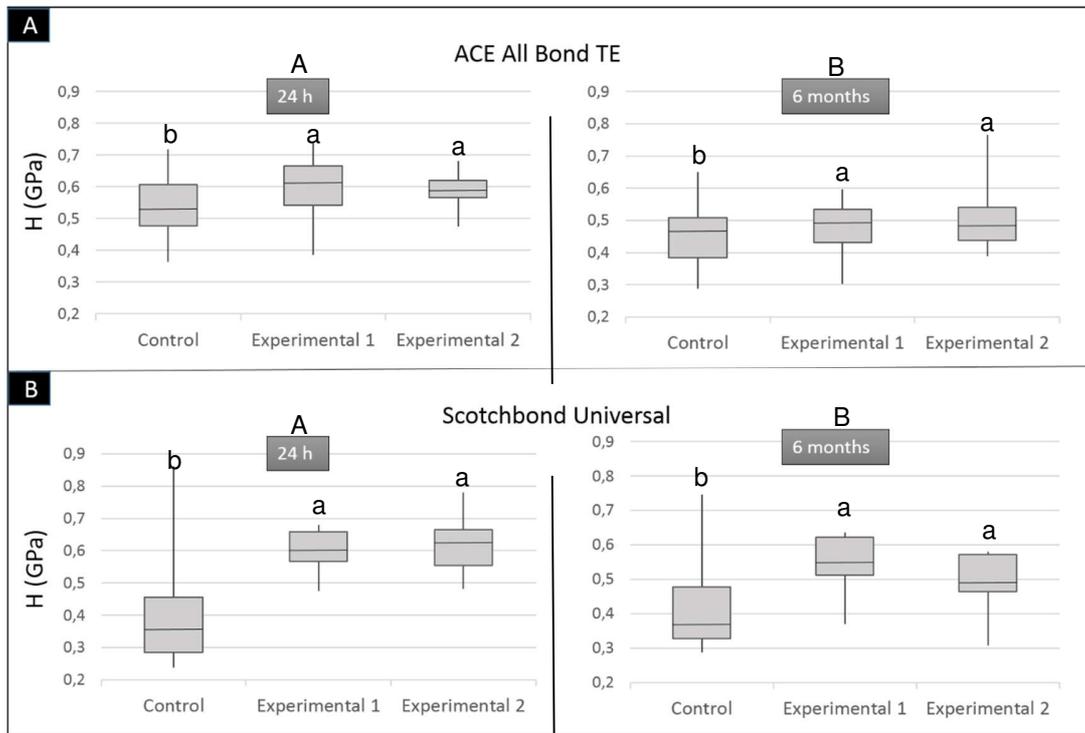


Fig. 3 – Graphs representing the hardness data for the Control, Experimental-1 and Experimental-2 groups, regarding the adhesive system after 24 h and 6 months of storage. Different upper-case letters indicate significant difference between the two periods and lower-case letters among the resin cements ($p=0.05$).

4. Discussion

Experimental resin cements containing AER were evaluated in combination with two commercial adhesive systems with acidic characteristic, regarding to the microtensile bond strength and nanomechanical properties of the cement layer. Previous studies (16, 18), reported that adding AER on the OH⁻ form to a self-cured unfilled resin, was able to avoid the incompatibility observed between acidic adhesives and dual or self-cured resin cements. However according to the results from the current study, it is likely that changes in the monomeric structure of the new simplified-step adhesive systems have led to the improvement on bond strength and lasting of the adhesively luted resin blocks.

The hypothesis that adding AER to the resin cement base would improve the cement layer properties was partially accepted. Higher E_r and H were observed for the experimental groups at the 24 h period. Adding alkaline AER resins to the resin cement base, may have increased the rate of polymerization of the self-cured resin cement, resulting in immediate higher nanomechanical properties. A previous study (22) reported significant reduction on the modulus of elasticity of adhesive interfaces after 6 months of storage using nanoindentation. Furthermore, a significant relationship between water sorption and a decrease on the modulus of elasticity of adhesive resins was reported (5). Although the indentations were performed at the resin cement layer, similar trend was observed after six months of water storage. Resin cements may not be as resistant to water degradation as restorative composite resins, however they exhibit a more hydrophobic behavior, in comparison to adhesive resins (23). For the experimental cements groups the reduction in E_r was significant. The anion exchange resin was triturated before mixing with the resin cement base to reduce the particle size and increase surface contact area. Considering that no treatment was performed on the AER surface to ensure that it would be chemically bonded to the methacrylate resin monomers, it visually became uniformly mixed to the base. Although the experimental cements had higher initial E_r possibly due to better polymerization at the first period evaluated, the addition of non-treated AER particles may have facilitated water penetration on the bulk cement layer, allowing it to diffuse and exert a softening effect at the resin cement.

As reported in a previous study (24), significant decrease in the surface nanohardness was observed in all groups. Hardness number represents the resistance of a material to plastic deformation and is an indirect method to evaluate the degree of conversion in composites (25). The reduction in the hardness number may be indicative of aging of the resin cement, causing it to be more susceptible to irreversible deformation. Resin cements are frequently used to lute ceramic materials. Taking into account that ceramic materials are not able to resist to plastic deformation (26), the integrity of the cement layer may play an important role for the success of the restoration. As reported in a recent study (19), changes in the resin

cement layer properties and adhesive failure may reduce the load bearing capacity of the ceramic restorations, thus leading to a catastrophic failure.

Because the SCU groups achieved higher bond strength than the ACE groups, the second hypothesis was partially accepted. In addition to the added reducing agents, the two adhesive systems employed in this study have a more hydrophobic characteristic after polymerization. The SCU is a universal adhesive that can be applied using the etch-and-rinse or self-etching mode. In order to avoid major discrepancies between the adhesives application mode, they were both applied following the etch-and-rinse approach. Former one step self-etch adhesives were known to have poor performance with high permeability leading to the formation of water channels (14) and low degree of conversion allowing to continuing etching even after light-curing (27). However, SCU is considered an ultra-mild self-etch adhesive, with pH 2.9 after mixing with the catalyst component (28). Thus, it may be less hydrophilic and achieve higher degree of conversion.

Self-etch adhesives have water in its composition to ionize the acidic monomers and etch the dentin surface. Water within the adhesive layer has shown to influence the degree of conversion in self-etch adhesives (27). When using the SCU adhesive under the etch-and-rinse approach, higher degree of conversion and microtensile bond strength were observed (28), likely due to the higher demineralization depth allowing the hydrophilic components to penetrate more deeply in dentin leaving the hydrophobic components closer to the surface of the adhesive layer and possibly less permeability at the adhesive layer (28). In addition to becoming more hydrophobic, the SCU adhesive has in its composition the MDP monomer claimed to chemically react with the tooth surface, either by binding to the collagen fibril hydrophobic domains (29) or by interacting with the hydroxyapatite resulting in Ca-MDP salts (30).

The incompatibility problem occurs due to the inactivation of the resin cement amine by the acidic monomers from simplified-step adhesive systems. Benzene sulphonic acid sodium salt was added to the adhesive systems composition, so they could act as a reducing agent generating free radicals and reducing the oxygen-

inhibited layer at the surface of the adhesive systems (15). The unreacted acidic monomers at the adhesive surface may cause an incompatibility reaction impairing the resin cement self-cure (7, 8, 11, 15). Water at the adhesive layer exerts a negative effect preventing the sulphonic acid sodium salts to avoid incompatibility (15). To overcome this situation, experimental resin cements were prepared capable of avoiding the unreacted acidic monomers at the adhesive system-resin cement interface from neutralizing the tertiary amine by adding the OH⁻ and OH⁺F⁻ forms of anion exchange resins. No difference in bond strength was observed between the control and experimental resin cements. Because higher degree of conversion and more hydrophobic characteristic was reported at the SCU surface (31), it is likely that less acidic monomers remained at the oxygen inhibited layer. Thus, the negative effects from the incompatibility reaction at the interface did not affect bond strength. However, the hydrophilic monomers that penetrated more deeply, within the demineralized dentin matrix, causing phase separation between the hydrophilic and hydrophobic components of the adhesive system (14, 27), may have established a porous area at the deeper areas of the hybrid layer that behaved as stress raisers as depicted in fig. 1 after analysis of the failure mode.

The third evaluated hypothesis was not confirmed because no significant reduction in bond strength was observed for any of the evaluated groups. Given that, significant reduction was observed on the nanomechanical properties of the resin cement layer after aging, in addition to the predominant failure occurring at the interface, it can be assumed that the microtensile bond strength of indirect resin restorations is affected predominantly by the adhesive system. The two adhesive systems evaluated may have a more hydrophobic characteristic than the previous simplified-step adhesives (31, 32). The 10-MDP monomer at the Scotchbond Universal adhesive (31) composition, is known to form a very stable interface, less susceptible to hydrolytic degradation (6, 33). Whereas for ACE All Bond TE adhesive, it is a highly cross-linking total-etch adhesive with the BPDM proprietary patented monomer (34) resulting in a more hydrophobic blend.

The bond strength test alone does not determine the rate of success or failure of bonded indirect restorations. As previously stated (19), the mechanical properties of the supporting adhesive and resin cement layers are equally important to ensure homogeneous dissipation of stresses within the tooth and the indirect restoration.

5. Conclusion

Based on the results of this study, adding AER to the resin cement base did not affect bond strength and improved the reduced modulus of elasticity and nanohardness of the resin cement. Water aging lowered the nanomechanical properties of the resin cement layer however bond strength remained stable.

Acknowledgements

The authors are grateful to Dr. Liang Chen and Bisco Inc. for helping with the experimental resin cements preparation and to Bisco Inc. and 3M ESPE for providing the adhesive systems. The research was sponsored by CAPES Foundation and FAPESP (grant #2012/02011-7).

References

1. Arrais CA, Rueggeberg FA, Waller JL, de Goes MF, Giannini M. Effect of curing mode on the polymerization characteristics of dual-cured resin cement systems. *Journal of Dentistry* 2008 Jun;**36**:418-26.
2. Meng X, Yoshida K, Atsuta M. Influence of ceramic thickness on mechanical properties and polymer structure of dual-cured resin luting agents. *Dental Materials* 2008 May;**24**:594-9.
3. Piva E, Correr-Sobrinho L, Sinhoreti MA, Consani S, Demarco FF, Powers JM. Influence of energy density of different light sources on Knoop hardness of a dual-cured resin cement. *Journal of Applied Oral Sciences* 2008 May-Jun;**16**:189-93.
4. Takubo C, Yasuda G, Murayama R, Ogura Y, Tonegawa M, Kurokawa H, et al. Influence of power density and primer application on polymerization of

- dual-cured resin cements monitored by ultrasonic measurement. *European Journal of Oral Sciences* 2010 Aug;**118**:417-22.
5. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, et al. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. *Biomaterials* 2005 Nov;**26**:6449-59.
 6. Feitosa VP, Leme AA, Sauro S, Correr-Sobrinho L, Watson TF, Sinhoreti MA, et al. Hydrolytic degradation of the resin-dentine interface induced by the simulated pulpal pressure, direct and indirect water ageing. *Journal of Dentistry* 2012 Dec;**40**:1134-43.
 7. Tay FR, Pashley DH, Yiu CK, Sanares AM, Wei SH. Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part I. Single-step self-etching adhesive. *Journal of Adhesive Dentistry* 2003 Spring;**5**:27-40.
 8. Sanares AM, Itthagarun A, King NM, Tay FR, Pashley DH. Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites. *Dental Materials* 2001 Nov;**17**:542-56.
 9. Franco EB, Lopes LG, D'Alpino P H, Pereira JC, Mondelli RF, Navarro MF. Evaluation of compatibility between different types of adhesives and dual-cured resin cement. *Journal of Adhesive Dentistry* 2002 Winter;**4**:271-5.
 10. Schittly E, Bouter D, Le Goff S, Degrange M, Attal JP. Compatibility of five self-etching adhesive systems with two resin luting cements. *Journal of Adhesive Dentistry* 2010 Apr;**12**:137-42.
 11. Cheong C, King NM, Pashley DH, Ferrari M, Toledano M, Tay FR. Incompatibility of self-etch adhesives with chemical/dual-cured composites: two-step vs one-step systems. *Operative Dentistry* 2003 Nov-Dec;**28**:747-55.
 12. Rueggeberg FA, Margeson DH. The effect of oxygen inhibition on an unfilled/filled composite system. *Journal of Dental Research* 1990 Oct;**69**:1652-8.

13. Chen L, Suh BI. Effect of hydrophilicity on the compatibility between a dual-curing resin cement and one-bottle simplified adhesives. *Journal of Adhesive Dentistry* 2012 Aug;**15**:325-31.
14. Ye Q, Park JG, Topp E, Wang Y, Misra A, Spencer P. In vitro performance of nano-heterogeneous dentin adhesive. *Journal of Dental Research* 2008 Sep;**87**:829-33.
15. Tay FR, Suh BI, Pashley DH, Prati C, Chuang SF, Li F. Factors contributing to the incompatibility between simplified-step adhesives and self-cured or dual-cured composites. Part II. Single-bottle, total-etch adhesive. *Journal of Adhesive Dentistry* 2003 Summer;**5**:91-105.
16. Finger WJ, Osada T, Tani C, Endo T. Compatibility between self-etching adhesive and self-curing resin by addition of anion exchange resin. *Dental Materials* 2005 Nov;**21**:1044-50.
17. Endo T, Finger WJ, Hoffmann M, Kanehira M, Komatsu M. The role of oxygen inhibition of a self-etch adhesive on self-cure resin composite bonding. *American Journal of Dentistry* 2007 Jun;**20**:157-60.
18. Kanehira M, Finger WJ, Hoffmann M, Komatsu M. Compatibility between an all-in-one self-etching adhesive and a dual-cured resin luting cement. *Journal of Adhesive Dentistry* 2006 Aug;**8**:229-32.
19. Lu C, Wang R, Mao S, Arola D, Zhang D. Reduction of load-bearing capacity of all-ceramic crowns due to cement aging. *Journal of the Mechanical Behavior of Biomedical Materials* 2013 Jan;**17**:56-65.
20. Oliver WC, Pharr GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *Journal of Materials Research* 1992;**7**:1564-83.
21. Ceballos L, Garrido MA, Fuentes V, Rodriguez J. Mechanical characterization of resin cements used for luting fiber posts by nanoindentation. *Dental Materials* 2007 Jan;**23**:100-5.

22. Dos Santos PH, Karol S, Bedran-Russo AK. Long-term nano-mechanical properties of biomodified dentin-resin interface components. *Journal of Biomechanics* 2011 Jun 3;**44**:1691-4.
23. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, et al. Water sorption/solubility of dental adhesive resins. *Dental Materials* 2006 Oct;**22**:973-80.
24. de Moraes RR, Marimon JL, Schneider LF, Sinhoreti MA, Correr-Sobrinho L, Bueno M. Effects of 6 months of aging in water on hardness and surface roughness of two microhybrid dental composites. *Journal of Prosthodontics* 2008 Jun;**17**:323-6.
25. Moosavi H, Hariri I, Sadr A, Thitthaweerat S, Tagami J. Effects of curing mode and moisture on nanoindentation mechanical properties and bonding of a self-adhesive resin cement to pulp chamber floor. *Dental Materials* 2013 Jun;**29**:708-17.
26. Rekow ED, Silva NR, Coelho PG, Zhang Y, Guess P, Thompson VP. Performance of dental ceramics: challenges for improvements. *Journal of Dental Research* 2011 Aug;**90**:937-52.
27. Wang Y, Spencer P. Continuing etching of an all-in-one adhesive in wet dentin tubules. *Journal of Dental Research* 2005 Apr;**84**:350-4.
28. Miletic V, Pongprueksa P, De Munck J, Brooks NR, Van Meerbeek B. Monomer-to-polymer conversion and micro-tensile bond strength to dentine of experimental and commercial adhesives containing diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide or a camphorquinone/amine photo-initiator system. *Journal of Dentistry* 2013 Oct;**41**:918-26.
29. Hiraishi N, Tochio N, Kigawa T, Otsuki M, Tagami J. Monomer-collagen interactions studied by saturation transfer difference NMR. *Journal of Dental Research* 2013 Mar;**92**:284-8.
30. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, et al. Self-assembled Nano-layering at the Adhesive interface. *Journal of Dental Research* 2012 Apr;**91**:376-81.

31. Munoz MA, Luque I, Hass V, Reis A, Loguercio AD, Bombarda NH. Immediate bonding properties of universal adhesives to dentine. *Journal of Dentistry* 2013 May;**41**:404-11.
32. Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR, Pashley DH. Adhesive permeability affects coupling of resin cements that utilise self-etching primers to dentine. *Journal of Dentistry* 2004 Jan;**32**:55-65.
33. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dental Materials* 2011 Jan;**27**:17-28.
34. Tay FR, Gwinnett JA, Wei SH. The overwet phenomenon in two-component acetone-based primers containing aryl amine and carboxylic acid monomers. *Dental Materials* 1997 Mar;**13**:118-27.

CAPÍTULO 2

Extra hydrophobic resin layer effect on bond strength, nanomechanical properties and micropermeability of adhesively luted indirect resin restoration

Ariene Arcas Leme, DDS MSc, Department of Restorative Dentistry, Dental Materials, Piracicaba Dental School, University of Campinas; 901 Limeira Av; CEP: 13414-903; PO BOX: 52; Piracicaba SP; Brazil.

Americo Bortolazo Correr, DDS PhD, Department of Restorative Dentistry, Dental Materials, Piracicaba Dental School, University of Campinas; 901 Limeira Av; CEP: 13414-903; PO BOX: 52; Piracicaba SP; Brazil.

Lourenço Correr-Sobrinho, DDS PhD, Department of Restorative Dentistry, Dental Materials, Piracicaba Dental School, University of Campinas; 901 Limeira Av; CEP: 13414-903; PO BOX: 52; Piracicaba SP; Brazil.

Ana Karina Bedran-Russo, DDS PhD, Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, Chicago, IL, USA. 801 South Paulina Street, room 551 Chicago, IL 60612.

Mario Alexandre Coelho Sinhoreti, DDS PhD, Department of Restorative Dentistry, Dental Materials, Piracicaba Dental School, University of Campinas; 901 Limeira Av; CEP: 13414-903; PO BOX: 52; Piracicaba SP; Brazil.

*Corresponding author:

Ariene Arcas Leme

Dental Materials, Department of Restorative Dentistry

Piracicaba Dental School, State University of Campinas-UNICAMP

901 Limeira Av.

Piracicaba, SP, Brazil

13414-903; PO BOX: 52;

Phone number: 55-67-99081986

Email: arienearcasleme@gmail.com

Running title: Bond strength, nano-indentation and micropermeability evaluation

Clinical relevance: Beneficial effects of adding an extra hydrophobic resin layer should be considered, although it did not improve bond strength, better nanomechanical properties of the cement layer were achieved.

SUMMARY

Objectives: This study evaluated the effect of applying an extra hydrophobic adhesive layer on bond strength, nanomechanical properties of the cement layer and micropermeability at the hybrid layer (HL) when luting resin composite restorations.

Material and Methods: The occlusal surface of twenty-eight human molars were ground flat to expose dentin. Luting procedures were performed with ACE All Bond TE (ACE) and Scotchbond Universal (SCU) with the application of an extra hydrophobic resin layer (AD) or not. Teeth were randomly assembled into 4 groups: G1= ACE; G2= ACE + AD; G3= SCU; G4=SCU + AD. The composite resin blocks were luted with Duolink cement and after 24 h resin-dentin beams were obtained and evaluated for microtensile bond strength (μ TBS), reduced modulus of elasticity (E_r) and nanohardness (H) of the cement layer and micropermeability at the adhesive interface. Data were analyzed with two-way Anova and Tukey's test with $\alpha=0.05$.

Results: No significant difference in μ TBS was observed with an additional hydrophobic resin layer ($p>0.05$). G3 (34.7 ± 13.3) and G4 (38.3 ± 7.2) attained higher bond strength in comparison to G1 (26.0 ± 13.9) and G2 (22.4 ± 4.3). Higher E_r was

observed for the cement layer bonded with ACE ($p=0.003$) and the additional hydrophobic layer resulted increased E_r ($p=0.001$) and H ($p=0.04$). Rhodamine infiltration was observed at the bottom of the HL, with no difference among the groups ($p>0.05$).

Conclusions: Extra hydrophobic adhesive layer improved the nanomechanical properties of the resin cement layer and did not improve bond strength. Microporosities were observed at the bottom of the hybrid layer.

Keywords: resin cements, adhesive systems, chemical cure, bond strength, nanoindentation, microporosity

INTRODUCTION

One current challenge of dental materials' manufacturers is to improve the simplified adhesive systems (i.e. two step etch-and-rinse and the one step self-etch adhesives) making them reliable and compatible to any restorative procedure. They are a blend of cross-linking and functional monomers, organic solvents, initiators and sometimes filler particles, presented as one or two bottles, the second bottle being usually the activator, to enable dual-cure.¹ The functional monomers are the ionizable acidic monomers, in general with hydrophilic characteristic.¹ The increased hydrophilicity of the adhesive systems, besides making them more prone to water degradation,^{2, 3} have lead to a sealing problem.⁴ They became permeable membranes after polymerization, allowing water to diffuse from the dentin towards the adhesive layer.⁴⁻⁶ In addition, the acidic monomers from the adhesive system can react with the nucleophilic amine from the self-cured resin cement, preventing it to react with the benzoyl peroxide impairing the self-cured resin polymerization.⁷ The mentioned problem can exert a more deleterious effect when used with delayed polymerization start, as occurs to self-cured resin cements.

Nowadays-simplified adhesive systems are claimed to be less hydrophilic⁸ and they come along with amine co-initiator compounds applied concomitantly, not

sensitive to the acidic media.^{9, 10} Adding sulfinate sodium salts improved the degree of conversion of simplified adhesive systems and self-cured resin cements.⁹ However, the degree of conversion tests were performed on the ATR diamond surface, underestimating the inherent humidity and the tooth substrate effect at the acid-etched dentin surface. Regarding dentin bonding, water can be the greatest edge¹¹ and adhesive permeability can accentuate the incompatibility problem observed between simplified adhesive systems and self-cured resin cement,¹² under the microtensile bond strength test. Therefore, application of an additional hydrophobic resin layer has been suggested.¹³ However, when using less hydrophilic adhesive systems, the efficacy of this extra hydrophobic resin layer can be questioned, besides the fact that it would increase the adhesive thickness¹⁴ at risk to jeopardize the adaptation of the indirect restoration.

In order to achieve long lasting success of an indirect restoration, all the system must work homogeneously, to avoid areas with excessive stress concentration.^{15, 16} The maintenance of the bonding is one important factor.¹⁵ All the mentioned problems can reduce the quality of the bonding interface, resulting in stress raisers areas, with potential to catastrophic failure.^{15, 16} Additionally, because indirect ceramic restorations cannot resist to plastic deformation, ensuring acceptable mechanical properties for the resin cement layer is also important for the clinical success of the restoration.¹⁵

The microtensile bond strength is a common test used to evaluate problems related to bonding of adhesively luted indirect restorations.^{7, 10, 17-19} It is a reliable test, however to provide more complete information, it can be associated to other methodologies. Confocal laser scanning microscopy enabled the evaluation of micropermeability of the hybrid layer.^{20, 21} The porosities are indicated by the presence of the fluorochrome.²⁰ The energy is absorbed by the fluorescent molecule and irradiated, therefore the image from the plane of focus that reach the detector can be measured.²⁰ In addition, it should be of interest to evaluate if the mentioned reactions taking place at the adhesive interface can influence the mechanical properties of the resin cement. Thus, a more specific approach evaluating the

nanomechanical properties of the resin cement layer, when an indirect restoration is simulated, can add important information regarding what happens within the cement layer. The technique to evaluate mechanical properties of materials in a submicron scale was possible due to the development of a mechanical properties microprobe that continuously measure force and displacement while an indentation is made.²² Load and depth sensing indentation (LDI) can be useful in measuring the mechanical properties of very thin films and layers²³⁻²⁵ and the most common parameters evaluated are the reduced modulus of elasticity (E_r) and nanohardness (H).

Therefore, the aim of this study was to evaluate the immediate bond strength, resin cement nanomechanical properties and micropermeability of adhesively luted indirect resin composite restorations, with adding or not an extra hydrophobic adhesive layer. The hypothesis tested were: the adhesive system and addition of an extra hydrophobic resin layer can affect the bond strength of indirect resin restoration, the resin cement nanomechanical properties and reduce micropermeability at the adhesive interface.

METHODS AND MATERIALS

Specimen preparation

After the protocol approval by the local ethics board committee (protocol #112/2011), 28 recently extracted human molars were used. Teeth were disinfected in 0.5% chloramine, cleaned and occlusal enamel was removed using a #180 grit SiC paper (Buehler Inc., Lake Bluff, IL, USA), resulting in a flat surface of coronal dentin. Teeth were randomly assembled into 4 groups according to the adhesive system applied and the application or not of an additional hydrophobic resin layer. The composition of the materials used in this study is depicted in Table 1.

Table 1 – Materials used, composition and manufacturer

Material	Composition	Manufacturer
Duo-Link	BASE: bis-GMA, Triethyleneglycol Dimethacrylate, UDMA, glass filler, tertiary amine CATALYST: bis-GMA, Triethyleneglycol Dimethacrylate, glass filler, benzoyl peroxide.	Bisco Inc., Schaumburg, IL, USA
ACE ALL Bond TE (ACE)	Ethanol, NTG-GMA Salt, bis-GMA, HEMA, BPDM	Bisco Inc., Schaumburg, IL, USA
ALL Bond Resin (ABR)	Bis-GMA, UDMA, HEMA	Bisco Inc., Schaumburg, IL, USA
Scotchbond Universal and Scotchbond Universal Dual Cure Activator (SCU)	Adhesive system: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water initiators and silane Activator: Ethyl alcohol and sodium p-toluenesulfinate, methyl ethyl ketone	3M ESPE, St. Paul, MN, USA
Scotchbond Multi-Purpose Plus Adhesive (SCA)	Bis-GMA, HEMA, tertiary amines and photoinitiator	3M ESPE, St. Paul, MN, USA

Abbreviations: Urethane dimethacrylate (UDMA), N-tolyglycine glycidyl methacrylate (NTG-GMA), 2-hydroxyethyl methacrylate (HEMA), bisphenol A diglycidyl methacrylate (bis-GMA), 10-methacryloyloxydecyl dihydrogen phosphate (MDP), biphenyl dimethacrylate (BPDM).

Composite resin blocks with 10 mm diameter and 5 mm thick were prepared to simulate the indirect restoration with the core build-up resin Bis-core (Bisco Inc., Schaumburg, IL, USA). A round metallic matrix was positioned over a glass slide and the core resin was incrementally inserted to fill the mold with a little excess. A mylar strip (Moyco Technologies, York, PA, USA) was hold with a 50 g weight and light-cured for 60 s with Optilux 501 (Demetron/Kerr, Danbury, CT, USA).

The bonding procedures were performed following the respective manufacturer's instructions as demonstrated in Table 2. Before the adhesive system application, the exposed dentin surface was wet polished for 30 s using a #600 grit SiC paper (Buehler) to standardize the smear layer.

Table 2 – Experimental groups and respective bonding procedures

Groups	Dentin treatment	Resin block treatment
Group 1 (ACE)	Phosphoric acid etching 15 s. One drop of each component of the ACE adhesive system were dispensed in a case and mixed for 5 s. The adhesive system was applied to the moist dentin surface under agitation for 10 s, excess solvent was volatilized for 15 s.	Phosphoric acid cleansing for 60 s, water rinsed and air dried. One layer of adhesive system was applied on the surface for 15 s. The excess solvent was volatilized for 15 s and light-cured for 20 s.
Group 2 (ACE+ABR)	The dentin surface was etched and the adhesive system ACE was applied the same manner as for Group 1. Additionally one layer of the All-Bond resin was applied and light cured for 20 s.	
Group 3 (SCU)	Phosphoric acid for 15 s. One drop of each bottle of the SCU adhesive was dispensed in a case and the adhesive system was applied to the moist dentin surface under agitation for 20 s, excess solvent was volatilized for 15 s.	Cleaned with phosphoric acid for 60 s, rinsed and air dried. One layer of adhesive system was applied on the surface for 20 s. The excess solvent was volatilized for 15 s and light-cured for 20 s.
Group 4 (SCU+SCA)	The dentin surface was etched and the SCU adhesive system was applied the same way as for Group 3, followed by one layer of the Adhesive resin from Scotchbond Multi-Purpose Plus and light-cured for 20 s.	

After bonding procedures in both dentin and at the resin block surface, the Duo-Link resin cement (Bisco Inc.) was dispensed on the resin block using an automix tip. The block was then positioned on the dentin surface and hold in position for 7 min. A 50 g weight was positioned over the luted resin to ensure that a standardized seating pressure was being exerted for all groups.

The restored teeth were stored for 24 h in a lightproof container at 37 °C under 100% humidity. Teeth were cut parallel to the long axis using a low speed precision saw Isomet 1000 (Buehler Inc.) and 1 mm² resin/dentin beams were obtained. The

beams were used for the immediate microtensile bond strength (μ TBS), assessment of the nanomechanical properties and micropermeability of the adhesive interface.

Microtensile bond strength

The resin/dentin beams were fixed to the device attached to the microtensile tester machine (Bisco Inc.) with a cyanoacrylate based adhesive (Loctite, Henkel Corp., Rocky Hill, CT, USA). The specimen was subjected to a tensile load at 1mm/min cross-head speed until failure. The maximum load at failure was recorded and bond strength was calculated dividing the maximum load by the cross sectional area of the bonded interface. The mean bond strength was obtained from each tooth and data was statistically analyzed using two-way Anova and Tukey's post-hoc test with significance level at $\alpha=0.05$.

The failure mode of fractured beams were observed using a stereoscope loupe (Zeiss, Göttingen, Germany) under 60 x magnification. The failures were classified as adhesive failure at the adhesive/resin or adhesive/dentin interface (A); cohesive failure at the resin block or the dentin (C) and mixed failure, comprising both adhesive at the interface and cohesive failures at the same specimen (M).

Nanomechanical properties evaluation of the resin cement layer

The nanomechanical properties of the resin cement layer was evaluated using a triboindenter (Hysitron Inc, Minneapolis, MN) equipped with a Berkovich fluid cell, that allowed the measurements to be performed under hydrated condition. From each tooth, two resin/dentin beams were embedded in epoxy resin (Buehler) and allowed to polymerize. The specimens were polished using #400, 600, 800 and 1200-grit SiC paper (Buehler Inc.) and diamond suspensions starting from 9, 6, 3, 1 and 0.05 μ m (Buehler Inc.). Immediately after polishing, a wax barrier was prepared to keep the beams immerse in water while indenting and the specimen was positioned on the stage inside the triboindenter chamber.

Before indenting the resin cement layer, an initial calibration was carried out using a fused silica sample with known elastic modulus ($E = 69.6$ GPa) and hardness

($H = 9.25$ GPa). After establishing the relationship between depth of indent and tip contact area, the reduced modulus of elasticity (E_r) and nanohardness (H) of the resin cement layer was obtained from the resulting force-displacement curve, following the method of Oliver and Pharr²². A standardized trapezoidal load function was used with 20-10-20s for the loading, holding and unloading segments respectively and a peak load of 1,000 μ N. Nine indentations were performed for each resin/dentin beam at the resin cement layer and the average E_r and H was obtained for each beam. The two-way Anova followed by Tukey's post-hoc test with the significance level as 5% was performed for E_r and H .

Confocal laser scanning microscopy evaluation

The micropermeability of the adhesive interface was evaluated using a solution of 0.1% w/v Rhodamine-B (RITC/Rhodamine B, Sigma, St Louis, MO, USA) dissolved in 0.1M Phosphate buffered solution (PBS, pH 7.2-7.4). Two beams from each tooth were embedded in epoxy resin (Buehler Inc.) and allowed to cure for 8 h. The specimens were polished using SiC papers and diamond suspensions following the sequence used to prepare the specimens for the nanoindentation test. The beams were then kept immerse in the Rhodamine/PBS solution for 1 h, in a lightproof container, to allow the fluorescent dye solution to infiltrate through the porosities at the adhesive interface. The excess solution was rinsed out and the specimens were observed in a confocal laser scanning microscope LSM 510 Meta (Zeiss, Göttingen, Germany) with the Plan-Neofluar 25x/0.8Imm corr DIC objective.

Two beams were observed per tooth and the image comprised an approximate distance of 360 μ m at the adhesive interface of each beam. In order to observe the auto-fluorescence from the dentin the Ar laser (488 nm) was used with 30% intensity. To observe the fluorescence from the Rhodamine/PBS solution that penetrated within the microporosities at the adhesive interface, the laser He-Ne (543 nm) was used at 5%. The pictures were observed with the Profile tool at the microscope software, which allowed converting the visual data in semi-quantitative data. Therefore, the amount of rhodamine that penetrated within the porosities was

calculated for each beam. The data was statistically evaluated using two-way Anova and Tukey's post-hoc test with $\alpha=0.05$.

RESULTS

Regarding the μ TBS, no significant interaction was observed between the factors ($p>0.05$). The SCU adhesive groups attained significant higher bond strength than ACE groups ($p=0.005$). No difference in μ TBS was observed when the additional adhesive resin layer was applied between the simplified adhesives and the self-cured resin cement ($p>0.05$). The most prevalent failure mode was at the adhesive interface, as depicted in Table 3.

Table 3 – Microtensile bond strength data (MPa), failure mode and the intensity of rhodamine absorption within the adhesive interface, for all groups

Groups	Bond Strength	Failure mode (%)	Rhodamine Intensity
G1 (ACE)	25.7±12.3 b	47.5 (A), 21.3 (M), 31.2 (C)	138.3±36.6 a
G2 (ACE+ABR)	22.4±4.3 b	73.3 (A), 13.3 (M), 13.4 (C)	127.3±84.3 a
G3 (SCU)	35.3±12.7 a	64.3 (A), 23.4 (M), 12.3 (C)	120.0±79.3 a
G4 (SCU+SCA)	38.3±7.2 a	50.0 (A), 21.2 (M), 28.8 (C)	83.2±51.6 a

Different lowercase letters means statistically significant difference among groups for each, microtensile bond strength and micropermeability, by the rhodamine intensity test. Failure modes: failure at the adhesive/resin or adhesive/dentin interface (A), cohesive failure at the resin block or the dentin (C) and mixed failure (M).

The H and E_r data are shown in Figure 1. No significant interaction was observed between the factors evaluated ($p>0.05$). No difference in H was observed ($p>0.05$) between the adhesive systems, but the application of an extra adhesive layer resulted in higher H ($p=0.04$). The resin cement layer bonded with ACE exhibited significant higher E_r ($p=0.003$). Applying the extra hydrophobic adhesive layer resulted in higher E_r at the resin cement layer ($p=0.001$) regardless of the adhesive system.

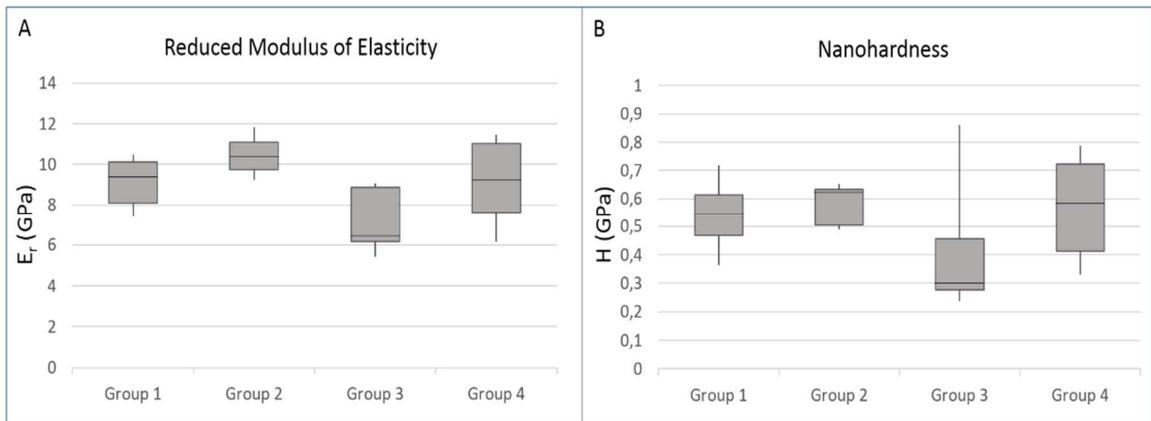


Figure 1- Box charts depicting nanoindentation data at the resin cement layer. In “A” the reduced modulus of elasticity (E_r) for all groups and “B” shows the nanohardness (H) data.

No statistically significant difference was observed on the micropermeability data (Table 3), regarding the adhesive system or the application or not of the additional hydrophobic resin layer ($p > 0.05$). Infiltration was observed predominantly at the bottom of the hybrid layer and no Rhodamine was detected at the adhesive system and resin cement interface (Figure 2).

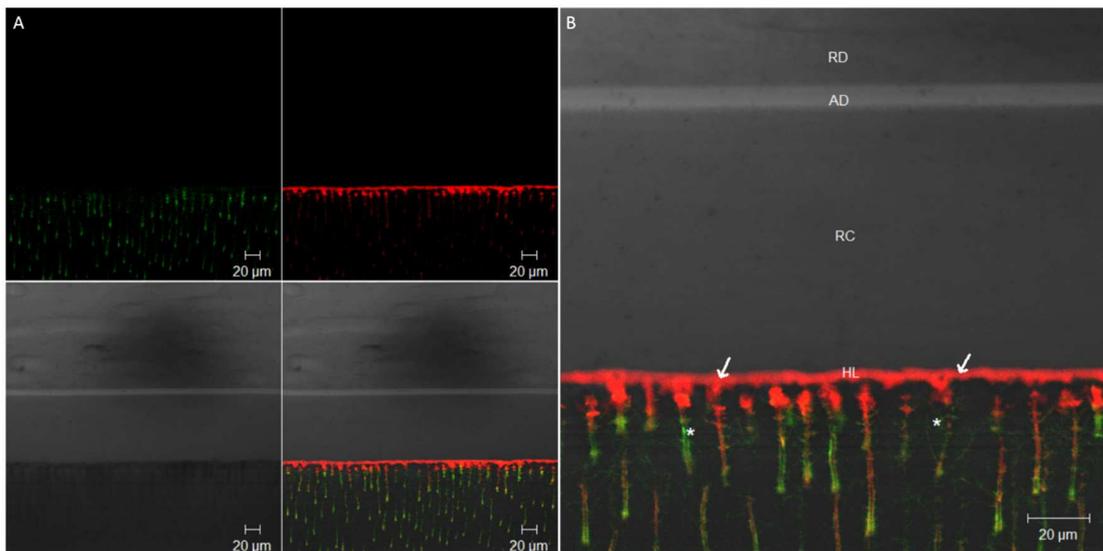


Figure 2 – Confocal laser scanning microscopy of the adhesive and resin cement interface of a specimen from Group 3 (SCU). Image “A” shows the interface in smaller magnification, depicting one single image from each laser Ar (488nm) appearing as green color, He-Ne (543 nm) as red and DIC image. Image “B”, is a higher magnification image from the same area, the different parts of the luted restoration can be observed: RD – resin block, AD – adhesive layer, RC – resin cement, HL – hybrid layer. The white arrows are pointing to the Rhodamine infiltration within the hybrid layer and asterisks indicate areas of collagen exposition, demonstrated by the collagen auto-fluorescence. Rhodamine infiltration was observed mainly at the hybrid layer, indicative of the occurrence of porosities at this zone.

DISCUSSION

In this study, the bond strength, micropermeability and nanomechanical properties of a self-cured resin cement used with simplified adhesives, and the effect of adding an extra hydrophobic adhesive layer was evaluated. The hypothesis that applying an additional hydrophobic resin layer would affect the bond strength was not confirmed. The hydrophobic resin layer is claimed to reduce adhesive permeability and act as a barrier for the unreacted acidic resin monomers, thus it

was expected to improve dentin bond strength of self-etch simplified adhesive systems.¹³ However, in agreement to a previous study,²⁶ the application of the hydrophobic resin layer did not improve the bond strength. Taking into account that the adhesive systems were light cured after the application of the hydrophobic resin layer, better quality of the hybrid layer was expected. However, the most fragile point was at the adhesive interface, mainly due to the presence of water,¹¹ which may have impaired the efficient polymerization of the resin at this point. This was also observed by the rodhamine infiltration, pointing out to the occurrence of microporosities at this area, regardless of the adhesive application technique performed. Moreover, according to the manufacturers, both simplified adhesive systems can be used in the self-cure mode. They have in their composition co-initiators^{9, 27} that can also help to initiate the resin cement polymerization, when it relies only in the self-cure activation.²⁷ These reasons may all have contributed to the similar performance encountered for the two luting techniques at the microtensile bond strength test.

The hypothesis that the bond strength would be influenced by the adhesive system was accepted. The higher bond strength observed for the groups luted with SCU, is possibly due to the monomeric composition. The MDP is an acidic monomer known to be very stable and with mild aggressiveness. The long carbonil chain has rendered this monomer hydrophobic¹ and its functional sites are believed to chemically bond with remaining hydroxyapatite forming Ca-MDP salts.²⁸ In addition, higher degree of conversion was observed on the surface of the adhesive layer when SCU was used in the etch-and-rinse mode.²⁹ The same authors²⁹ speculated that because SCU is considered an ultra-mild self-etching adhesive, when it was applied after phosphoric acid, more efficient etching of the dentin surface was observed. Therefore, the water and other hydrophilic components would flow better through the dentin matrix, and the hydrophobic components would remain at the surface, originating a more hydrophobic layer at the adhesive surface, with better degree of conversion. The phase separation was previously reported with simplified-step self-etch adhesives,³⁰ however as a foible characteristic. In this case, considering water

flow towards the adhesive system surface, it is likely that making the adhesive surface more hydrophobic, would avoid water coming from dentin to be entrapped at the adhesive system and resin cement junction, causing the resin cement unable to stay in contact with the adhesive.

Innovative staining techniques, allowing evaluation of the micropermeability have been suggested.^{20, 21} The technique employed in this study is an adaptation of the original micropermeability technique, which consists of placing a solution of Rhodamine B inside the pulp chamber and evaluating the fluorescent tracer that penetrated through the resin tags to reach lateral branches and microporosities.²¹ With this technique, it is possible to evaluate porosities within the hybrid layer with minimal specimen preparation. In order to avoid major discrepancies between both adhesives, they were applied using the etch-and-rinse approach. In a previous study²⁵ the confocal images depicted higher rhodamine infiltration occurring at the bottom of the hybrid layer, which is in agreement to what was observed in this study. Dentin was acid etched before adhesive system application, opening the dentin tubules and exposing a great amount of collagen fibrils. Thus, it is possible that water from the dentin tubules was allowed to flow from underlying dentin^{4, 5, 31, 32} and impaired polymerization reaction mainly at the bottom of the hybrid layer.¹¹ In a simplified adhesive system, the use of a hydrophobic resin as a sealer showed able to avoid water flowing throughout the adhesive layer.³³ However, in this current study, microporosity occurring throughout the adhesive layer and reaching the resin cement was not observed, regardless of using or not a hydrophobic resin layer.

Interestingly, although decreased nanomechanical properties were observed with the nanoindentation test at the resin cement layer for the groups without the extra hydrophobic resin, it did not impair the microtensile bond strength. The decrease in bond strength previously reported due to the incompatibility^{7, 10, 18, 34} was not observed in this study, but still we encountered significant lower E_r and H for the resin cement layer in contact with the not light-activated adhesive system, compared to the group where the extra hydrophobic layer was applied. This fact can be due to two different reasons. One is the poor quality of polymerization of the resin cement

within the areas close to the adhesive system, due to: water coming from the dentinal tubules,^{4, 5, 32} diffused through the adhesive layer and reaching the resin cement layer;⁶ the possible acid-base reaction between the acidic monomers and tertiary amine from the resin cement;^{7, 10, 18, 19, 35} and the incomplete solvent volatilization from the adhesive blends,³⁶ as they were mixed during the luting procedures. Because lower degree of conversion was reported in adhesive systems with excessive remaining solvent,³⁷ it can be speculated that by mixing them, the solvent solution may have impaired the resin cement polymerization as well. According to the microporosity evaluation under confocal microscope, no fluorescent dye infiltration was observed at the resin cement and adhesive system interface. The second hypothesis for the lower nanoindentation values observed for these groups is the formation of a transition zone between the adhesive, which in this case were not previously light-cured, and the resin cement. It can be speculated that part of the resin cement that is in contact with the adhesive system were mixed, resulting in a zone of gradual increase of the E_r and H. The modulus of elasticity is an important parameter to be evaluated for adhesive restorations, it can be representative of the aging effects²⁴ and influence on the stress distribution within the adhesive interface.¹⁵ However, in this study, the specimens were not evaluated for a long-term fatigue degradation, thus the assumption that the difference observed on the modulus of elasticity between the groups could reflect in premature failure under a clinical situation cannot be affirmed.

The previously reported incompatibility problems are more likely to occur when lower pH adhesive systems are employed,^{18, 35} potentiated by the adhesive system permeability, allowing water to come from dentin to the adhesive system surface.⁶ In addition, the type of tertiary amine present at the resin cement should be considered. The most nucleophilic amine is usually in self-cure systems.¹⁰ The contemporary more hydrophobic simplified adhesive systems with the ternary catalysts, may have suppressed the negative effects observed by the low bond strength of these systems. However, further studies should investigate the effect of

the decreased modulus of elasticity observed for the resin cement layer, after long-term aging and mechanical fatigue challenge.

CONCLUSIONS

It can be concluded that:

- Bond strength of adhesively luted composite resin specimens was affected by the adhesive system.
- Application of an extra hydrophobic resin layer resulted in increased nanomechanical properties of the resin cement layer.
- The microtensile bond strength was not affected by the application of an extra hydrophobic resin layer.

Acknowledgements

The authors are grateful to Bisco Inc. and 3M ESPE for providing the materials used in this study. The research was sponsored by CAPES Foundation and FAPESP (grant #2012/02011-7).

References

1. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P & Van Meerbeek B (2007) Systematic review of the chemical composition of contemporary dental adhesives *Biomaterials* **28(26)** 3757-3785.
2. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR & Pashley DH (2005) Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity *Biomaterials* **26(33)** 6449-6459.

3. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK & Carrilho MR (2006) Water sorption/solubility of dental adhesive resins *Dental Materials* **22(10)** 973-980.
4. Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR & Pashley DH (2004) Adhesive permeability affects coupling of resin cements that utilise self-etching primers to dentine *Journal of Dentistry* **32(1)** 55-65.
5. Tay FR & Pashley DH (2003) Have dentin adhesives become too hydrophilic? *Journal of the Canadian Dental Association* **69(11)** 726-731.
6. Tay FR, Pashley DH & Peters MC (2003) Adhesive permeability affects composite coupling to dentin treated with a self-etch adhesive *Operative Dentistry* **28(5)** 610-621.
7. Cheong C, King NM, Pashley DH, Ferrari M, Toledano M & Tay FR (2003) Incompatibility of self-etch adhesives with chemical/dual-cured composites: two-step vs one-step systems *Operative Dentistry* **28(6)** 747-755.
8. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J & Van Landuyt KL (2011) State of the art of self-etch adhesives *Dental Materials* **27(1)** 17-28.
9. Arrais CA, Giannini M & Rueggeberg FA (2009) Effect of sodium sulfinate salts on the polymerization characteristics of dual-cured resin cement systems exposed to attenuated light-activation *Journal of Dentistry* **37(3)** 219-227.
10. Tay FR, Suh BI, Pashley DH, Prati C, Chuang SF & Li F (2003) Factors contributing to the incompatibility between simplified-step adhesives and self-cured or dual-cured composites. Part II. Single-bottle, total-etch adhesive *Journal of Adhesive Dentistry* **5(2)** 91-105.
11. Wang Y & Spencer P (2005) Continuing etching of an all-in-one adhesive in wet dentin tubules *Journal of Dental Research* **84(4)** 350-354.
12. Chen L & Suh BI (2012) Effect of hydrophilicity on the compatibility between a dual-curing resin cement and one-bottle simplified adhesives *Journal of Adhesive Dentistry* **15(4)** 325-331.

13. Reis A, Albuquerque M, Pegoraro M, Mattei G, Bauer JR, Grande RH, Klein-Junior CA, Baumhardt-Neto R & Loguercio AD (2008) Can the durability of one-step self-etch adhesives be improved by double application or by an extra layer of hydrophobic resin? *Journal of Dentistry* **36(5)** 309-315.
14. Arrais CA, Miyake K, Rueggeberg FA, Pashley DH & Giannini M (2009) Micromorphology of resin/dentin interfaces using 4th and 5th generation dual-curing adhesive/cement systems: a confocal laser scanning microscope analysis *Journal of Adhesive Dentistry* **11(1)** 15-26.
15. Lu C, Wang R, Mao S, Arola D & Zhang D (2013) Reduction of load-bearing capacity of all-ceramic crowns due to cement aging *Journal of the Mechanical Behavior of Biomedical Materials* **17** 56-65.
16. May LG, Kelly JR, Bottino MA & Hill T (2012) Effects of cement thickness and bonding on the failure loads of CAD/CAM ceramic crowns: multi-physics FEA modeling and monotonic testing *Dental Materials* **28(8)** e99-109.
17. Arrais CA, Giannini M, Rueggeberg FA & Pashley DH (2007) Effect of curing mode on microtensile bond strength to dentin of two dual-cured adhesive systems in combination with resin luting cements for indirect restorations *Operative Dentistry* **32(1)** 37-44.
18. Sanares AM, Itthagarun A, King NM, Tay FR & Pashley DH (2001) Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites *Dental Materials* **17(6)** 542-556.
19. Tay FR, Pashley DH, Yiu CK, Sanares AM & Wei SH (2003) Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part I. Single-step self-etching adhesive *Journal of Adhesive Dentistry* **5(1)** 27-40.
20. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Carvalho RM & Pashley DH (2006) A new technique for assessing hybrid layer interfacial micromorphology and integrity: two-photon laser microscopy *Journal of Adhesive Dentistry* **8(5)** 279-284.

21. Sauro S, Pashley DH, Mannocci F, Tay FR, Pilecki P, Sherriff M & Watson TF (2008) Micropermeability of current self-etching and etch-and-rinse adhesives bonded to deep dentine: a comparison study using a double-staining/confocal microscopy technique *European Journal of Oral Sciences* **116(2)** 184-193.
22. Oliver WC & Pharr GM (1992) An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments *Journal of Materials Research* **7(6)** 1564-1583.
23. Ceballos L, Garrido MA, Fuentes V & Rodriguez J (2007) Mechanical characterization of resin cements used for luting fiber posts by nanoindentation *Dent Mater* **23(1)** 100-105.
24. Dos Santos PH, Karol S & Bedran-Russo AK (2011) Long-term nano-mechanical properties of biomodified dentin-resin interface components *Journal of Biomechanics* **44(9)** 1691-1694.
25. Sauro S, Osorio R, Watson TF & Toledano M (2012) Assessment of the quality of resin-dentin bonded interfaces: an AFM nano-indentation, μ TBS and confocal ultramorphology study *Dental Materials* **28(6)** 622-631.
26. De Vito Moraes AG, Francci C, Carvalho CN, Soares SP & Braga RR Microshear bond strength of self-etching systems associated with a hydrophobic resin layer *Journal of Adhesive Dentistry* **13(4)** 341-348.
27. Takubo C, Yasuda G, Murayama R, Ogura Y, Tonegawa M, Kurokawa H & Miyazaki M (2010) Influence of power density and primer application on polymerization of dual-cured resin cements monitored by ultrasonic measurement *European Journal of Oral Sciences* **118(4)** 417-422.
28. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J & Van Meerbeek B (2004) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **83(6)** 454-458.
29. Miletic V, Pongprueksa P, De Munck J, Brooks NR & Van Meerbeek B (2013) Monomer-to-polymer conversion and micro-tensile bond strength to dentine

- of experimental and commercial adhesives containing diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide or a camphorquinone/amine photo-initiator system *Journal of Dentistry* **41(10)** 918-926.
30. Ye Q, Park JG, Topp E, Wang Y, Misra A & Spencer P (2008) In vitro performance of nano-heterogeneous dentin adhesive *Journal of Dental Research* **87(9)** 829-833.
31. Tay FR, Gwinnett JA & Wei SH (1997) The overwet phenomenon in two-component acetone-based primers containing aryl amine and carboxylic acid monomers *Dental Materials* **13(2)** 118-127.
32. Tay FR, Pashley DH, Garcia-Godoy F & Yiu CK (2004) Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part II. Silver tracer penetration evidence *American Journal of Dentistry* **17(5)** 315-322.
33. Brackett WW, Ito S, Tay FR, Haisch LD & Pashley DH (2005) Microtensile dentin bond strength of self-etching resins: effect of a hydrophobic layer *Operative Dentistry* **30(6)** 733-738.
34. Suh BI, Feng L, Pashley DH & Tay FR (2003) Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part III. Effect of acidic resin monomers *Journal of Adhesive Dentistry* **5(4)** 267-282.
35. Schittly E, Bouter D, Le Goff S, Degrange M & Attal JP (2010) Compatibility of five self-etching adhesive systems with two resin luting cements *Journal of Adhesive Dentistry* **12(2)** 137-142.
36. Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, Carvalho RM, Pashley DH & Tay FR (2005) Solvent and water retention in dental adhesive blends after evaporation *Biomaterials* **26(34)** 6863-6872.
37. Bail M, Malacarne-Zanon J, Silva SM, Anauate-Netto A, Nascimento FD, Amore R, Lewgoy H, Pashley DH & Carrilho MR Effect of air-drying on the solvent evaporation, degree of conversion and water sorption/solubility of

dental adhesive models *Journal of Materials Science: Materials in Medicine*
23(3) 629-638.

CONSIDERAÇÕES GERAIS

Foram avaliados dois métodos com o objetivo de evitar os efeitos negativos da reação adversa entre sistemas adesivos simplificados e cimentos resinosos de ativação química ou dupla.

Para verificar o efeito da adição de resina de troca aniônica (AER) em evitar essa incompatibilidade, foi preparado um cimento resinoso experimental, com composição similar a do cimento Duolink e foi adicionada AER nas formas OH e OH+F. Em estudo prévio (Kanehira *et al.*, 2006) foi observado que o efeito de desprotonação das AER está em função da área de superfície específica do polímero usado para as trocas iônicas. Assim, foi sugerido que a redução no tamanho dos grãos, tornaria possível a desprotonação efetiva com menor concentração de AER. Para verificar se a incorporação da resina não acarretaria prejuízo às propriedades do cimento foram realizados testes mecânicos, como resistência à flexão e microdureza Knoop, bem como a possível interferência no grau de conversão. Assim, cimentos experimentais com diferentes concentrações de AER (0, 2, 4, 8, 16% em peso) foram avaliados em estudo piloto e foi selecionado o material com concentração que obteve o melhor desempenho, no caso 4%.

Neste estudo (capítulo 1), não houve diferença na resistência da união entre os grupos fixados com o cimento controle (sem adição de AER) e os cimentos com adição de AER. A ação de desprotonar os monômeros ácidos pela amina aromática terciária está relacionada com a espessura da camada superficial do adesivo com polimerização inibida pela presença de oxigênio, resultando assim em

maior ou menor quantidade de monômeros ácidos presentes na superfície. Foram encontradas melhores propriedades nanomecânicas (módulo de elasticidade reduzido e nanodureza) para os cimentos com AER, no período inicial. Durante os testes preliminares para a seleção do cimento de concentração adequada foi observado, na análise do grau de conversão, que a reação de polimerização química do cimento com adição de AER ocorreu em menos tempo que o cimento controle. As AERs podem ser utilizadas para neutralizar o pH, agindo como uma solução sólida tampão (Li *et al.*, 2008). No presente estudo, as AER foram convertidas para forma alcalina, com pH em torno de 10, quando em solução aquosa, o que pode ter aumentado a taxa de polimerização do cimento resinoso, justificando assim os maiores valores do módulo de elasticidade reduzido (E_r) e nanodureza (H). Entretanto, essa melhora nas propriedades não implicou em aumento na resistência da união. Assim, o sistema adesivo e a qualidade da camada adesiva parece influenciar mais significativamente a resistência da união do que a camada de cimento resinoso.

Conforme observado no capítulo 2 deste estudo, a aplicação de uma camada extra de resina hidrófoba não implicou em aumento significativo na resistência da união. Reis *et al.*, em 2008, ao reportar aumento na resistência da união, creditaram este resultado a redução da permeabilidade da camada de adesivo após aplicação de uma camada de resina hidrófoba. De acordo com os resultados observados neste estudo acredita-se que os sistemas adesivos estudados apresentam característica mais hidrófoba e pH menos ácido (ACE=3,8 e SCU=2,8) em comparação aos materiais avaliados em estudo anterior (pH entre 0,8 e 2,0),

realizado por Brackett *et al.* (2005), podendo assim, mascarar o efeito positivo relatado em estudos anteriores após a aplicação de uma camada intermediária de resina hidrófoba. Ainda, é possível que tenha havido a separação de fase entre os componentes no sistema adesivo (Wang e Spencer, 2005), permitindo que os componentes com característica mais hidrófila, se difundissem mais profundamente na matriz dentinária desmineralizada, deixando maior concentração de componentes hidrófobos na superfície da camada de adesivo, na área de interface sistema adesivo e cimento resinoso. Assim, devido a dificuldade de volatilizar solventes (Yiu *et al.*, 2005) e a água presentes na formulação dos adesivos, pode ter se formado uma camada mais porosa, com predomínio de monômeros hidrófilos na base da camada de adesivo e monômeros hidrófobos mais concentrados na superfície. Esta observação justifica também os resultados observados após avaliação da infiltração por Rodamina, tendo em vista que também não foi observada diferença na quantidade de infiltração entre os grupos. Entretanto, ao se realizar o teste de microtração, a probabilidade de falha é maior em regiões que apresentam defeitos, como porosidades por exemplo, as quais podem existir, independente da aplicação ou não da camada superficial de resina hidrófoba.

Nesse estudo foram verificadas as propriedades nanomecânicas da camada de cimento resinoso, quando foi simulada *in vitro* uma restauração indireta de resina composta. O método empregado para a avaliação do módulo de elasticidade e dureza da camada de cimentação foi possível com base no gráfico de tensão-deformação do material durante a indentação. Segundo o método proposto por Oliver e Pharr (1992), o cálculo é baseado na profundidade da indentação realizada

com ponta Berkovich, em relação a força máxima. Conhecendo a profundidade da indentação e a forma da ponta, é possível calcular a área de contato durante a força máxima. No método comumente usado, os dados são obtidos do completo ciclo de carregamento e descarregamento, sendo o módulo de elasticidade obtido dos dados de descarregamento, de acordo com um modelo que relaciona a área de contato durante a força máxima com o módulo de elasticidade. Como o cimento resinoso possui característica pseudoplástica, foi adicionado aos ciclos de carregamento e descarregamento, um ciclo de espera, no qual a ponta é mantida sob força máxima constante. Assim, a recuperação durante o período de descarregamento será somente elástica. A dureza pode ser utilizada como forma indireta de avaliação do grau de conversão. Os valores de dureza encontrados (0,4 a 0,7 GPa) estão de acordo com a literatura para cimentos resinosos (Moosavi *et al.*, 2013). Os dados de módulo de elasticidade, além de serem utilizados para verificar o envelhecimento do material (Dos Santos *et al.*, 2010), podem ser indicativos do comportamento das tensões na interface, quando submetida a estresse mecânico.

Nos capítulos um e dois, a utilização do cimento resinoso com ativação química em contato direto com os sistemas adesivos simplificados, assim como a não adição de AER ao cimento resinoso, resultou em menores valores de módulo de elasticidade reduzido e nanodureza. Contudo, é interessante o fato que mesmo obtendo-se uma camada de cimentação com propriedades inferiores, a diferença na resistência da união ocorreu principalmente, pelas características individuais dos sistemas adesivos utilizados.

CONCLUSÃO

Os procedimentos utilizados para a redução da incompatibilidade entre sistemas adesivos simplificados e cimentos resinosos de ativação dupla mostraram-se capazes de melhorar as propriedades nanomecânicas da camada de cimento resinoso polimerizado quimicamente, quando associada a sistemas adesivos simplificados. Entretanto, esta melhora não refletiu em aumento na resistência da união.

REFERÊNCIAS

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

Darr AH, Jacobsen PH. Conversion of dual cure luting cements. *J Oral Rehab* 1995; 22: 37-42.

Franco EB, Lopes LG, D'Alpino P H, Pereira JC, Mondelli RF, Navarro MF. Evaluation of compatibility between different types of adhesives and dual-cured resin cement. *J Adhes Dent* 2002; 4: 271-5.

Li Q, Li W, Wang D, Liu B, Tang H, Yang M, Liu Q *et al.* pH Neutralization while succinic acid adsorption onto anion-exchange resins. *Appl Biochem Biotechnol* 2010; 160: 438-445.

Piva E, Correr-Sobrinho L, Sinhoreti MA, Consani S, Demarco FF, Powers JM. Influence of energy density of different light sources on Knoop hardness of a dual-cured resin cement. *J Appl Oral Sci* 2008; 16: 189-93.

Rueggeberg FA, Margeson DH. The effect of oxygen inhibition on an unfilled/filled composite system. *J Dent Res* 1990; 69: 1652-8.

ANEXO 1

Carta de aprovação do Comitê de Ética em Pesquisa.

06/02/2012

Comitê de Ética em Pesquisa - Certificado



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



CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Inativação da presa química de cimentos resinosos**", protocolo nº 112/2011, dos pesquisadores Ariene Arcas Topal Paes Leme e Mário Alexandre Coelho Sinhoretí, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 28/10/2011.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project "**Self-cure of resin cements inactivation**", register number 112/2011, of Ariene Arcas Topal Paes Leme and Mário Alexandre Coelho Sinhoretí, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 10/28/2011.

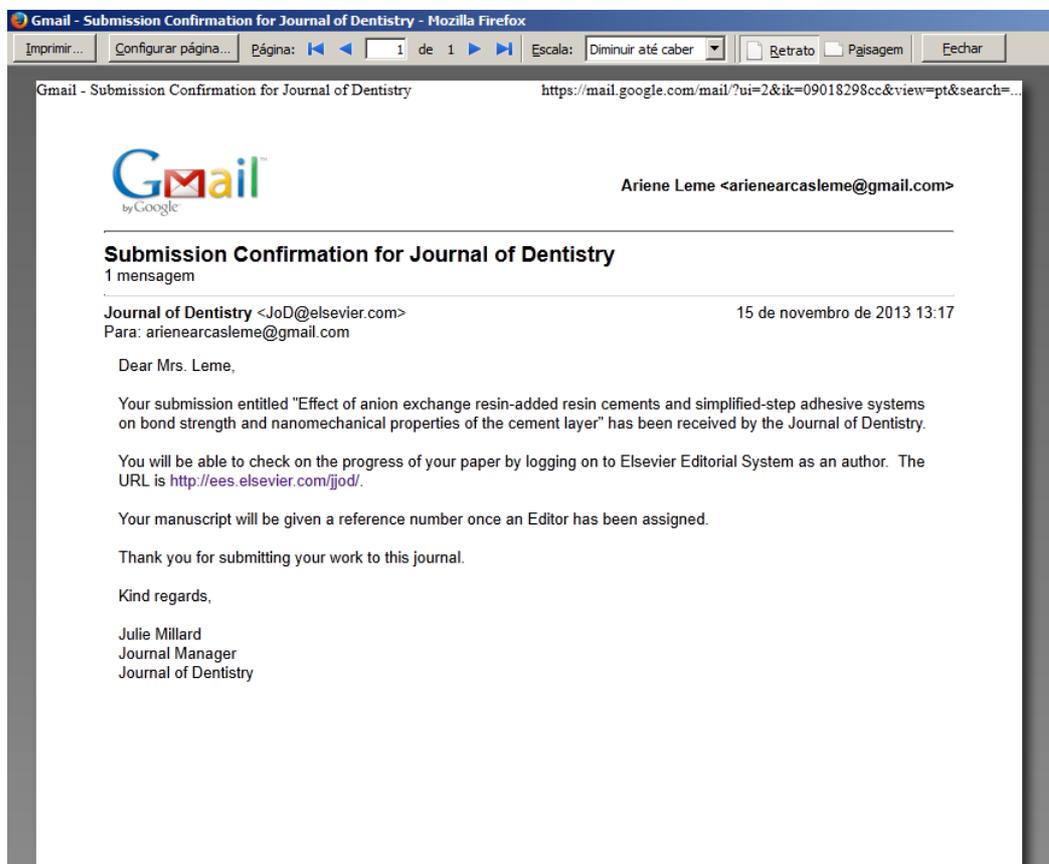

Prof. Dra. Lívia Maria Andakó Tenuta
Secretária
CEP/FOP/UNICAMP


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

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

Anexo 2

Carta de envio do Capítulo 1 para publicação.



APÊNDICE

ILUSTRAÇÃO DA METODOLOGIA



Figura 1 – Materiais utilizados para os procedimentos de cimentação: os sistemas adesivos (A) ACE ALL Bond TE (Bisco Inc.), (B) Scotchbond Universal (3M ESPE) e (C) os cimentos experimentais Controle, Experimental-1 e Experimental-2.

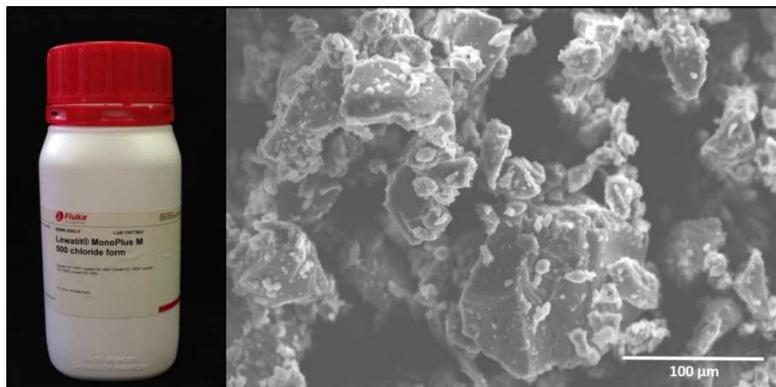


Figura 2 –Resina de troca aniônica (AER) observada em microscópio eletrônico de varredura após ter o tamanho das partículas reduzidos, para possibilitar a incorporação aos cimentos resinosos experimentais.

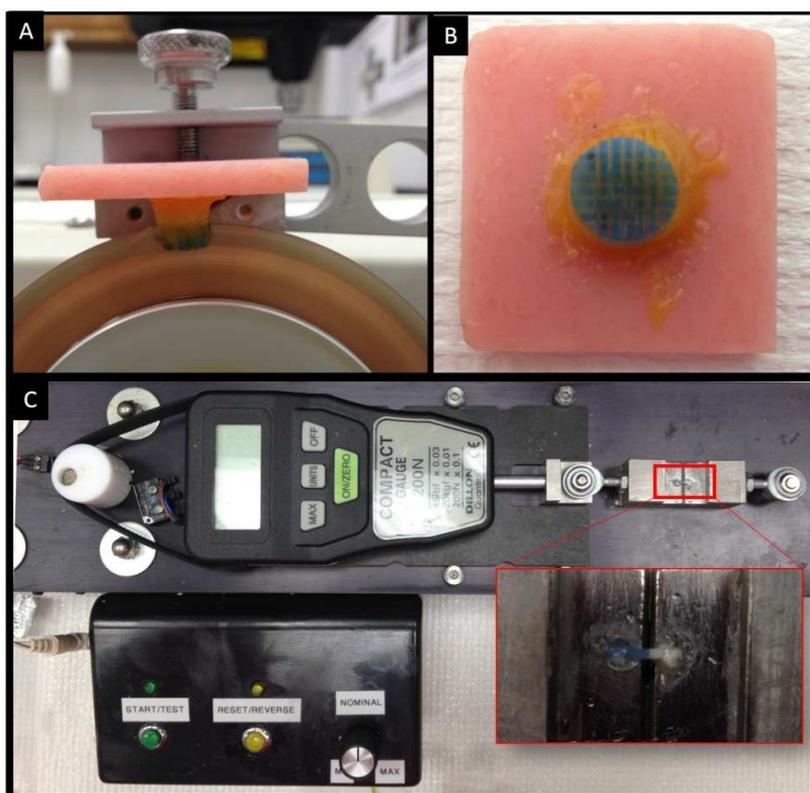


Figura 3 – Amostras para o ensaio de resistência da união. Em A e B, corte dos dentes para obtenção dos palitos. Em C, a máquina de microtração e, em maior aumento, a amostra fixada ao dispositivo pronta para o ensaio.

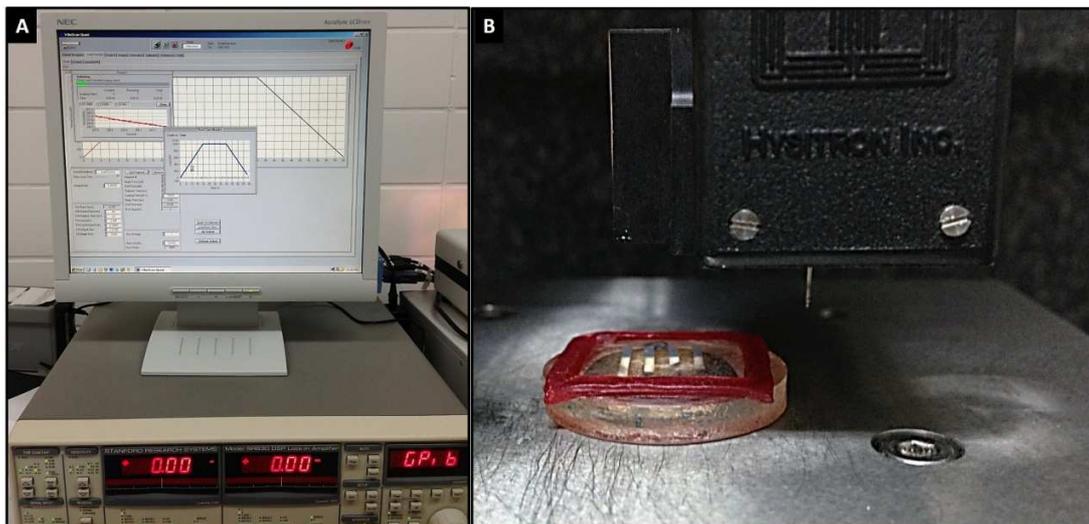


Figura 4 – Em (A) os gráficos de força e deslocamento durante o ensaio de nanoindentação, com o Triboindenter (Hysitron Inc.) equipado com uma ponta Berkovich fluid cell (B), que permitiu a avaliação das amostras imersas em água destilada a 25° C.

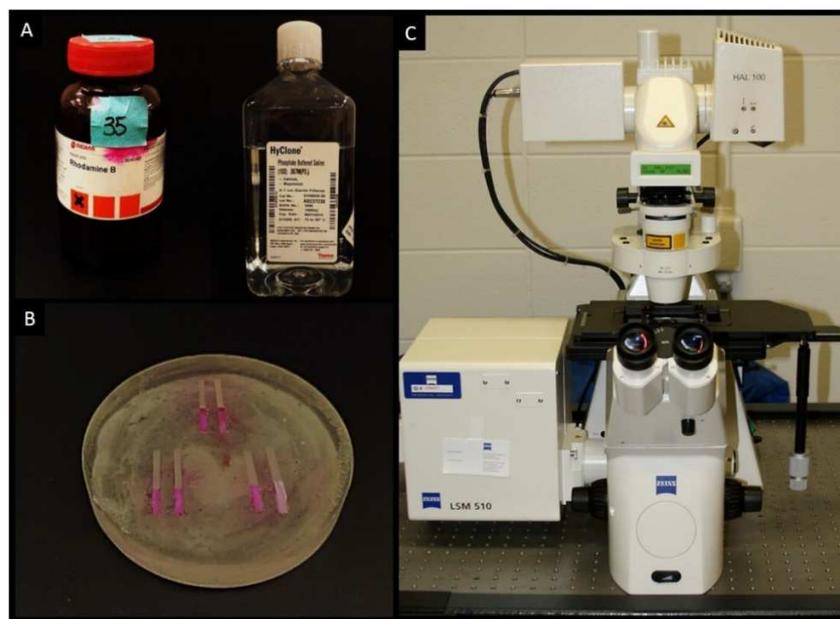


Figura 5 – Em A, solução de Rodamina 0,1% w/v (RITC/Rhodamine B, Sigma), dissolvida em 0,1 M *Phosphate buffered solution* (PBS, pH 7,2-7,4), usada para infiltrar as amostras (B). Em C, o microscópio de varredura confocal laser modelo LSM 510 (Zeiss).