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ESTRATÉGIAS BIOMIMÉTICAS PARA MELHORAR A ADESIVIDADE E O DESEMPENHO MECÂNICO DE RESTAURAÇÕES DE RESINAS COMPOSTAS AOS TECIDOS DENTINÁRIOS SADIOS E CLAREADOS

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Tese apresentada ao Programa de Pós Graduação em Odontologia Integrada da Universidade Estadual de Maringá para obtenção do título de Doutora.

Área de concentração: Odontologia Integrada

Orientador: Prof. Dr. Mauro Luciano Baesso

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Amaral ALMU. Estratégias biomiméticas para melhorar a adesividade e o desempenho mecânico de restaurações de resinas compostas aos tecidos dentinários sadios e clareados [tese]. Maringá: Universidade Estadual de Maringá; 2018

RESUMO

Objetivo: Investigar os efeitos de estratégias restauradoras biomiméticas nas propriedades químicas e mecânicas da interface adesiva entre restaurações de resina composta e a dentina sadia e clareada. **Métodos:** Foram realizados quatro estudos com metodologias diferentes. 1) Com o teste de microtração (μTBS) e a microscopia eletrônica de varredura (MEV) foram avaliados o pré-aquecimento do sistema adesivo, a inclusão do Biovidro® 45S5 (BAG) na solução do primer, e a combinação de ambas as técnicas na utilização de um sistema adesivo convencional de três passos sobre a dentina após uma semana e seis meses de armazenamento; 2) Utilizando-se o μTBS, o MEV e a espectroscopia micro-Raman (MRS) foram investigadas as modificações na resistência de união, as características físico-químicas da dentina e a espessura da interface adesiva após tratamento com o biovidro realizado com o adesivo de 3 passos; 3) Com o μTBS e a MRS analisou-se a capacidade do BAG e de um Biossilicato de promoverem remineralização dentinária, modificações na resistência de união e efeitos na interface adesiva. Isto foi feito no processo de remineralização durante o uso de um adesivo convencional de dois passos nos tecidos dentinário sadio e clareado; 4) A resistência acelerada à fadiga foi utilizada para avaliar incisivos tratados endodonticamente (ITE) após serem submetidos à técnica de clareamento interno e restaurados com base de cimento de ionômero de vidro (CIV), base de resina composta reforçada com fibras de vidro e com pino intraradicular de fibra de vidro. **Resultados:** A utilização do sistema adesivo pré-aquecido aumentou a resistência de união dentinária no tempo inicial, a qual manteve-se constante após seis meses. O tratamento remineralizador aumentou a composição mineral da dentina, modificou a composição estrutural da interface adesiva e aumentou a resistência de união entre o sistema adesivo e o tecido dentinário sadio e clareado. A substituição do tecido dentinário pelo CIV na restauração dos ITE clareados demonstrou ser uma alternativa conservadora e satisfatória do ponto de vista mecânico. **Conclusão:** O uso de estratégias biomiméticas durante o procedimento restaurador melhorou significativamente o desempenho das restaurações adesivas diretas em dentina.

Palavras-chave: Adesão, Dentina, Teste de Microtração, Análise Espectral Raman, Microscopia Eletrônica de Varredura, Resistência à Fadiga Acelerada.

Amaral ALMU. Improvement of the adhesive and mechanical proprieties of composite resin restorations to healthy and bleached dentin tissues with biomimetic strategies [tesis]. Maringá: State University of Maringá; 2018.

ABSTRACT

Objective: To investigate the effects of biomimetic restorative strategies on chemical and mechanical properties of the adhesive interface between composite resin restorations and healthy and bleached dentin. **Methods:** Four studies with different methodologies were done: 1) Microtensile test (μTBS) and scanning electron microscopy (SEM) were used to test heathy dentin specimens treated with an etch-and-rinse 3-step adhesive system in different conditions: pre-heated, containing Bioglass® 45S5 (BAG) in its primer solution and preheated with BAG in its primer; specimens were tested after one week and six months of storage. 2) μTBS, SEM and micro-Raman spectroscopy (MRS) were used to investigate dentin bond strength, dentin physical-chemical modifications and adhesive interface thickness after dentin remineralization treatment with BAG performed during the treatment with a 3-step adhesive system. 3) With the methods of μTBS and MRS, BAG and Biosilicate the ability to promote healthy and bleached dentin remineralization were investigated using a 2-step adhesive system. In addition, changes at adhesive interface and modifications on dentin bond strength were analyzed. 4) The accelerated fatigue technique was used to evaluate bleached endodontically treated incisors (ETI) restored with three distinct internal materials: glass-ionomer cement (GIC) base, short-fiber reinforced composite resin base and luted fiberglass post. **Results:** The use of the pre-heated adhesive system increased dentin bond strength after one week and this increased value remained the same after six-month storage. Remineralization treatment increased the mineral composition of dentin tissue, modified the structural characteristic of the adhesive interface and improved the bond strength between the adhesive system and the healthy and bleached dentin tissue. The replacement of dentin tissue by GIC as the restorative treatment of bleaching ETI was shown to be a conservative and mechanically satisfactory alternative. **Conclusion:** The use of biomimetic strategies during the restorative procedures significantly improved the performance of dentin direct adhesive restorations.

Key words: Adhesion; Dentin; Raman Spectral Analysis; Microtensile Bond Strength; Scanning Electron Microscopy; Accelerated Fatigue Resistance.

SUMÁRIO

1. CAPÍTULO DE CONTEXTUALIZAÇÃO

1.1 ODONTOLOGIA BIOMIMÉTICA

Tratamentos odontológicos minimamente invasivos são procedimentos realizados com o objetivo de restaurar os elementos dentários quanto a sua função, propriedades mecânicas e ópticas com um desgaste mínimo de estrutura dentária sadia (WHITE, EAKLE, 2000; RAINEY, 2001). As técnicas restauradoras dentro do conceito da mínima invasão, preservam o esmalte e a dentina, diminuem o risco de exposição pulpar e reduzem as chances de fratura dentária. Esta preservação dos tecidos dentários hígidos vem ao encontro com os princípios da Odontologia Biomimética, na qual os procedimentos restauradores devem ser realizados com materiais que tenham características estéticas e funcionais semelhantes aos dos tecidos dentários e sejam capazes de mimetizar o tecido perdido, restabelecendo a anatomia das camadas de dentina e de esmalte (MAGNE, BELSER, 2002).

Considerando que a combinação entre o esmalte e a dentina proporciona ao dente uma relação ideal entre rigidez, força e resiliência (MAGNE, BELSER, 2002), o efeito de mimetismo ao dente natural deve ser almejado durante os procedimentos restauradores a fim de evitar a perda do equilíbrio das propriedades físicas e mecânicas destes tecidos. O objetivo principal do uso de abordagens terapêuticas biomiméticas é restabelecer os tecidos dentários perdidos proporcionando uma união funcional e estável dos materiais restauradores com o remanescente dentário.

Quanto aos materiais restauradores disponíveis no mercado odontológico, o cimento de ionômero de vidro (CIV) tem sido considerado o melhor substituto ao tecido dentinário por apresentar módulo de elasticidade semelhante ao da dentina, adesividade aos tecidos dentários e liberação de flúor

(MALHOTRA et al., 2009). Por outro lado, em função de suas excelentes propriedades mecânicas e ópticas as cerâmicas odontológicas consistem em materiais alternativos ao esmalte dentário. Além disto, com o desenvolvimento da Odontologia Adesiva, o processo de hibridização entre o tecido dentinário e os monômeros adesivos tem sido comparado com a complexa união entre o esmalte e a dentina na junção amelo-dentinária (MAGNE, BELSER, 2002).

Independente do material restaurador selecionado, ao aplicar-se o princípio biomimético, entende-se que as abordagens restauradoras não devem ser realizadas para obter-se a restauração mais forte possível, mas sim a restauração que tenha as propriedades físicas e mecânicas mais compatíveis com as do tecido dentário subjacente.

1.2 ADESÃO AO TECIDO DENTINÁRIO

O desenvolvimento das técnicas adesivas tem influenciado a Odontologia Restauradora moderna (VAN MEERBEEK et al., 2010). Embora a técnica adesiva possibilite a confecção de restaurações minimamente invasivas, a substituição de restaurações adesivas diretas insatisfatórias constitui grande parte do trabalho operacional na prática odontológica atual (NIU et al., 2014). Devido à sua composição estrutural heterogênea, o tecido dentinário representa o substrato mais sensível aos procedimentos restauradores e com menor longevidade de união (LOGUERCIO et al., 2008). A união entre a resina e a dentina é predominantemente micromecânica, ocorrendo por meio da formação da camada híbrida que se caracteriza pela infiltração dos monômeros adesivos no substrato dentinário parcial ou completamente desmineralizado, promovendo a proteção do emaranhado de fibras colágenas expostas na dentina (NAKABAYASHI, 1992)

Os sistemas adesivos convencionais de 3 passos são considerados os adesivos padrão ouro até o presente momento em função de sua alta resistência adesiva (50 MPa) e de sua ótima estabilidade de

união em função do tempo (VAN MEERBEEK et al., 2010; DE MUNK et al., 2012). Nesta classe de adesivos a formação da camada hibrida é dependente da etapa de condicionamento ácido que além de limpar a superfície dentinária removendo a camada de detritos, desmineraliza a dentina superficial (DE MUNK et al., 2010) expondo as fibrilas de colágeno (BRESCHI et al., 2010).

Contudo, a literatura relata que a camada híbrida ideal não é alcançada quando os adesivos convencionais são utilizados uma vez que os monômeros resinosos do adesivo não conseguem se difundir ao longo da região de dentina desmineralizada, que é em geral em torno de 5 µm de espessura (SPENCER, SWAFFORD, 1999). Esta discrepância entre a profundidade de condicionamento e de infiltração dos monômeros (VAN MEERBEEK et al., 2003; PASHLEY et al., 2011) está associada com a perda da integridade da camada híbrida, devido a hidrólise dos componentes hidrófobos do adesivo pela presença de água na região não infiltrada (ITO et al., 2005) bem como com a degradação do colágeno não protegido pelas enzimas metaloproteinases (MMPs) que são ativadas pelo condicionamento ácido (PASHLEY et al., 2004).

1.3 ADESÃO AO TECIDO DENTINÁRIO CLAREADO

Devido ao alto risco de falhas biomecânicas, o procedimento restaurador de dentes tratados endodonticamente consiste em um grande desafio clínico na odontologia restauradora contemporânea (DIETSCHI et al., 2008; JULOSKI et al., 2012; SAMRAN et al., 2015). O acesso endodôntico e o preparo dos condutos radiculares promovem perda excessiva de tecido dentário, modificando consequentemente a retenção das restaurações dentárias (SANTOS-FILHO et al., 2014). Além disto, o clareamento intracoronário, uma opção conservadora para melhorar a estética de dentes submetidos ao tratamento endodôntico com alterações de cor, contribui com o aumento da taxa de falha destas restaurações. A modificação estrutural da dentina em função da ação dos agentes clareadores durante

o procedimento de clareamento intracoronário diminui a resistência dentária à fratura e aumenta a taxa de falha de procedimentos restauradores de dentes tratados endodonticamente.

A aplicação do agente clareador diretamente sobre a dentina promove modificações estruturais e morfológicas (JIANG et al., 2007; UYSAL et al., 2009) diminuindo a eficácia da adesividade deste tecido. A característica oxidativa dos produtos clareadores provoca desnaturação proteica (LADO et al., 1983) e aumenta a degradação do colágeno pela ativação das MMPs (TOLEDANO et al., 2011). Além disto, o baixo pH dos agentes clareadores promove um efeito de condicionamento ácido (PRICE et al., 2000) do tecido dentinário promovendo uma redução do componente mineral da dentina (JIANG et al., 2007). A análise da microtração da dentina clareada demonstrou que o tratamento clareador reduz a resistência de união dentinária (TAM et al., 2007). Esta alteração na adesividade dentinária tem sido atribuída tanto às modificações estruturais provenientes da ação do clareador quanto à inibição de polimerização causada pela presença residual de moléculas de oxigênio e radicais livres resultantes do processo clareador (JIANG et al., 2007; TOLEDANO et al., 2011).

De acordo com os princípios biomiméticos, a utilização de pinos intraradiculares na restauração de dentes tratados endodonticamente deve ser evitada uma vez que a alta resistência à fratura destes pinos associada à fragilidade do remanescente dentário radicular resulta em perdas dentárias devido à ocorrência de falhas catastróficas comprovadas por testes de resistência a fatiga (MAGNE et al., 2017, LAZARI et al., 2017). Considerando as modificações estruturais e mecânicas no tecido dentinário clareado, o uso do CIV como material de preenchimento intracoronário durante o procedimento restaurador pode apresentar-se como uma alternativa eficaz do ponto de vista biomimético. Além de possuir o mesmo coeficiente de expansão térmico dentinário e de unir-se quimicamente com a dentina, o uso do CIV representa uma boa escolha porque este material é de fácil manipulação e inserção, proporciona um selamento marginal satisfatório e apresenta coloração e opacidade apropriados.

1.4 ESTRATÉGIAS EXPERIMENTAIS PARA PREVENIR A DEGRADAÇÃO DA SUPERFÍCIE ADESIVA

A durabilidade da interface entre a resina composta e o tecido dentinário consiste em um dos maiores desafios da odontologia adesiva contemporânea. Os dois principais problemas envolvidos na degradação desta interface adesiva consistem na hidrólise dos monômeros adesivos e na degradação das fibrilas de colágeno. A fim de minimizar estes mecanismos e prevenir a deterioração da interface adesiva, algumas estratégias experimentais têm sido investigadas, entre elas: o aumento do grau de conversão dos sistemas adesivos; o uso de inibidores das enzimas colagenolíticas; utilização de agentes promotores de ligações cruzadas e a técnica de adesão com etanol. Estas estratégias atuam de maneiras diferentes, mas com o mesmo objetivo, melhorar as propriedades mecânicas da interface entre a resina e a dentina. Contudo, a literatura tem demonstrado que estes mecanismos não aumentam a habilidade dos monômeros resinosos em infiltrar a matriz de colágeno desmineralizada, pois não conseguem eliminar a presença da água nos compartimentos das fibrilas do colágeno (MALACARNE-ZANON et al., 2009; PARK et al., 2010). Além disto, não proporcionam uma proteção de longo prazo contra a degradação do colágeno desmineralizado pela ação das MMPs (MONTAGNER et al., 2014), tanto pela incapacidade de remover as moléculas de água (SADEK et al., 2010) quanto em função da incapacidade de alterar a dureza intrínseca da molécula do colágeno (LIAO et al., 2005).

A mineralização biomimética é uma estratégia que utiliza princípios da nanotecnologia para imitar o que ocorre na biomineralização (TAY E PASHLEY, 2008). Esta técnica de remineralização dentinária tem se mostrado uma alternativa eficaz para a preservação da integridade e consequentemente da longevidade da interface adesiva (RYOU et al., 2011; PROFETA et al., 2012). O uso de agentes promotores da remineralização proporciona um mecanismo de desidratação progressiva, substituindo a água intra e extrafibrilar, promovendo a formação de uma camada de

hidroxiapatita na superfície dentinária (TIRAPELLI et al., 2010; WANG et al., 2011), refazendo assim a função de proteção das apatitas intra e extrafibrilares sobre o colágeno (MAZZONI et al., 2006; BRACKETT et al., 2011; PROFETA et al., 2013).

Diferentes técnicas de remineralização têm sido utilizadas para promover a remineralização da interface entre a resina e o tecido dentinário. A técnica de remineralização pode ser realizada após a hibridização por meio do mecanismo de difusão pela imersão da interface em meios compostos por substâncias remineralizadoras (análogos biomiméticos duplos com cimento Portland, fluído corporal simulado, nanopartículas poliméricas carregadas de cálcio, soluções de fosfato, Cl_2Zn e ZnO) (NIU et al., 2014). Por outro lado, a remineralização tem sido testada de forma mais clínica com aplicação do agente remineralizador durante as etapas do procedimento adesivo, pela utilização de soluções contendo agentes remineralizadores na dentina condicionada previamente a aplicação do sistema adesivo (Biovidro® 45S5, Biosilicato®) ou pela inclusão de substâncias bioativas nos monômeros adesivos (micropartículas de cimento Portland, Biovidro® 45S5, análogos de fosfoproteínas, hidroxiapatita de policarboxilato e fosfato de tricálcio) (Liu et al., 2011).

Dentre os agentes remineralizadores, o Biovidro® 45S5 consiste no material padrão ouro por apresentar o maior índice de bioatividade (IB:12.5) (HENCH et al. 1971). O Biosilicato®, que consiste em um vidro cerâmico, foi desenvolvido com o objetivo de combinar a alta bioatividade do Biovidro 45S5 com boa resistência mecânica e a dureza das cerâmicas (CROVACE et al., 2015). A ação remineralizadora destes dois agentes é dependente do contato com um meio fluído e acontece em cinco estágios (HENCH et al. 1971; CROVACE et al., 2015). No primeiro estágio acontece a substituição dos cátions de sódio (Na^+) da substância bioativa pelos cátions de hidrogênio e/ou hidrônio (H^+/H_3O^+) provenientes do meio aquoso. Esta troca iônica promove um aumento localizado e momentâneo do pH, e esta mudança resulta na quebra da ligação do óxido de silicone (Si-O-Si). Em consequência da ruptura

desta ligação ocorre a liberação de hidróxido de silício ($Si(OH)_4$) caracterizando o segundo estágio da reação. Se o pH for menor que 9,5 o hidróxido de silício sofre condensação formando uma camada de sílica gel na superfície dentinária, correspondendo ao terceiro estágio da remineralização. A estrutura aberta desta camada de sílica gel permite a continuação das trocas iônicas entre as partículas do agente bioativo e do meio aquoso. Desta forma, íons de cálcio e fosfato (Ca^{2+} e PO_4^{3-}) destes dois meios irão se unir e formar uma camada amorfa de fosfato de cálcio ($Ca_3(PO_4)_2$) na superfície da sílica gel, fato este que consiste no quarto estágio da remineralização. Após o aumento da espessura desta camada amorfa de sílica-gel com fosfato de cálcio, ocorre a incorporação das espécies de carbonato (CO_3^{2-}), que corresponde ao quinto e último estágio de remineralização, que resulta do processo de cristalização e de formação da apatita hidrocarbonatada, que é quimicamente e estruturalmente semelhante a fase mineral encontrada no tecido mineral.

O processo de biomineralização permite a formação de ligações eletrostáticas, iônicas e/ou ponte de hidrogênio entre o colágeno desmineralizado e os compostos silanóis (PASHLEY et al., 2011; NIU et al., 2014). Estes compostos restringem as atividades das MMPs tanto pela formação de um complexo de peso molecular elevado (Ca/P-MMP) (LIU et al., 2011, BEDRAN-RUSSO et al.,2014), como pela criação de um ambiente alcalino ideal (PASHLEY et al., 2004; SADEK et al., 2010, BRESCHI et al., 2008; SAURO et al., 2009) resultante da liberação de Na^+ e Ca^{2+}. Outra vantagem, devido ao aumento do pH consiste no efeito bacteriostático na interface, uma vez que materiais restauradores contendo partículas bioativas apresentaram efeito bacteriostático sobre uma vasta variedade de bactérias aeróbias (BOECKH et al., 2002). Diferentemente das outras estratégias que visam a prevenção da degradação da camada híbrida, a remineralização dentinária tem se demonstrado uma alternativa eficaz uma vez que além de induzir a formação da fase mineral perdida durante o tratamento ácido da superfície dentinária, também promove a remoção das moléculas de água residuais

na interface, reduzindo a ocorrência de hidrólise dos monômeros adesivos assim como da degradação das fibrilas de colágeno.

1.5 OBJETIVOS

De acordo com os princípios da odontologia biomimética, a preservação da estrutura dentária sadia deve ser preconizada por meio da utilização de técnicas restauradoras minimamente invasivas. A realização dos procedimentos restauradores deve preconizar o uso de materiais dentários capazes de mimetizar o tecido perdido e o emprego de estratégias biomiméticas para prevenir a degradação da interface adesiva. Desta forma, a presente tese teve como objetivo analisar os efeitos da utilização de estratégias restauradoras biomiméticas nas propriedades químicas e mecânicas da interface adesiva entre restaurações de resina composta e os tecidos dentinários sadios e clareados. O estudo foi dividido em etapas, resultando em quatro artigos com objetivos específicos:

- Artigo 1: Avaliar a influência do pré-aquecimento do sistema adesivo, a inclusão do Biovidro® 45S5 na solução do primer, bem como a combinação de ambas as técnicas para investigar a resistência de união de um sistema adesivo de três passos com o tecido dentinário sadio após uma semana e seis meses de armazenamento. Artigo submetido para o periódico Journal of Prosthetic Dentistry.

- Artigo 2: Investigar as modificações na resistência de união dentinária, as características físico-químicas da dentina e a espessura da interface adesiva após tratamento remineralizador dentinário com solução do Biovidro® 45S5, realizado durante as etapas de hibridização de um sistema adesivo de três passos. Artigo submetido para o periódico Journal of Adhesive Dentistry.

- Artigo 3: Analisar a capacidade do Biovidro® 45S5 e de um Biossilicato® de promover remineralização dentinária, modificações na resistência de união dentinária e efeitos terapêuticos na

interface adesivo-dentina; quando utilizados como agentes remineralizadores durante o processo de hibridização com um adesivo convencional de dois passos aplicado no tecido dentinário sadio e clareado. Artigo submetido para o periódico Dental Materials.

- Artigo 4: Comparar a resistência acelerada à fadiga e os padrões de fratura de incisivos tratados endodonticamente submetidos à técnica de clareamento interno e restaurados com três técnicas diferentes, 1) base com cimento de ionômero de vidro substituindo a dentina envolta por resina composta, 2) base com resina composta reforçada com fibras de vidro substituindo a dentina envolta por resina composta e 3) restauração de resina composta sobre pino intraradicular de fibra de vidro. Artigo submetido para o periódico Journal of Dentistry.

REFERÊNCIAS

BEDRAN-RUSSO, A.K. et al. Dentin biomodification: strategies, renewable resources and clinical applications. **Dent Mater,** v. 30, n. 1, p. 62-76, 2014.

BOECKH, C.; SCHUMACHER, E.; PODBIELSKI, A.; HALLER, B. Antibacterial activity of restorative dental biomaterials in vitro. **Caries Res,** v. 36, n. 2, p. 101-107, 2002.

BRACKETT, M.G. et al. The critical barrier to progress in dentine bonding with the etch-and-rinse technique. **J Dent,** v. 39, n. 3, p.238-248, 2011.

BRESCHI, L. et al. Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. **Dent Mater,** v. 26, p. 320-325, 2010.

BRESCHI, L. et al. Dental adhesion review: aging and stability of the bonded interface. **Dent Mater,** v. 24, p. 90-101, 2008.

CROVACE, M.C. et al. Biosilicate® — A multipurpose, highly bioactive glass-ceramic. In vitro, in vivo and clinical trials. **J Non Cryst Solids**, p. 90-110, 2015.

DE MUNCK, J. et al. Enzymatic degradation of adhesive-dentin interfaces produced by mild self-etch adhesives. **Eur J Oral Sci,** v. 118, p. 494-501, 2010.

DE MUNCK, J. et al. Meta-analytical review of parameters involved in dentin bonding. **J Dent Res,** v. 9, p.351-357, 2012.

DIETSCHI, D.; DUC, O.; KREJCI, I.; SADAN, A. Biomechanical considerations for the restoration of endodontically treated teeth: a systematic review of the literature, Part II (Evaluation of fatigue behavior, interfaces, and in vivo studies). **Quintessence Int,** v. 39, n. 2, p. 117-129, 2008.

HENCH, L.L. et al. Bonding mechanisms at the interface of ceramic prosthetic materials. **J Bio Mater Research,** v. 5, n. 6, p. 117-141, 1971.

ITO, S. et al. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. **Biomaterials,** v. 26, p.6449-6459, 2005.

JIANG, T. et al. Effects of hydrogen peroxide on human dentin structure. **J Dent Res,** v. 86, p. 1040-1045, 2007.

JULOSKI, J. et al. Ferrule effect: a literature review. **J Endod,** v. 38, n. 1, p. 11-19, 2012.

LADO, E.A.; STANLEY, H.R.; WEISMANN, M.I. Cervical resorption in bleached teeth. **Oral Surg Oral Med Oral Pathol,** v. 55, p. 78-80, 1983.

LAZARI, P.C.; DE CARVALHO, M.A.; DEL BEL CURY, A.A.; MAGNE. P. Survival of extensively damaged endodontically treated incisors restored with different types of posts-and-core foundation restoration material. **J Prosthet Dent,** v. S0022-3913. N. 17, 30368-30372, 2017.

LIAO, J.; YANG, L.; GRASHOW, J.; SACKS, M.S. Molecular orientation of collagen in intact planar connective tissues under biaxial stretch. **Acta Biomater, v.** 1, p. 45-54, 1:45-54, 2005.

LIU, Y. et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. **J Dent Res,** v. 90, n. 8, p. 953-968, 2011.

LOGUERCIO, A.D. et al. Durability of enamel bonding using two-step self-etch systems on ground and unground enamel. **Oper Dent,** v. 33, p. 79-88, 2008.

MAGNE, P.; BELSER, U. Bonded porcelain restorations in the anterior dentition: a biomimetic approach**. Quintessence Publishing Company,** 2002.

MAGNE, P.; LAZARI, P.C.; CARVALHO, M.A.; JOHNSON, T.; DEL BEL CURY, A.A. Ferrule-Effect Dominates Over Use of a Fiber Post When Restoring Endodontically Treated Incisors: An In Vitro Study. **Oper Dent,** v. 42, n. 4, p. 396-406, 2017.

MALACARNE-ZANON, J. et al. Effects of ethanol addition on the water sorption/solubility and percent conversion of comonomers in model dental adhesives. **Dent Mater,** v. 25, p. 1275-1284.

MALHOTRA, S.; HEGDE, M.N. Analysis of marginal seal of ProRoot MTA, MTA Angelus biodentine, and glass ionomer cement as root end filling materials: An in vitro study. **J Oral Res Rev,** v. 7, p. 44-49, 2009.

MAZZONI, A. et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. **Biomater,** v. 27, p. 4470-4476, 2006.
MONTAGNER, A.F.; SARKIS-ONOFRE, F.; PEREIRA-CENCI, T.; CENCI, M.S. MMP Inhibitors on Dentin Stability: A Systematic Review and Meta-analysis. **J Dent Res,** v. 93, n. 8, p. 733-743, 2014.

NAKABAYASHI, N.; ASHIZAWA, M.; NAKAMURA, M. Identification of a resin-dentin hybrid layer in vital human dentin created in vivo: durable bonding to vital dentin. **Quintessence Int**, v. 23, n. 2, p. 135-141, 1992.

NIU, L.et al. Biomimetic remineralization of dentin. **Dent Mater,** v. 31, n. 1, p. 77-96, 2014.

PARK, J. et al. Effect of photoinitiator system and water content on dynamic mechanical properties of a light-cured bisGMA/HEMA dental resin. **J Biomed Mater Res A,** v. 93, p. 1245-1251, 2010.

PASHLEY, D.H. et al. Collagen degradation by host-derived enzymes during aging. **J Dent Res,** v. 83, p. 216-221, 2004.

PASHLEY, D.H. et al. State of the art etch-and-rinse adhesives. **Dent Mater,** v. 27, n. 1, p. 1-16, 2011.

PRICE, R.B.T.; SEDAROUS, M.; HILTZ, G.S. The pH of tooth whitening products. **J Can Dent Assoc,** v. 66, p. 421-426, 2000.

PROFETA, A.C. et al. Bioactive effects of a calcium/sodium phosphosilicate on the resin-dentine interface: a microtensile bond strength, scanning electron microscopy, and confocal microscopy study. **Eur J Oral Sci,** v. 120, n. 4, p. 353-362, 2012.

PROFETA, A.C. et al. Experimental etch-and-rinse adhesives doped with bioactive calcium silicate-based micro-fillers to generate therapeutic resin-dentin interfaces. **Dent Mater,** v. 29, n. 7, p. 729-741, 2013.

RAINEY, J.T. Understanding the applications of microdentistry. **Compend Contin Educ Dent,** v. 22, n. 11A, p. 1018-1025, 2001.

RYOU, H. et al. Effect of biomimetic remineralization on the dynamic nanomechanical properties of dentin hybrid layers. **J Dent Res**, v. 90, n. 9, p. 1122-1128, 2011.

SADEK, F.T. et al. One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. **Dent Mater,** v. 26, n. 4, p. 380-386, 2010.

SAMRAN, A.; AL-AFANDI, M.; KADOUR, J.A.; KERN, M. Effect of ferrule location on the fracture resistance of crowned mandibular premolars: An in vitro study. **J Prosthet Dent,** v. 114, n. 1, p. 86-91, 2015.

SANTOS-FILHO, P.C. at al. Influence of ferrule, post system, and length on stress distribution of weakened root-filled teeth. **J Endod,** v. 40, n. 11, p. 1874-1878, 2014.

SAURO, S. et al. Two-photon laser confocal microscopy of micropermeability of resin–dentin bonds made with water or ethanol wet bonding. **J Biomed Mater Res B Appl Biomater,** v. 90, p. 327-337, 2009.

SPENCER, P.; SWAFFORD, J.R. Unprotected protein at the dentin-adhesive interface. **Quintessence Int,** v. 30, p. 501-507, 1999.

TAM, L.E.; NOROOZI, A. Effects of direct and indirect bleach on dentin fracture toughness. **J Dent Res,** v. 86, p. 1193-1197, 2007.

TAY, F.R.; PASHLEY, D.H. Guided tissue remineralisation of partially demineralised human dentine. **Biomater,** v. 29, p. 1127-1137, 2008.

TIRAPELLI, C. et al. A novel bioactive glass-ceramic for treating dentin hypersensitivity. **Braz Oral Res,** v. 24, n. 4, p. 381-387, 2010.

TOLEDANO, M.; YAMAUTI, M.; OSORIO, E.; OSORIO, R. Bleaching agents increase metalloproteinases-mediated collagen degradation in dentin. **J Endod,** v. 37, p. 1668-1672, 2011.

UYSAL, T.; ER, O.; SAGSEN, B.; USTDAL, A.; AKDOGAN, G. Can intracoronally bleached teeth be bonded safely? **Am J Orthod Dentofacial Orthop,** v. 136, p. 689-694, 2009.

VAN MEERBEEK, B. et al. Adhesion to enamel and dentin: current status and future challenges. **Oper Dent,** v. 28, n. 3, p. 215-235, 2003.

VAN MEERBEEK, B. et al. Relationship between bond-strength tests and clinical outcomes. **Dent Mater**, v. 26, p. e100-e121.

WANG, Z. et al. Dentine remineralization induced by two bioactive glasses developed for air abrasion purposes. **J Dent,** v. 39, n. 11, p. 746-756, 2011.

WHITE, J.M.; EAKLE, W.S. Rationale and treatment approach in minimally invasive dentistry. **J Am Dent Assoc,** v. 131, n. 9, p. 1250-1252, 2000.

2. ARTIGO 1:

Thermal and bioactive optimization of a 3-step etch-and-rinse dentin adhesive

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2.1 ABSTRACT

Statement of problem. Resin-dentin bonds limited durability is considered adhesive restorations major problem. Therefore, some clinical strategies have been developed to improve hybrid layer stability over time. **Purpose.** The influence of preheating and inclusion of a bioactive glass on the 1-week and 6-month microtensile bond strength (μTBS) of a three-step etch-and-rinse adhesive system was evaluated. **Material and Methods.** Dentin discs of 80 molars were assigned into 8 groups: G1/G1+: control group, G2/G2+: adhesive preheated at 68 ºC, G3/G3+: 0.05 mg of Bioglass® 45S5 (BAG) (particle size: 3μm) added to primer, G4/G4+: adhesive and BAG-modified primer preheated at 68 ºC. Beams were fabricated for microtensile testing at 1 week (G1-G4) and at 6 months (G1+/G4+). μTBS data were analyzed with a 2-way analysis of variance. Scanning electron microscopy (SEM) was used to analyze the failure mode and evaluate intact dentin-composite interfaces. **Results.** Bond strength mean values at 1 week were statistically different with G2 at 69.80 MPa superior to all other groups (P<.001) G1 at 58.24 MPa, G3 at 60.68 MPa and G4 at 60.97 MPa were not statistically different (P >.123). G2 remained the highest at 6 months (G2+: 68.32 MPa), and no decrease in μTBS was observed with time (P =.081). Failure modes were mostly adhesive. Intact dentin-composite interface SEM analysis showed a more uniform and continuous hybrid layer for G2/G2+ groups. **Conclusion.** Adhesive system preheating significantly increased the 1-week and 6-month μTBS. Inclusion of BAG did not have any effect on the results.

Keywords: Adhesion, Resin-dentin interface, Bond strength, Preheated, Bioglass.

2.2 CLINICAL IMPLICATIONS

The preheating of restorative materials increases their physical and mechanical properties. This applies also to a filled 3-step etch-and-rinse adhesive system, already known as the gold standard system, the bond strength of which was improved 20% by preheating.

2.3 INTRODUCTION

The lack of durability of dentin-resin bonding is still a challenge in clinical restorative dentistry resulting frequent restoration replacements due to its short lifetime. Three-step etch-and-rinse adhesive systems are among the most studied and proven adhesives, and a filled one (Optibond FL; Kerr, Orange, CA) seems to be best representative of such adhesives with not only the highest microtensile bond strength (μTBS) (ca. 50 MPa) but also the greatest stability at 1 year.[1] However, the ideal reference for mimicking dentin bonding (i.e., the biomimetic approach)[2] remains the human dentinoenamel junction, the μTBS of which amounts 51.5 MPa.[3] One of the main tenant in durability of the dentin bond is the complete sealing of the demineralized collagen network and avoidance of nanoleakage within the hybrid layer.[4] The total-etch technique removes the smear layer but create an additional challenge because of the greater depth of collagen demineralization (ca 8-12 μm)[5] compared to self-etching adhesives. Although self-etching adhesive systems were developed to decrease etch-and-rinse adhesives adverse effects, this simplified system failed to completely infiltrate the demineralized dentin on the base of the hybrid layer and have not been able to match the values of gold standard 3-step etch-and-rinse adhesive system.[6]

For in vitro testing, the adhesive system is normally used at room temperature (20-25 ^{o}C), which is inferior to body temperature. Such a parameter (temperature) might be significant in several practical applications. A trend in restorative dentistry is to use preheated composite resins in order to improve

the conversion rate,[7] facilitate their placement and increase their flow. Self-etch and self-adhesive cements also seems to improve significantly their μTBS when preheated.[8] Preheating of a dentin adhesive system, yet, remain to be tested but could be significant in improving the seal of the hybrid layer through enhanced flowability and interdiffusion (deeper penetration into dentin).

Remineralization techniques represent another approach for improving the stability of hybrid layer. This principle has been used successfully for bone tissue engineering in medicine, for more than 50 years, due to bioactive glass ability to bond to mineral tissue, promoting bone repair and regeneration. [9, 10] At the level of dentin, the inclusion of bioactive elements may influence bonding through the precipitation or growth of hydroxyapatite crystals between and within the collagen fiber.[11, 12, 13] Three-step etch-and-rinse experimental adhesives[14] including Bioglass® 45S5 (BAG) in the resin component (not the primer) demonstrated improved stability at 6 months. μTBS values of such system, however, did not exceed 27 MPa. Another study also used the bioactive elements inside the resin with unsatisfying μTBS results after 3 months already (8-15 MPa).[15] No attempts have been made so far to include BAG into an existing commercial dentin bonding system, except by adding an additional step between the etching and priming steps.[16] The application of bioactive solutions (BAG and Biosilicate®) to the etched dentin before applying a 2-step etch and rinse adhesive (Optibond Solo; Kerr, Orange, CA) significantly increased μTBS. An alternative approach, not yet tested, would be to include the bioactive agent into the primer of the adhesive system and combining it to the preheating technique, hence saving one-step.

Therefore, the aim of this study is to evaluate the influence of preheating or inclusion of BAG, as well as the combination of both on the bond strength of a three-step etch-and-rinse adhesive using the non-trimming μTBS. The first null hypothesis was that no difference in bond strength would be found between the control group (adhesive at room temperature) and the three experimental groups

(preheated adhesive, bioactive primer and combination of both). The second null hypothesis was that no difference in μTBS would be found after 6 months of storage.

2.4 MATERIALS AND METHODS

Upon approval from the Ethical Review Committee of the University of Southern California, (protocol HS-17-00703), eighty extracted sound human molars were used. Molars were stored at 4 °C in thymol solution up to 1 month before use. Flat mid-coronal dentin surfaces were created using a low–speed diamond saw (Isomet; Buehler Ltd, Lake Bluff, Ill). Any remaining enamel was removed by finishing with 600-grit SiC paper (Gatorgrit; Ali Industries, Fairborn, OH) under water. The teeth were assigned to 8 experimental groups (10 teeth each) (Table 1).

Table 1. Experimental groups with different mode of use of the adhesive system (AdS).

	G1	G1+	G2	G2+	G3	G3+	G4	G4+
AdS Preheating for 15min. /68 °C	No (AdS applied at room temperature)		Yes		No (AdS applied at room temperature)		Yes	
Addition of .05 mg of Bioglass® 45S5 to AdS bottle 1	No		No		Yes (in AdS Primer rocket)		Yes (in AdS Primer rocket)	
Delay before MTBS testing	1 week	6 months	1 week	6 months	1 week	6 months	1 week	6 months

In group G1 and G1+, a 3-step etch-rinse adhesive unidose system (Optibond FL; Kerr, Orange,

CA) was used according to the manufacturer's instructions, polymerized for 20 s at 1.000 mW/cm² with an LED unit (VALO Curing Light, Ultradent Products, Inc, South Jordan, UT, USA), and immediately restored using 5 layers of 1mm-think composite resin (Filtek Z100, 3M-ESPE). Each layer was polymerized 20 s at 1,000 mW/cm^2 (VALO Curing Light, Ultradent Products, Inc, South Jordan, UT, USA).

Groups G2 and G2+ were restored similarly to G1/G1+ but the adhesive rockets (primer and resin, Optibond FL) were preheated for 15 min in a composite resin preheater (Calset, Addent, Danbury CT) at 68 ºC just before its application. Application mode and restoration were similar to G1/G1+.

Group G3 and G3+ were restored similar to G1/G1+ but the adhesive system was modified by adding 0.05 mg of BAG (45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_2 – wt.%) [17] powder to the primer rocket unidose system. The adhesive application mode and restoration were similar to group G1/G1+.

Group G4 and G4+ were restored similarly to group G3/G3+ (including the BAG) but the adhesive rockets (bioactive primer and resin) were preheated for 15 min in a composite resin preheater (Calset, Addent, Danbury CT) at 68 ºC just before its application. Adhesive application mode and restoration were similar to group G1/G1+.

All restored specimens were stored in distilled water at room temperature for 24 h to 1 week hours before testing (G1 to G4) or for 6 months (G1+ to G4+, water replaced weekly). Each restored specimen was radially sectioned through the tooth-restoration interface with approximately 0.9-mm-thick slices. Each slice was sectioned again to obtain tooth-restoration beams with a cross-sectional area of approximately 0.8 mm^2. Measurements were made with digital calipers (Zaas Precision; Amatools). Ten beams were prepared from each tooth. Resin-dentin beams were placed in the grips of the testing jig with cyanoacrylate glue (Super Bonder; Henkel/Loctite Corporation) and were tested in a universal test machine (Bisco Dental, Illinois, USA) at 0.5 mm/min until failure. The maximum load

at fracture (N) and the cross-sectional area of each failed beam was used to calculate μTBS values in Mega Pascal (MPa) [18].

After the test, the specimens were placed onto aluminum diskcs, metallized with gold-palladium alloy (Ion Revestidor,; IC-50;, Shimadzu Biotech) and observed under scanning electron microscopy (SEM) (Superscan SS-550, Shimadzu Corporation, Kyoto, Japan) (×100). Failure modes of each beam was determined as: (1) adhesive failure, if the fracture site was located between the adhesive and dentin or if the fracture site continued from the adhesive into either the composite resin or dentin (mixed type) or (2) substrate failure, if the fracture occurred exclusively within the composite resin or dentin.

The mean microtensile bond strength from the 10 beams was used as a single measurement, yielding 10 measurements per group. Statistical analyzes were done using the R i386 3.0.2 software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria). Data normality was confirmed by Shapiro-Wilk test. Bond strength data obtained from the 8 experimental groups were analyzed with a 2-way analysis of variance (ANOVA) (dentin bonding strategy and storage time). Statistical significance was set in advance at the .05 level. Post-hoc comparison was done using the Tukey test.

The dentin surface of 5 fractured beams (interfacial failure) from each tooth were air dried, sputter-coated with gold/palladium (Bal-Tec, Capovani Brothers Inc., New York) and examined using SEM (Superscan SS-550, Shimadzu Corporation, Kyoto, Japan).

Two unused slabs for each group were also prepared for SEM analysis (Quanta FEG 250, ThermoFisher Scientific, Oregon, USA). Those additional specimens were embedded into thermoactivated acrylic resin (JET; colorless classic) in a metallographic filler (PRE-30mi Embossing Press; Arotec). Each section was polished with wet abrasive SiC papers, gently decalcified with 37% phosphoric acid for 30 s, de-proteinized with 2% NaOCl solution for 1 minute, ultrasonicated in 96%

ethanol for 2 min, air-dried and then sputter-coated with gold/palladium. Intact dentin-resin interfaces were observed at different magnifications in order to evaluate resin tags and hybrid layer formation.

2.5 RESULTS

μTBS results are presented in Table 2. The 2-way ANOVA (Table 3) indicated a significant effect for the dentin bonding strategy (*P* <.001) but not the storage time (*P* =.081). The interaction term was not significant (*P*=.340). The preheated group (G2) with 69.80 MPa was significantly stronger than the others. G1 (58.24 MPa), G3 (60.68 MPa) and G4 (60.97 MPa) did not differ from each other (*P*>.123). G2+ remained the highest at 6 months (68.32 MPa), and no decrease in μTBS was observed with time.

Table 2: Mean and standard deviation (SD) of microtensile bond strength values (MPa) obtained for the different experimental groups.

Group	**μTBS - mean ± SD**	
	1 week test	**6 month test (+)**
G1	58.2 ± 6.7 A	60.5 ± 5.3 A
G2	69.8 ± 7.8 B	68.3 ± 3.7 B
G3	60.6 ± 4.1 A	64.0 ± 3.3 AB
G4	60.9 ± 4.6 A	65.6 ± 6.5 AB

Values with identical letters indicate no significant difference using Two-way ANOVA test (P > .05).

Table 3: Two-way ANOVA.

Source	***df***	**Type III Sum of Squares**	**Mean Square**	**F**	***P***
Dentin bonding strategy	3	984.96	328.32	10.63	<.001
Storage time	1	96.97	96.97	3.14	0.081
Interaction	3	105.29	35.09	1.13	0.340

P<.05 indicates significant difference

Failure modes indicated that most failures were adhesive for all groups (Fig. 1). SEM analysis demonstrated that adhesive failures were complex, typically involving various substrate with areas of failed adhesive resin possibly involving the hybrid layer and areas of cohesively failed dentin (Figure 2 and 3).

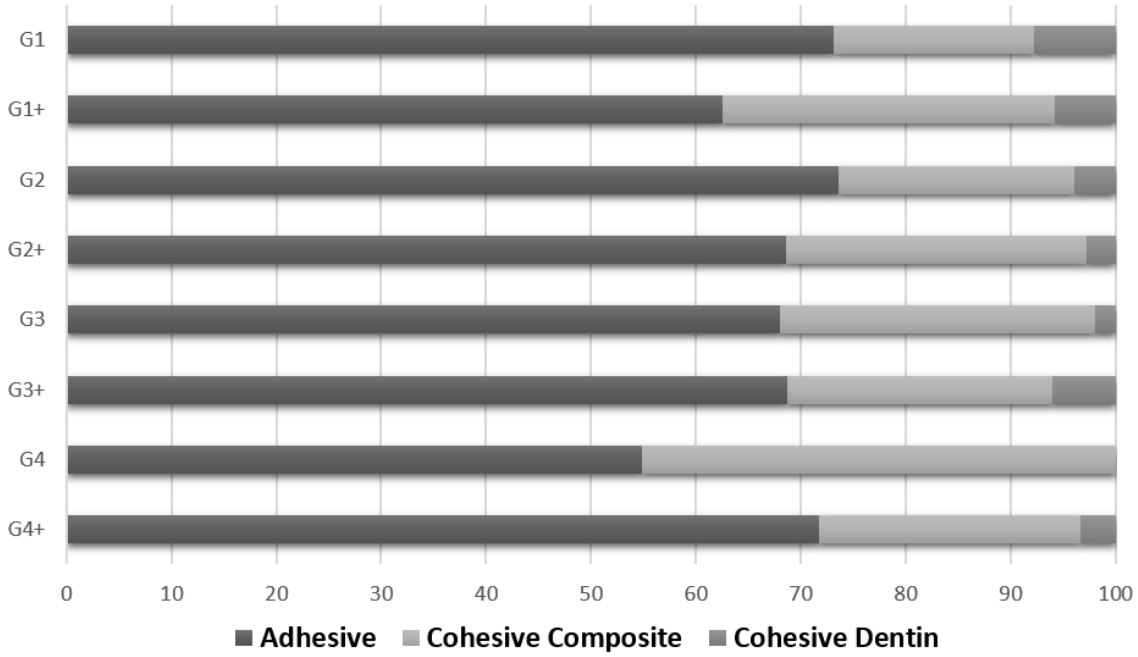

Figure 1: Failure mode distribution for each experimental group.

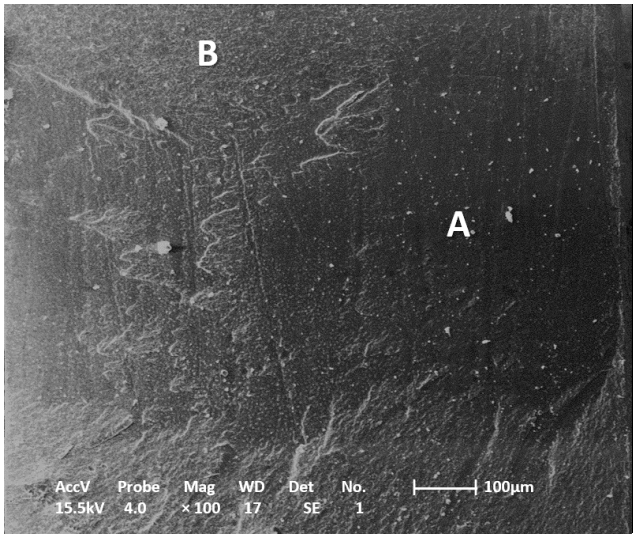

Figure 2: SEM of fractured surface of specimens (×100). Specimen of group G2 (preheated Optibond FL), which fractured at 70 MPa, displaying adhesive failure at the level of the hybrid layer (A) and within the adhesive (B).

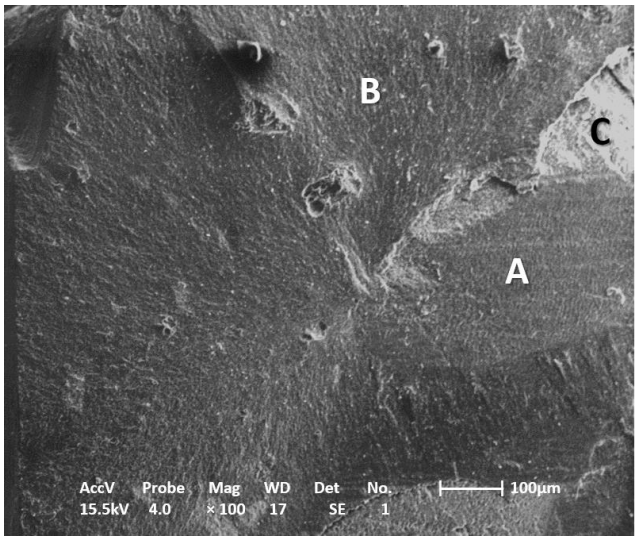

Figure 3: SEM of fractured surface of specimens (×100). Specimen of group G1 (control), which fractured at 57 MPa, displaying mixed failure at the hybrid layer (A), adhesive layer (B) and within dentin (C).

The SEM analysis of intact dentin-composite interfaces (Fig. 4) revealed a hybrid layer with better defined resin tag formation when the adhesive system was preheated before it application (Figs. 4B and 4D). This layer was more uniform and continuous when the adhesive was preheated (Fig. 4B) but not when mixed with BAG (Fig. 4D). The bioactive glass could be observed as bright crystals within the hybrid layer and tags (Figs. 4C and 4D).

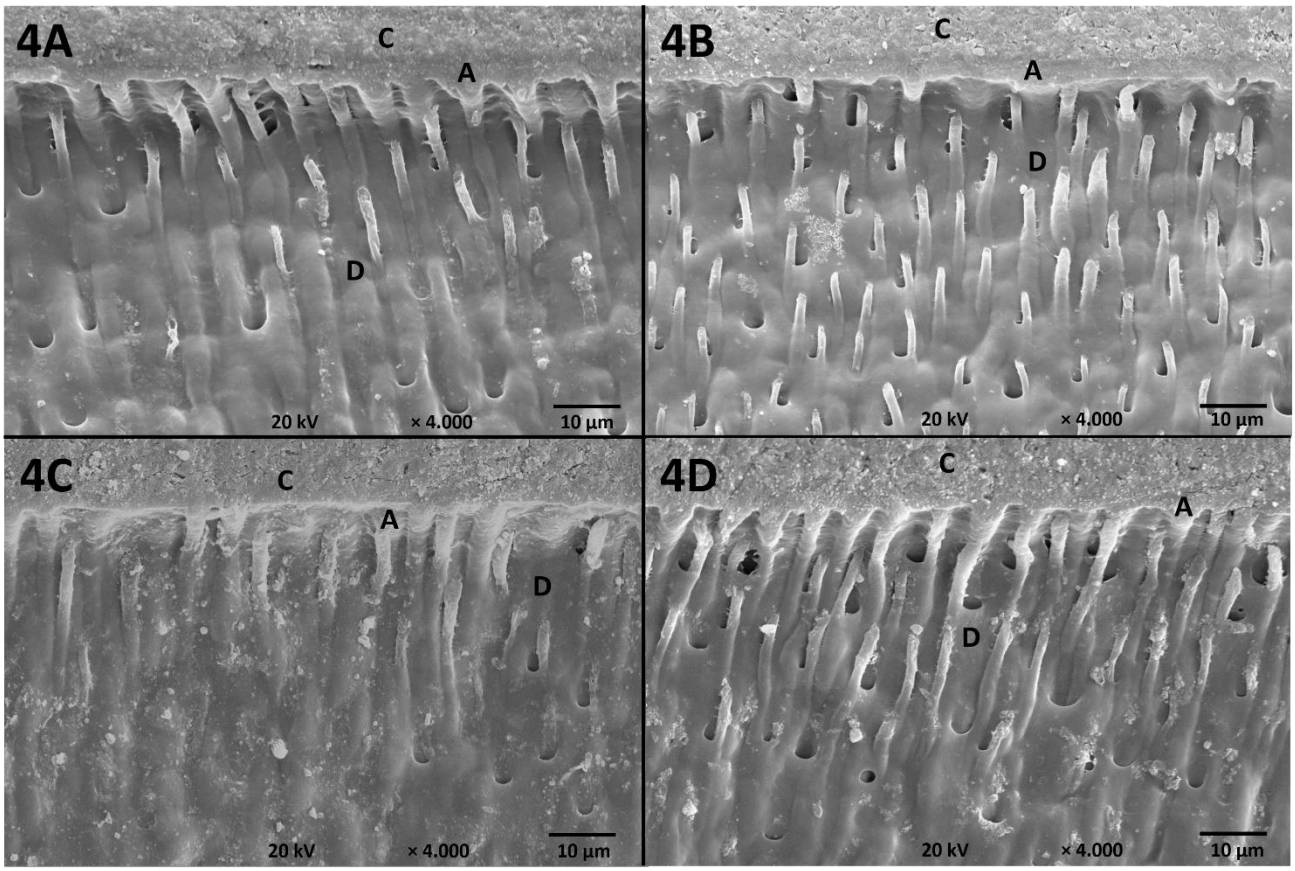

Figure 4: SEM of intact dentin (D) - adhesive (A) - composite resin (C) interface of G1+ (4A), G2+ (4B), G3+ (4C) and G4+ (4D). In groups G1+ and G2+ dentin tubules were not totally infiltrated by adhesive (4A and 4C). When the preheated adhesive system was used, the hybrid layer showed longer adhesive tags (4B and 4D). In group G2+, when the adhesive was preheated but not mixed with BAG, the hybrid layer was more uniform and continuous and presented more defined resin tags (4B). BAG particles were observed as bright crystals in both groups G3+ and G4+ (4C and 4D).

2.6 DISCUSSION

The first null hypothesis that no difference in bond strength would be found between the control group (adhesive at room temperature) and the three experimental groups (preheated adhesive, Bioglass-modified primer and combination of both) can be rejected. While BAG groups did not differ from the

control, preheating of the adhesive resulted in a significant improvement. The second null-hypothesis was accepted because storage time did not have any effect on μTBS.

μTBS is the most common way of testing dentin bonding agents. It is normally performed at room temperature, that is 20-25 ^{o}C, well below body temperature. In this experiment a significant increase in temperature caused an increase in μTBS. Hence, in-vivo μTBS might benefit from the increased body temperature. There is, however, no existing literature about preheating dentin bonding agents. A sensitive matter is that preheated materials such as restorative composites can lose heat rapidly during the transfer into the mouth.[19] The temperature drop should be minimized by reducing the time between removal from the heating device and the application in the mouth. The resin preheater device used in this study is compact and portable and can be placed in a convenient location to optimize the application time. Another element that is paramount in the preheating technique is the use of a unidose adhesive system. The small single-use rocket-shaped containers (one for the primer and one for the filled adhesive) can be easily stored into the heating device and are totally sealed. Hence, according to the manufacture orientation, it is safe to preheat the primer for less than 20 min and the bond for less than 2 h prior to application. The same system exists in a bottle kit. The bottles, however, are too large to fit in the heating device and the effect of repeated heating on the more volatile components and shelf-life stability of the primer remain unknown. The tested adhesive system is more viscous than other systems because it is filled 48% in weight with radiopaque glass and barium particles (fumed silicon dioxide, barium aluminoborosilicate and disodium hexafluorosilicate). First marketed in 1992 in form of a dual-cure version, then in 1994 in form of a filled light-cure only version (hence the acronym FL), this 3-step etch-and-rinse adhesive systems remains a gold standard in adhesion strength and stability with a value of 50 MPa and the least amount of bond strength loss at 1 year.[1] As a comparison, a study using the same methodology (μTBS) evaluated the « biologic » bond of the

natural dentinoenamel junction between 47.7 et 51.5 MPa.[3] With the additional 20 MPa of bond strength obtained by preheating in the present study, it can be concluded that this specific adhesive has the potential to mimic the dentinoenamel junction. SEM analysis clearly showed that the use of the preheated adhesive system promoted a more defined hybrid layer with deeper resin tags. The preheating, while enhancing the interdiffusion and penetration of the adhesive into the demineralized dentin, should not have a negative effect to the pulp. The temperature of primer/adhesive can drop significantly during transfer/application and other studies have demonstrated that the major temperature increase is produced by the polymerization light itself .[19, 20] The μTBS values presented in this study might not even be the highest possible because the specimens were polished with 600-grit SiC sandpaper before bonding procedures. It is known that a total-etch system will further benefit from a rougher dentin surface such as obtained with diamond bur cutting prior to bonding.[21]

The performance of the etch-and-rinse adhesive in the present work could be significantly optimized by the use of preheating, but not when combined with the bioactive glass. On the other hand, the application of the same bioactive glass improved μTBS significantly (from 58 MPa to 78 MPa) when performed in a separate step in-between etching and priming.[16] BAG is a biocompatible calcium/sodium phosphate-phyllosilicate (45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_2 -%wt.), and presents the highest bioactivity index (IB = 12.5) compared to other bioactive materials. BAG is also considered a gold standard within bioactive materials.[22] The lack of μTBS improvement in the present study might be due to the "encapsulation" of the bioactive glass in the resin network. The remineralization process requires exchange of ions (Na^+, Ca^{2+}, PO_4^{3-}, F^-) from the Bioglass silicate network with the surrounding dentin fluid.[14] This process induces calcium phosphate (Ca/P) precipitation and its subsequent crystallization into hydroxyapatite in the mineral tissue surface. [15] The bioactive glass forms a stable bond to the tissue once biomimetic remineralization is achieved. [13, 23]

This process even produces electrostatic, ionic and/or hydrogen bonding between the demineralized collagen and Bioglass silanols, which in turn may reduce collagen degradation.[13,14, 24] It might be that all those interactions have been limited by the good seal and interdiffusion obtained with the 3-step etch-and-rinse adhesive system used in the present study. Because the remineralization seems to require some sort of nanoleakage (fluid exchanges) in order to succeed, the real benefit of bioactive glasses might be better revealed with contemporary adhesive systems suffering from hydrolytic degradation by water sorption, such as simplified adhesives. In such systems, the resin monomers are unable to completely infiltrate acid-etched dentin and form a stable hybrid layer.[24] This deficiency is therefore the perfect environment for remineralization of the water-filled collagen matrix and inclusion of MMP-inhibitors (MMPs being particularly active in this environment). The present experiment also demonstrated that the addition of the bioactive particles to the primer did not affect either positively or negatively the 6-month MTBS the 3-step etch-and-rinse adhesive, even when combined with preheating. Further research is indicated to confirm the best strategy (sequence, method) for the application of BAG to benefit a 3-step total etch-and-rinse adhesive. The high values of all μTBS at 6 months is extremely encouraging and calls for further analysis of the stability of those values.

2.7 CONCLUSIONS

Within the limitations of the in vitro study, it can be concluded that preheating of the filled three-step etch-and-rinse adhesive enhanced its interdiffusion and penetration into the demineralized dentin. This optimized hybrid layer resulted in significantly increased 24-hour and 6-month μTBS. Inclusion of BAG in the primer component did not yield any improvement in μTBS. None of the groups lost their bond strength after 6 months.

REFERENCES

1. De Munck J, Mine A, Poitevin A, Van Ende A, Cardoso MV, Van Landuyt KL, Peumans M, Van Meerbeek B. Meta-analytical review of parameters involved in dentin bonding. J Dent Res 2012; 91:351-357.
2. Magne P, Belser U. Bonded Porcelain Restorations in the Anterior Dentition – A Biomimetic Approach. Understanding the intact tooth and the biomimetic principle. Chicago: Quintessence, 2002; 23-56.
3. Urabe I, Nakajima S, Sano H, Tagami J. Physical properties of the dentin-enamel junction region. Am J Dent 2000; 13:129-135.
4. Tay FR, Pashley DH. Water treeing-a potential mechanism for degradation of dentin adhesives. Am J Dent 2003; 16:6-12.
5. Anchieta RB, Machado LS, Sundfeld RH, Reis AF, Giannini M, Luersen MA, Janal M, Rocha EP, Coelho PG. Effect of partially demineralized dentin beneath the hybrid layer on dentin-adhesive interface micromechanics. J Biomech 2015; 26; 48:701-707.
6. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int. 1999; 30:501-507.
7. Daronch M, Rueggeberg FA, Moss L, de Goes MF. Clinically relevant issues related to preheating composites. J Esthet Restor Dent 2006; 18:340-350.
8. Cantoro A, Goracci C, Papacchini F, Mazzitelli C, Fadda GM, Ferrari M. Effect of pre-cure temperature on the bonding potential of self-etch and self-adhesive resin cements. Dent Mater 2008; 24:577-583.
9. Hench, Larry L. Chronology of bioactive glass development and clinical applications. NJGC 2013; 03, 02:67.
10. Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. J Adv Pharm Technol Res 2013; 2:78-83.
11. Bertassoni LE, Habelitz S, Kinney JH, Marshall SJ, Marshall GW Jr. Biomechanical perspective on the remineralization of dentin. Caries Res 2009; 43:70-77.
12. Efflandt SE, Magne P, Douglas WH, Francis LF. Interaction between bioactive glasses and human dentin. J Mater Sci Mater Med 2002; 13:557-65.
13. Fernando D, Attik N, Pradelle-Plasse N, Jackson P, Grosgogeat B, Colon P. Bioactive glass for

dentin remineralization: A systematic review. Mater Sci Eng C Mater Biol Appl 2017; 76:1369-1377.

14. Profeta AC, Mannocci F, Foxton RM, Thompson I, Watson TF, Sauro S. Bioactive effects of a calcium/sodium phosphosilicate on the resin-dentine interface: a microtensile bond strength, scanning electron microscopy, and confocal microscopy study. Eur J Oral Sci 2012; 120:353-362.
15. Sauro S, Osorio R, Watson TF, Toledano M. Therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface. J Mater Sci Mater Med 2012; 23:1521-1532.
16. Ubaldini LMU, De Oliveira B, Sato F, Soares VO, Pascotto RC, Baesso ML. Resin-dentin interface remineralization effects on bond strength. J Dent Res 2017; 96 (Spec Iss A), Abstr. No. 3257.
17. Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J Bio Mater Research 1971; 5(6): 117-141.
18. Magne P, So WS, Cascione D. Immediate dentin sealing supports delayed restoration placement. J Prosthet Dent 2007; 98:166-174.
19. Daronch M, Rueggeberg FA, Hall G, De Goes MF. Effect of composite temperature on in vitro intrapulpal temperature rise. Dent Mater 2007; 23:1283-1288.
20. Rueggeberg FA, Daronch M, Browning WD, DE Goes MF. In vivo temperature measurement: tooth preparation and restoration with preheated resin composite. J Esthet Restor Dent 2010; 22:314-322.
21. Zimmerli B, De Munck J, Lussi A, Lambrechts P, Van Meerbeek B. Long-term bonding to eroded dentin requires superficial bur preparation. Clin Oral Investig 2012; 16:1451-1461.
22. Crovace MC, Souza MT, Chinaglia CR, Peitl O, Zanotto ED. Biosilicate®—A multipurpose, highly bioactive glass-ceramic. In vitro, in vivo and clinical trials. J. Non-Cryst. Solids 2016; 432:90-110.
23. Niu L, Zhang W, Pashley DH, Breschi L, Mao J, Chen J,Tay FR. Biomimetic remineralization of dentin. Dent Mater 2104 31(1):77-96.
24. Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res 2011; 90:953-968.

3. ARTIGO 2:

Resin-dentin interface mechanical and physic-chemical changes after remineralization treatment with Bioglass 45S5

3.1 ABSTRACT

Purpose: This study evaluated Bioglass 45S5 (BAG) remineralization effects on dentin bond strength, resin-dentin physic-chemical properties and interface thickness. **Materials and Methods:** Dentin specimens were assigned into 2 groups (n=10): CG, control group and RG, remineralized group, in which a BAG solution was applied on etched dentin surface before bonding application. Dentin-bonded specimens were prepared using a 3-step etch-and-rinse adhesive and composite resin. Beams were obtained and tested with microtensile bond strength (μTBS). One non used beam per tooth was submitted to micro-Raman spectroscopy (MRS) and another to scanning electron microscopy (SEM). MRS 3D-maps characterized interface physic-chemical composition and diffusion zone thickness. SEM evaluated interface pattern and thickness. Data were analyzed with Student's t-test or 2-way ANOVA and Tukey-Kramer post-hoc test (α=.05). **Results:** μTBS mean value of RG (45.3 ± 8.4 MPa) was superior to CG (38.3 ± 6.4 MPa) (P=.048). Dentin remineralization treatment increased relative mineral concentration, reduced organic matrix mineral content and augmented amide I components. Adhesive penetration was reduced on remineralized dentin, however, its chemical interaction with phosphate and its degree of conversion were improved. Diffusion zone in CG (3.1 ± 0.7 μm) did not differ from RG (2.5 ± 0.8 μm) (P=.68) and interface thickness values of CG (3.5 ± 0.6μm) showed no difference from RG (3.0 ± 0.6 μm) (P=.74). **Conclusion:** Dentin mineral growth protected collagen fibrils leading to an improvement on adhesive properties. Although adhesive penetration into remineralized dentin decreased, its interface thickness did not change. Remineralization treatment

resulted in higher resin-dentin bond strength values.

Keywords: Bioactive Glass, Dentin remineralization, Etch-and-rinse adhesives, Resin-dentin interface, Bond strength, Raman Spectroscopy.

3.2 INTRODUCTION

Resin-dentin bonding longevity is still a challenge in clinical restorative dentistry. When compared to amalgam restorations, composite resins fillings present a double failure rate and a half clinical lifetime. [1] Three-step etch-and-rinse adhesive systems are considered the gold standard due to their higher mechanical properties and better stability over time. [2,3] Hybrid layer created by etch-and rinse adhesives is dependent on their phosphoric acid etching step which cleans the prepared surface removing the smear layer and demineralizes the superficial dentin [4] exposing the collagen matrix [5] to posterior impregnation with the adhesive monomers. Literature shows that the ideal hybrid layer, a continuous 3D polymer/collagen network, is not reached since monomers cannot diffuse into the 5 microns of demineralized dentin tissue leaving collagen fibrils unprotected. [6,7] Resin-dentin interface loss of integrity has been reported to be caused by hybrid layer failure because of water-sorption-induced hydrolysis of the hydrophilic adhesive resin components [8] and collagen fibrils degradation through endogenous matrix metalloproteinases (MMPs) activated by acidic conditioning. [9]

Self-and-etching adhesive systems were developed aiming to eliminate the problems resulted from the acid conditioning step of etch-and-rinse adhesives. [10] The use of mid self-etching acidic monomers decreases the amount of demineralized dentin that are not diffused by adhesive monomers, nevertheless, a deficient infiltration area is still found on the base of the hybrid layer. [6] Considering

that etch-and-rinse adhesives mechanical properties are superior to those of self-and-etch adhesives, some clinical strategies have been developed to protect collagen fibrils and thus improve the stability of the hybrid layer created by etch-and-rinse bonding agent. Chlorhexidine is the most used MMPs inhibitor [11] and when applied on dentin surface after acid etching decreases the immediate degradation of the hybrid layer, [12,13] however, its beneficial outcome has not been reproducible after longer periods of aging. [14] Collagen fibrils can also be protected from degradation using collagen cross-linkers during adhesive restorative procedures. [15] Although both of those methods focus on collagen integrity, hybrid layer hydrolysis is not reduced by them since they are not able to remove the intrafibrillar collagen water. [16] Ethanol-wet bonding technique was developed to chemically dehydrate the demineralized collagen matrix; however, it has been demonstrated that ethanol solvent is incapable of completely replacing the residual intrafibrillar water by monomers resins. [16]

Dentin remineralization is described as an effective strategy for increasing the durability of resin-dentin bonds [17,18] since it dehydrates collagen fibrils by replacing the residual water in hybrid layers with apatite crystallites [16,19] and restores the protection function of collagen creating interfibrillar and intrafibrillar apatites. [20,21] Different post-bonding and during-bonding techniques, [22,23] as well as remineralization agents have been used to promote resin-dentin remineralization. [24,25,26] BAG is a biocompatible calcium/sodium phosphate-phyllosilicate (45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_2 – wt.%) [27] that presents the highest bioactivity index (IB = 12.5). [28] Its remineralization process involves exchange of ions (Na^+, Ca^{2+}, PO_4^{3-}, F^-) from the BAG silicate network with the surrounding body fluid. This process induces calcium phosphate (Ca/P) precipitation and its subsequent crystallization into hydroxyapatite in the mineral tissue surface [29] producing electrostatic, ionic and/or hydrogen bonding between the demineralized collagen and BAG silanols. [30]

Resin-dentin remineralization is well established in literature; however, no study has evaluated the relationship between dentin mechanical properties, dentin chemical changes and adhesive interface/diffusion zone after remineralization treatment. Therefore, the purpose of this study was to investigate how a during-bonding dentin remineralization technique with a BAG solution affects dentin mechanical behavior, dentin physic-chemical characteristics and adhesive interface thickness. The null hypothesis to be tested are 1) dentin bond strength is not altered after remineralization treatment, 2) there is no difference in dentin physic-chemical characteristics in the presence or absence of remineralization and 3) dentin remineralization does not change adhesive interface and diffusion zone thickness.

3.3 MATERIALS AND METHODS

3.3.1 Specimen preparation, dentin bonding and remineralization treatment

Upon approval from the Research Ethics Committee of State University of Maringa (Research protocol: 50615715.1.0000.0104), twenty healthy human molars were used. Flat mid-coronal dentin 5 mm discs were created using a low–speed diamond saw (Isomet; Buehler Ltd, Lake Bluff, III) under water-cooling. The exposed dentin surfaces were wet polished using 600-grit SiC paper (Carborundum Abrasives, Saint-Gobain) for 1 min to remove any remaining enamel and create flat surface with homogeneous and standard smear layer. The specimens were then divided into two groups (n=10): control group (CG) and remineralized group (RG) (Figure 1).

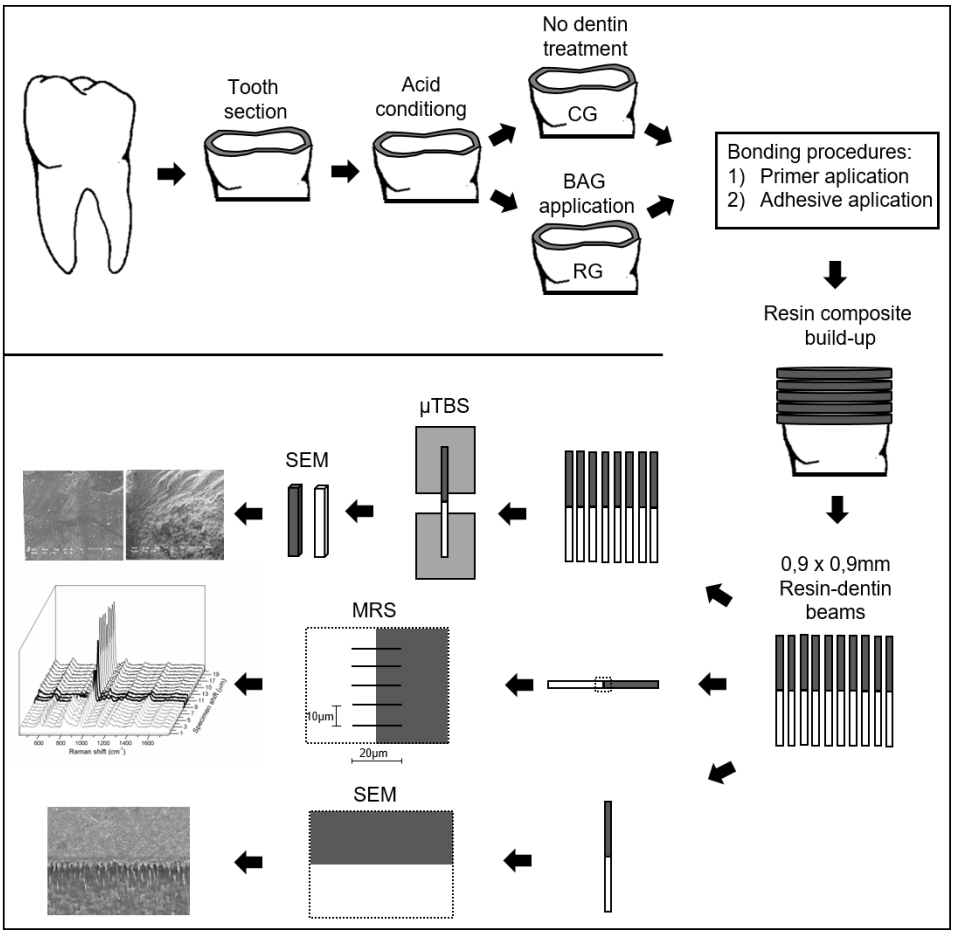

Figure 1: Schematic drawing illustrating the study setup and specimen preparation.

Specimens were etched with 35% phosphoric acid for 10 s, to produce a 5-6-µm-thick layer of completely demineralized collagen surface, followed by copious water rinse for 1 min. After acid conditioning, the remineralization treatment was carried out in RG specimens to induce new mineral formation before the adhesive application. BAG (particle size 3 µm) was used as the remineralization agent in a solution form, in which 0.015 g of its powder was diluted in 1.35 ml of distilled water. [31] A micropipette (Monocanal VVCS-10, Digipet) was used to apply 10 µl of this solution in the moist dentin surface and using a microbrush the solution was slightly rubbed in dentin for 30 s. The surface was then washed with deionized water for 15 s and air-dried for 2 s to remove the excess of water.

Bonding procedures were done with a 3 steps etch-and-rinse adhesive (Adper Scotchbond Multi-Purpose, 3M ESPE). Primer and bond agents were used according to the manufacturer's instructions and light-cured for 20 s at 1.000 mW/cm² with a LED unit (Translux Power Blue; Heraeus Kulzer). The specimens were immediately restored using 5 layers of 1mm-thick composite resin (Filtek Z250; 3M ESPE). Each layer was light-cured 20 s at 1.000 mW/cm^2 and then a final curing of 60 s was done. Specimens were stored in artificial saliva [28] for 24 h at 37 °C before testing.

3.3.2 Microtensile bond strength (μTBS) test and scanning electron microscopy (SEM) analysis of the failure modes

Each specimen was sectioned perpendicular to the adhesive interface to obtain bonded sticks with cross-sectional areas of approximately 0.9 mm^2. Peripheral beams with residual enamel were excluded from the study and 2 beams per group were set apart for the interface analysis with MRS and SEM, therefore, 8 bonded beams were obtained per tooth. The exact width of each beam was measured with a digital caliper (Zaas Precision; Amatools).

Resin-dentin beams were placed in the grips of the testing jig with cyanoacrylate glue (Super Bonder; Henkel/Loctite Corporation) and were tested in a universal test machine (EZ Test; Shimadzu) at 0.5 mm/min until failure. The maximum load at fracture (N) and the cross-sectional area of each failed beam was used to calculate μTBS values in Mega Pascal (MPa). The μTBS value was then computed for each tooth by averaging the values of the 8 resin-dentin beams from that tooth. Bond strength results were statistically analyzed with Shapiro-Wilk normality test and Student's t-test ($P<.05$) using the R i386 3.0.2 software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria). Pretest failures were not considered in the statistical analysis.

Fractured surfaces of the tested beams were placed onto aluminum discs, sputter-coating with

gold-palladium alloy (Ion Revestidor; IC-50, Shimadzu Biotech) and observed under SEM (Superscan SS-550, Shimadzu Corporation) with a 100× magnification. Failure patterns were classified as (1) adhesive fracture, if the fracture site was located between the adhesive and dentin; (2) mixed, when fracture involved different regions, such as dentin-adhesive interface, dentin and composite resin; (3) cohesive dentin fracture, and (4) cohesive fracture in the composite resin.

3.3.3 Micro-Raman spectroscopy (MRS) analysis

One untested beam for each tooth and for both groups (n=10) had its bonded interface analyzed by MRS. No pre-treatment was conducted, before the measurement specimens were only washed with distilled water in an ultrasonic bath for 10 min. Chemical mapping of the interface was performed with a confocal Raman microscope (Senterra Bruker Optik; GmbH). The spectra were excited by a 785 nm laser source and recorded in the spectral range of 450 to 1800 cm^{-1}. The laser power (100 mW) was focused on the specimen with a 100× magnification lens. Spatial resolution was 3 to 5 cm^{-1}, and integration time of the detector was 3 s. Each final curve resulted from the mean of 60 spectra. The interface line mapping was obtained starting in the dental composite region, across each resin-dentin specimen. Raman spectra were acquired in steps of 1µm using the x-y-z stage from 5 lines of 20 µm long, with 10 µm space between the lines, resulting in 100 scanning spectra per specimen. In order to avoid dehydration of the specimens, gauze soaked in distilled water was kept in contact with the dentin during MRS data collection.

Physic-chemical spectra modifications due to remineralization treatment were investigated in the region between the dentin and adhesive system. The following ratios were calculated for every Raman mapping point on the z-axis and were plotted as a function of spatial position, starting on the first specimen of the interface going to the adhesive part of the specimen.

1) Relative presence of mineral, mineral-to-matrix ratio (961 cm^{-1}/1003 cm^{-1}): [32,33] intensity ratio of phosphate peak (961 cm^{-1} -ν1 phosphate symmetric stretch) and phenyl group (1003 cm^{-1} - aromatic ring of phenylalanine residues in collagen).

2) Organic matrix mineral content (1450 cm^{-1}/961 cm^{-1}): [34] intensity ratio of the peaks that represent dentin organic CH group (1450 cm^{-1}) and dentin mineral component (961cm^{-1} -ν1 phosphate symmetric stretch).

3) Organization of collagen (1667 cm^{-1}/1246 cm^{-1}): [23] intensity ratio of dentin amide I (1667 cm^{-1}) and amide III (1246 cm^{-1}).

4) Chemical interaction (961 cm^{-1}/1458 cm^{-1}): [35] intensity ratio of the peaks that represent the mineral component in dentin (961 cm^{-1} -ν1 phosphate symmetric stretch) and the CH_2 group of methacrylate monomers (1458 cm^{-1} - deformation δ of CH_2).

5) Degree of conversion (DC) of adhesive (1637 cm^{-1}/1608 cm^{-1}): [36] intensity ratio of the peak associated with C=C of methacrylate (1637 cm^{-1}) and the peak related to C-C in phenyl of adhesive monomer (1608 cm^{-1}).

6) Adhesive /Bis-GMA penetration (1113 cm^{-1}/1667 cm^{-1}): [37,43], intensity ratio of the peak associated with C-O-C of adhesive (1113 cm^{-1}) and the peak related to amide I (1667 cm^{-1}).

Statistical analyses were performed using the ratios calculated for the first interface specimens (specimen at zero position in Figures 4A-4B) with Shapiro-Wilk and Student's t-test ($P<.05$) using the R i386 3.0.2 software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria). Statistical differences were identified by evaluating the effect of remineralization treatment through the comparison of CG and RG.

Diffusion zone thickness was obtained by the sigmoidal Boltzmann fitting, applied on the Raman band peak. The sigmoidal fitting was applied on the Raman band peak intensities centered at

1113 cm^{-1} (νC─O─C), which is representative of the carbon chain in the resin monomer present in the adhesive system, [38] and analyzed its permeation into dentin tissue. This analytical fitting procedure is described in details in a previous publication. [39] The Boltzmann fitting was applied in each of the 5-line scans from each specimen and the final diffusion zone depth was its average for each tooth.

3.3.4 Resin-dentin interfaces SEM analysis

One untested beam for each tooth was also prepared for SEM analysis (Quanta FEG 250, ThermoFisher Scientific) (n=10). Those additional specimens were embedded into thermoactivated acrylic resin (JET; colorless classic) in a metallographic filler (PRE-30mi Embossing Press; Arotec). Each section was polished with wet abrasive SiC papers (#400, #600, #1200, #2000 and #4000, Carborundum Abrasives, Saint-Gobain), gently decalcified with 37% phosphoric acid for 30 s, de-proteinized with 2% NaOCl solution for 1 min, ultra-sonicated in 96% ethanol for 2 min, air-dried and then sputter-coated with gold/palladium. Intact resin-dentin interfaces were observed at 2000× magnification to enable not only the evaluation of resin tags and hybrid layer formation but also the measurement of the interface dimensions. SEM images were processed and analyzed by an image software (UTHSCSA Image tool) and the interface thicknesses were calculated in micrometers using the distance tool from 10 different regions along the bonded interface of each specimen. The final interface thickness was obtained from the average of those 10 measurements.

Interface thickness measurements (SEM images) and diffusion zone values (MRS Boltzmann adjustment) were checked for normality using the Shapiro-Wilk test and then were compared using a 2-way ANOVA test followed by Tukey's test (R i386 3.0.2 software - R statistical software, R Foundation for Statistical Computing, Vienna, Austria) ($P > .05$).

3.4 RESULTS

3.4.1 μTBS test and SEM analysis of the failure modes

Statistical analysis of μTBS results showed significant difference between the two tested groups (P=.048) (Table 1). Remineralization treatment increased dentin bond strength values from 38.3 ± 6.4 MPa to 45.3± 8.4 MPa. The SEM analysis of the fractured surfaces revealed that most failures in both groups were at the adhesive interface (Table 1). The types of failures occurring in CG and RG specimens are illustrated in figures 2A-2D.

Table 1 – Mean and standard deviation (SD) of dentin μTBS (MPa).

Group	μTBS
CG	38.3 ± 6.4 A (5/75) [49/43/6/2]
RG	45.3 ± 8.4 B (0/80) [54/35/10/1]

First row indicates the mean ± SD in MPa, different capital letters indicate significant statistical difference (P<.05). Second row reports number of premature failures and number of tested beams. Third row shows the percentage of failure modes [adhesive/mixed/cohesive in dentin/cohesive in composite resin].

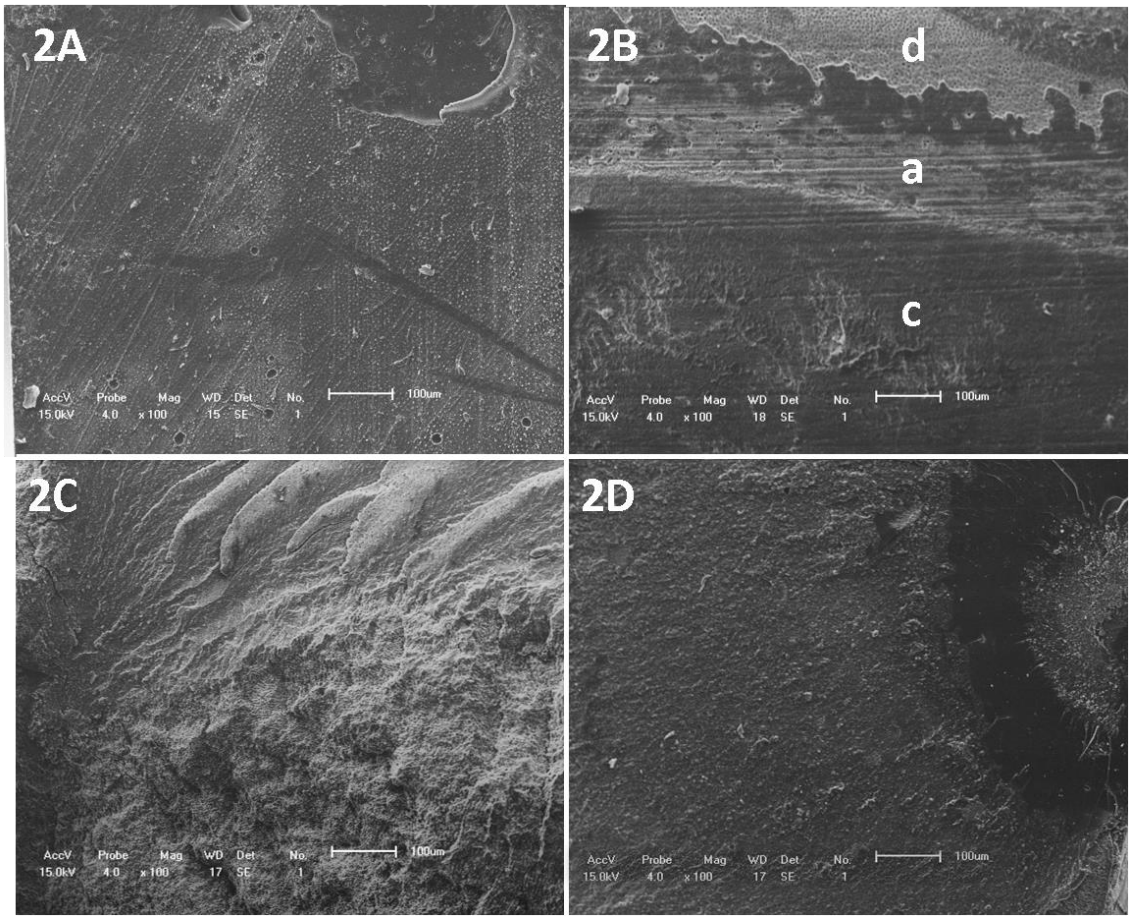

Figure 2: SEM failure analysis of fractured specimens (×100). (A) Adhesive failure along the dentin surface for CG. (B) Mixed failure for RG displaying adhesive layer [a], dentin surface [d] and cohesive failure in composite resin [c]. (C) Cohesive failure within dentin for RG. (D) Cohesive failure within composite resin for CG.

3.4.2 MRS physic-chemical analysis

Interface 3D chemical mapping is presented in figures 3A and 3B. Three major components were identified in the mapping spectra: dentin, adhesive and dentin-adhesive interface. The peak-ratio analysis (Fig. 4A and 4B) revealed that remineralization treatment resulted in significantly physic-chemical modifications at interface spectra. Considering dentin compound, BAG

application promoted an increase in the relative mineral concentration (P=.036), a reduction on the prevalence of organic matrix mineral content (P=.002) and a change in the nature of collagen with the increase of amide I peak intensity (P=.016). Dentin remineralization also changed some monomer adhesive characteristics. The adhesive chemical interaction ratio (P=.003) and the adhesive DC (P=.033) were significantly higher for RG. Dentin remineralization resulted in lower Bis-GMA adhesive monomer penetration (P=.001).

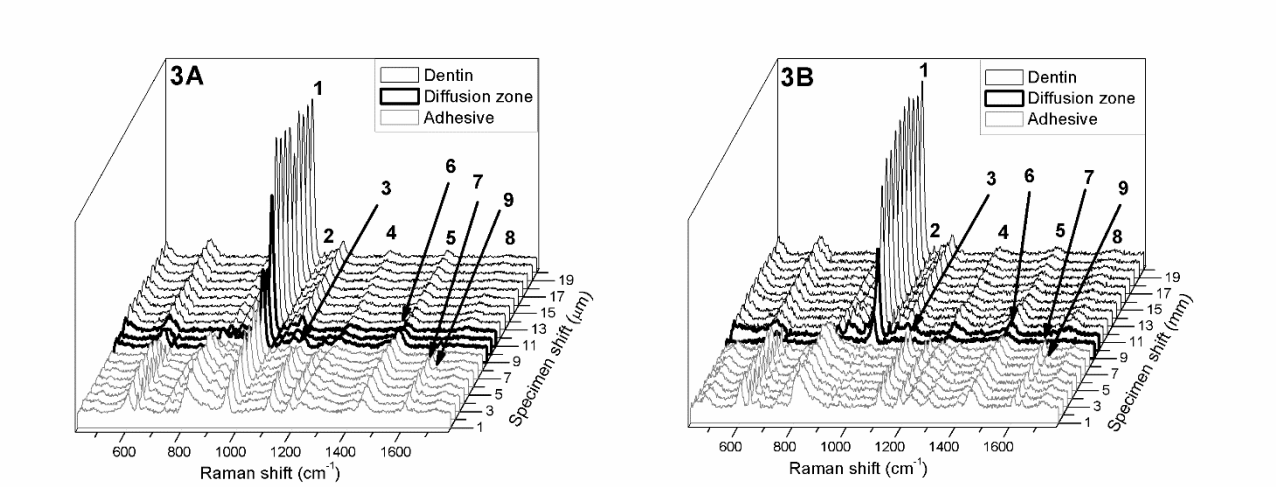

Figure 3: Representative MRS 3D-spectra acquired across adhesive-dentin interface of groups CG (A) and RG (B). Each measurement is represented by a numbered line on the z-axis (specimen shift). The gray lines represent the adhesive, the black, bold lines represent the resin-dentin interface, and the black, slim lines the dentin. The numbers indicate the Raman peaks used for the spectra physic-chemical analysis: 1) 961 cm^{-1}; 2) 1003 cm^{-1}; 3) 1113 cm^{-1}; 4) 1246 cm^{-1}; 5) 1450 cm^{-1}; 6) 1458 cm^{-1}; 7) 1608cm^{-1}; 8) 1637 cm^{-1}; 9) 1667 cm^{-1}.

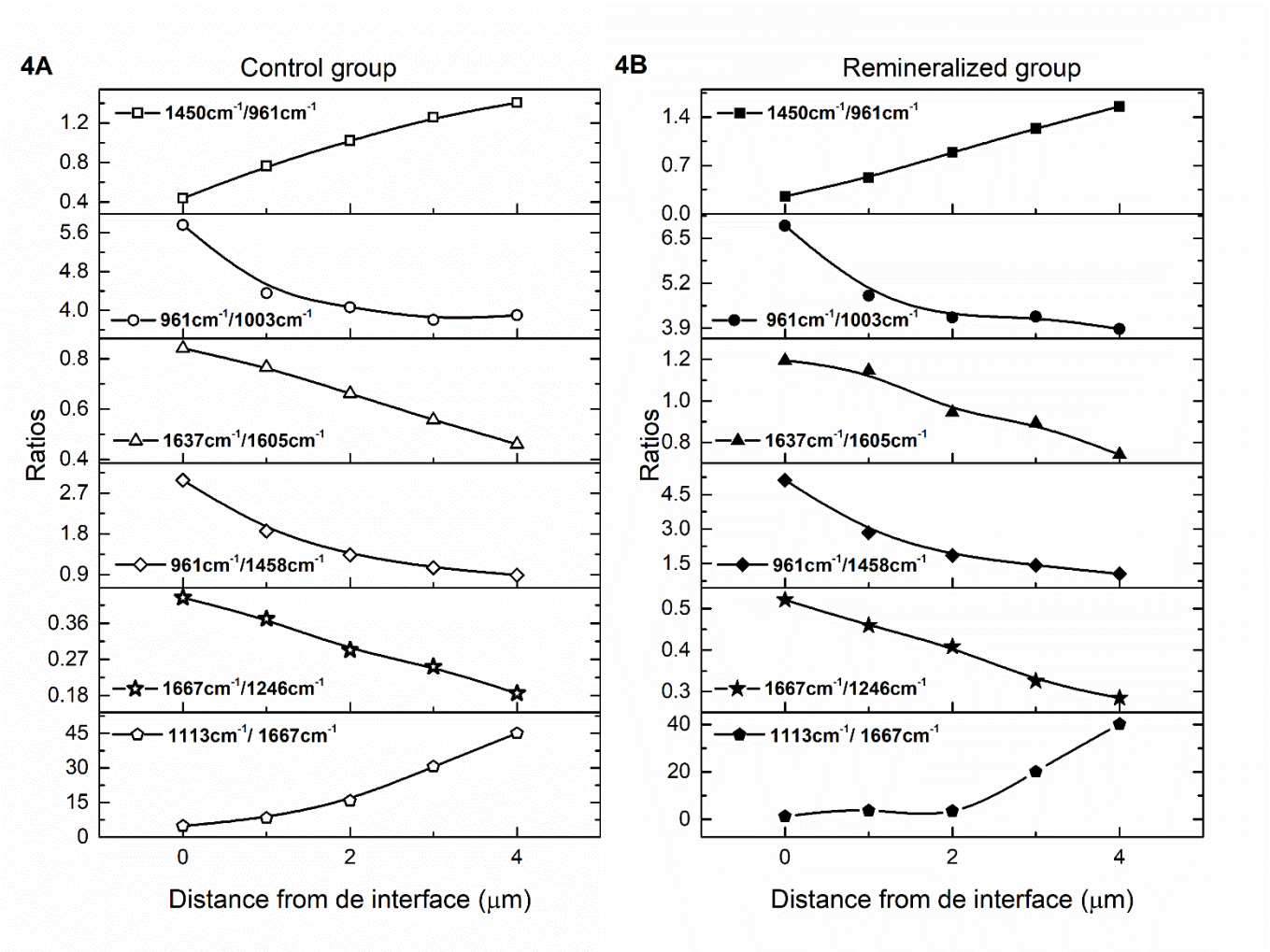

Figure 4: MRS physic-chemical analysis of resin-dentin interface as a function of spatial position. Figure 4A represent CG and figure 4B the RG. Profiles of organic matrix mineral content (R1450 cm^{-1}/961 cm^{-1}); relative mineral concentration (R961 cm^{-1}/1003 cm^{-1}); adhesive DC (R1637 cm^{-1}/1605 cm^{-1}); chemical interaction ratio (R961 cm^{-1}/1458 cm^{-1}); band intensity ratio of amide I to amide III (R1667 cm^{-1}/1246 cm^{-1}) and adhesive monomers penetration as a function of spatial position of Bis-GMA ratios (R1113 cm^{-1}/1667 cm^{-1}).

3.4.3 Adhesive diffusion zone (MRS) and interface (SEM) thickness comparison

Boltzmann analysis (Figure 5) showed lower values for the adhesive-dentin diffusion zones of RG (2.5 ± 0.8 µm) when compared to CG (3.1 ± 0.7 µm) (Table 2). SEM images (Figure 6) analysis also revealed lower interface thickness values for RG (3.0 ± 0.6 µm) than for CG (3.5 ± 0.6µm) (Table 2). Statistical analysis, however, demonstrated no significant difference between adhesive diffusion zones of RG and CG (P=.68) neither among interface thickness of RG nor CR (P=.74). Although the resulted values were higher when SEM images analysis were done, once again there was no significant difference between those two different methods to estimate the adhesive interface (P=.92).

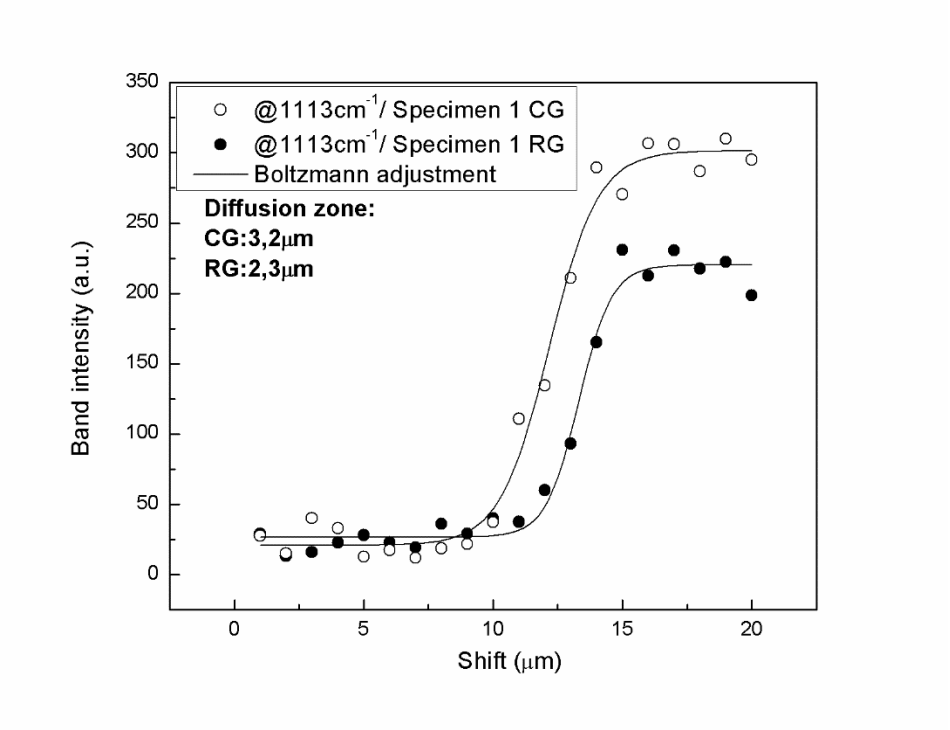

Figure 5: Representative plot of the diffusion zone of groups CG and RG. Raman mapping spectra at 1113 cm^{-1} (νC─O─C) was plotted according to the specimen shift position and fitted to the Boltzmann function.

Table 2 – Mean and standard deviation (SD) of adhesive diffusion zone (MRS) and interface thickness (SEM) expressed in micrometers (µm).

Group	MRS	SEM
CG	3 ± 0.6 AB	3.5 ± 0.6 A
RG	2.5 ± 0.8 B	3.0 ± 0.6 AB

Values are mean ± SD in µm. Different capital letters indicate significant statistical difference (P<.05).

SEM analysis of intact dentin-composite interfaces (Figures 6A and 6B) showed differences in hybrid layer of CG and RG. Remineralized specimens interface presented a more continuous hybrid layer with major part of dentin tubular spaces filled with adhesive projections (Figure 6B). Bioactive glass was observed as bright crystals within the hybrid layer and resin tags (Figure 6B).

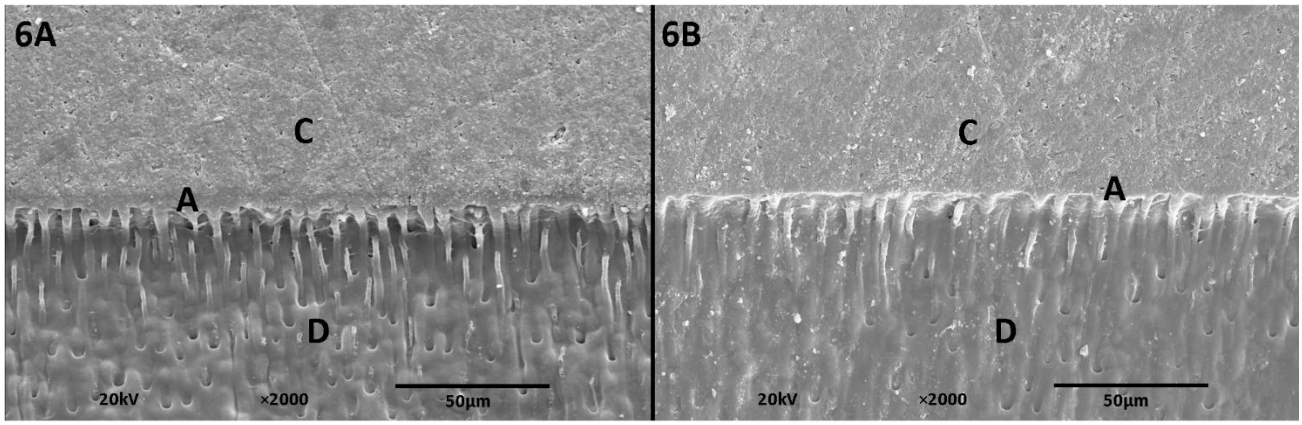

Figure 6: SEM of intact dentin (D) - adhesive (A) - composite resin (C) interface of CG (A) and RG (B) (×2000). In some regions of the CG interface it is possible to visualize gaps between dentin tubules and adhesive tags (A). In RG, the hybrid layer is more continuous and dentin

tubules seem to be better infiltrated with adhesive system (B). BAG particles are observed as bright crystals (B).

3.5 DISCUSSION

A clear and consistent effect of the dentin remineralization during bonding procedures has been demonstrated in both mechanical and physicochemical properties of resin-dentin interface. Dentin treatment with BAG solution after the acid conditioning improved its bond strength, promoted an increase in the inorganic compounds of dentin mineral matrix and resulted in collagen fibrils protection since it decreased the amount of organic matrix components exposed at the dentin interface and changed collagen framework configuration. In addition, the remineralization treatment modified the interaction between the adhesive and dentin tissue since it enhanced the chemical bonding of adhesive with mineral compounds of dentin and improved the DC of adhesive monomers. Because of the mineral formation, the investigated treatment promoted a decrease in the adhesive monomers penetration into dentin, however, it did not change significantly the adhesive diffusion zone nor the thickness of adhesive interface.

The first null hypothesis of this study was rejected, as the remineralization treatment promoted an improvement in dentin μTBS. This reinforcement at the resin-dentin interface was a consequence of mineral deposition since dentin mechanical properties are directly affected by the degree and quality of its mineral content. [40] This was the first remineralization study using BAG solution as an additional bonding step. BAG has been used in immersion solutions, as abrasion powder and mixed with adhesive agents. Microtensile studies [21,41,42] revealed that dentin treatment with experimental primers and adhesives containing biomimetic analogs or micro-fillers (Portland cement, BAG, Ca/P) did not change μTBS values in the 24 h analysis but provided durable resin-dentin bonds when long term analyzes

were done. The higher μTBS immediate results obtained in this study, differently from when BAG was added in the adhesive compounds can be an indicative that its components are more reactive when not associated with other chemical molecules. Dentin acid conditioning is another variable that seems to affect μTBS of resin-dentin remineralized interface. Even after remineralization treatment, dentin bond strength was significantly lower than control group when demineralization step was applied during a prolonged time [41] or using different or strong acids than those applied in the etch-and-rinse protocol. [42]

As a consequence of dentin demineralization, mineral components around organic molecules are eliminated leaving the collagen fibrils unprotected and surrounded by water. When this residual water is removed with air spay or organic solvents, the collagen fibrils collapses making adhesive infiltration a difficult process, exposing these fibrils to degradation enabling hybrid layer integrity damage. [43] The second hypothesis that there is no difference in dentin physic-chemical properties in the presence or absence of remineralization was rejected since MRS investigation proved the remineralization effect of dentin treatment with BAG. Minerals could efficiently precipitate in the previous partially demineralized dentin surface, promoting an increase in phosphate ion concentration and in the apparent relative mineral concentration. [28,51] These MRS findings are associated with the formation of hydroxyapatite on dentin surface. [44]

Changes in dentin organic compounds play an important role in the remineralization process. [23] Demineralized collagen network provides active sites for mineral precipitation around its fibrils. [24,45] Hydroxyapatite formation protected the non-denatured collagen from hydrolytic and enzymatic degradation and restored its mechanical behavior. [22] This protection effect was reflected in the decrease of exposed organic matrix mineral content at the interface surface (ratio 1450 cm^{-1}/961 cm^{-1}). MRS band of amide I is considered the best spectra region for studying protein conformation and

secondary structure. [46] Additionally, the ratio of the amides I to III is used to investigate the organization of collagen. In this study dentin remineralization was also characterized through the modifications in the MRS spectral region of amide since amide I to amide III ratio were higher on RG spectra. [23]

Although remineralization treatment was done previously to adhesive system application, it enhanced monomers chemical bonding with dentin and increased monomers polymerization. Etch-and-rinse micromechanical retention is improved when methacrylate monomers and/or carboxylic esters chemically interact with etched inorganic dentin compounds. [3] Chemical interaction ratio (961 cm^{-1}/1458 cm^{-1}) was higher when the BAG solution was applied on etched dentin, demonstrating that methacrylate monomers are more susceptible to interact with dentin when mineral compounds are present in larger quantity. The results of this study are in accordance with those of Toledano et al., 2014; [23] since the adhesive DC increased due to dentin remineralization. Mineral formation seems to increase monomer polymerization ratio as a consequence of its dehydration effect since hybrid layer residual water removal makes adhesive monomers more susceptible to realize C=C bonds. Adhesive monomer to polymer conversion is related to adhesive bond physicochemical properties and influences the stability of the resin-dentin bond. [47] Thus, the reduction of adhesive penetration ratio and the better results of μTBS values after remineralization treatment could also contributed to DC results. When the adhesive DC is obtained inside the hybrid layer, the polymerization reaction is measured considering not only monomers conversion but also the interactions of the adhesive material with the dentin tissue. [48]

Even though remineralization treatment increased the amount of mineral matrix on dentin surface, this outcome did not significantly decrease adhesive diffusion zone nor resin-dentin interface thickness, leading to the acceptance of the third hypothesis of this study. Literature is controversial

about remineralization effect on the thickness of resin-dentin interface. Remineralization was described to facilitate and increase the penetration of adhesive monomers since it prevented dentin collagen collapse. [50] On the contrary to this, another study concluded that mineral deposition promoted dentin tubules sealing, decreasing interface thickness. [22] Corroborating with the present study, other investigations reported that hybrid layer thickness did not change after dentin remineralization because mineral deposition occurred around dentinal tubular orifices, between collagen fibrils, resulting in a less-porous hybrid layer. [16,25] Boltzmann analysis is a chemical method to estimate the adhesive diffusion zone into dentin since it measurement is done using the sigmoidal behavior of a specific adhesive monomer MRS band at the interface region, [46] while SEM investigation is a quantitative imaging method that uses resin-dentin images to determine the interface thickness. The comparison between Boltzmann analysis and SEM investigation revealed similar thickness values for both techniques demonstrating that the MRS can be used as an effective tool to estimate interface thickness.

Hybridization efficiency is not related to the interface thickness but is assigned to its infiltration characteristic and its bond quality; [49] therefore, incomplete permeation of adhesive monomers into demineralized dentin enables hybrid layer degradation. [5] Thus, mineral formation resulted from remineralization treatment is an alternative to protect denuded collagen fibrils and eliminate residual water on etched dentin, enhancing the durability of resin-dentin bonds. Although remineralization treatment did not increase hybrid layer thickness, it promoted a decrease in the adhesive penetration ratio. This ratio is calculated by the subtraction of MRS band intensity of Bis-GMA adhesive monomer (1113 cm^{-1}) by amide I (1667 cm^{-1}). Mineral deposition promotes the bonding between hydroxyapatite and unprotected collagen, which leads to the increase of mineral compounds and to the reduction of organic- matrix mineral content available at the interface. MRS analysis revealed changes in the structural composition of collagen, a substitution of amide III for amide I, therefore, the increase in

amide I intensity also promoted the reduction on Bis-GMA penetration ratios. [23]

In vitro studies may be limited to simulate clinical conditions. When extracted dentin specimens are used the loss of connection between dentin and pulp tissues might affect organic dentin tissue compounds and its link with the mineral compounds. Thus, remineralization chemical dynamics found in this investigation could be different if vital teeth were analyzed. Considering that saliva compounds are readily available to remineralize dental tissue, the use of artificial saliva can also be listed as a limitation in a remineralization study. Nevertheless, both control and experimental groups were immersed in saliva and the remineralization effect was observed only in remineralized specimens. The objective of the present study was to evaluate immediate dentin remineralization effect; however, further studies are in progress to investigate the resin-dentin bond stability over time when remineralization is done with BAG solution in a during-bonding technique.

3.6 CONCLUSIONS

Within the limitations of this study, it can be concluded that BAG solution applied on etched dentin previously bonding procedures promoted remineralization at the resin-dentin interface. Although interface thickness was not changed after remineralization treatment, dentin new mineral growth improved both physic-chemical and mechanical properties of resin-dentin interface treated with an etch-and rinse adhesive system. Because of mineral collagen fibrils protection, adhesive monomers penetration into dentin was reduced, however, its chemical interaction with phosphate and its conversion ratio into polymers were enhanced. Consequently, dentin remineralization associated with adhesive properties improvements resulted in higher resin-dentin bond strength values.

3.7 CLINICAL RELEVANCE

Dentin remineralization treatment with Bioglass 45S5 induces mineral growth, removes residual extra and intrafibrillar water and thus promotes collagen fibrils protection. Resin-dentin bond strength is improved due to remineralization effects on dentin physic-chemical composition and adhesive system properties.

REFERENCES

1. Opdam NJM, Bronkhorst EM, Loomans BAC, Huysmans M. 12-year Survival of Composite vs. Amalgam Restorations. Journal of Dental Research 2010;89:1063-1067.
2. De Munck J, Mine A, Poitevin A, Van Ende A, Cardoso MV, Van Landuyt KL, Peumans M, Van Meerbeek B. Meta-analytical review of parameters involved in dentin bonding. J Dent Res 2012;91:351-357.
3. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond-strength tests and clinical outcomes. Dent Mater 2010;26:e100-121.
4. De Munck J, Mine A, Van den Steen PE, Van Landuyt KL, Poitevin A, Opdenakker G, Van Meerbeek B. Enzymatic degradation of adhesive-dentin interfaces produced by mild self-etch adhesives. Eur J Oral Sci 2010;118:494-501.
5. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, Ruggeri A Jr, Tay FR, Dorigo Ede S, Pashley DH. Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. Dent Mater 2010;26:320-325.
6. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int. 1999;30:501-507.
7. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay

A. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1-16.

8. 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. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005;26:6449-6459.
9. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83:216-221.
10. Tay FR, Gwinnett JA, Wei SH. Micromorphological spectrum from overdrying to overwetting acid-conditioned dentin in water-free acetone-based, single-bottle primer/adhesives. Dent Mater 1996;12:236-244.
11. Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res 2006;85:384.
12. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86:529-533.
13. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjäderhane L, Mazzoni A, Breschi L, Foulger S, Pashley DH. Host-derived loss of dentin matrix stiffness associated with solubilization of collagen. J Biomed Mater Res B Appl Biomater 2009;90:373-80.
14. Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, Cenci MS. MMP Inhibitors on Dentin Stability: A Systematic Review and Meta-analysis. J Dent Res 2014;93:733-743.
15. Al-Ammar A, Drummond JL, Bedran-Russo AK. The use of collagen cross-linking agents to enhance dentin bond strength. J Biomed Mater Res B Appl Biomater 2009;91:419-424.
16. Sadek FT, Castellan CS, Braga RR, Mai S, Tjäderhane L, Pashley DH, Tay FR. One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. Dent

Mater 2010;26:380-386.

17. Kim YK, Gu LS, Bryan TE, Kim JR, Chen L, Liu Y, Yoon JC, Breschi L, Pashley DH, Tay FR. Mineralisation of reconstituted collagen using polyvinylphosphonic acid/polyacrylic acid templating matrix protein analogues in the presence of calcium, phosphate and hydroxyl ions. Biomaterials 2010;31:6618-6627.
18. Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res 2011;90:953-968.
19. Magne D, Weiss P, Bouler JM, Laboux O, Daculsi G. Study of the maturation of the organic (type I collagen) and mineral (nonstoichiometric apatite) constituents of a calcified tissue (dentin) as a function of location: a Fourier transform infrared microspectroscopic investigation. J Bone Miner Res 2001;16:750-757.
20. Kinney JH, Habelitz S, Marshall SJ, Marshall GW. The importance of intrafibrillar mineralization of collagen on the mechanical properties of dentin. J Dent Res 2003;82:957-961.
21. Profeta AC, Mannocci F, Foxton R, Watson TF, Feitosa VP, De Carlo B, et al. Experimental etch-and-rinse adhesives doped with bioactive calcium silicate-based micro-fillers to generate therapeutic resin-dentin interfaces. Dent Mater 2013;29:729-741.
22. Tay FR, Pashley DH. Biomimetic remineralization of resin-bonded acid-etched dentin. J Dent Res 2009;88:719-724.
23. Toledano M, Aguilera FS, Cabello I, Osorio R. Remineralization of mechanical loaded resin-dentin interface: a transitional and synchronized multistep process. Biomech Model Mechanobiol 2014;13:1289-1302
24. Toledano M, Aguilera FS, Osorio E, Cabello I, Osorio R. Microanalysis of thermal-induced

changes at the resin-dentin interface. Microsc Microanal 2014;20:1218-1233.

25. Ryou H, Niu LN, Dai L, Pucci CR, Arola DD, Pashley DH, Tay FR. Effect of biomimetic remineralization on the dynamic nanomechanical properties of dentin hybrid layers. J Dent Res 2011;90:1122-1128.
26. Toledano M, Cabello I, Vílchez MA, Fernández MA, Osorio R. Surface microanalysis and chemical imaging of early dentin remineralization. Microsc Microanal 2014;20:245-256.
27. Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J Bio Mater Research 1971;5:117-141.
28. Crovace MC, Souza MT, Chinaglia CR, Peitl O, Zanotto ED. Biosilicate®—A multipurpose, highly bioactive glass-ceramic. In vitro, in vivo and clinical trials. Journal of Non-Crystalline Solids 2016;432: 90-110.
29. Sauro S, Watson TF, Mannocci F, Miyake K, Huffman BP, Tay FR, et al. Two-photon laser confocal microscopy of micropermeability of resin–dentin bonds made with water or ethanol wet bonding. Journal of Biomedical Materials Research B: Applied Biomaterials 2009;90:327–337.
30. Niu L, Zhang W, Pashley DH, Breschi L, Mao J, Chen J, Tay FR. Biomimetic remineralization of dentin. Dent Mater 2104;31:77-96.
31. Tirapelli C, Panzeri H, Soares RG, Peitl O, Zanotto ED. A novel bioactive glass-ceramic for treating dentin hypersensitivity. Braz Oral Res 2010; 24:381-387.
32. Schwartz AG, Pasteris JD, Genin GM, Daulton TL, Thomopoulos S. Mineral distributions at the developing tendon enthesis. PLoS One 2012;7:e48630.
33. Karan K, Yao X, Xu C, Wang Y. Chemical profile of the dentin substrate in non-carious cervical lesions. Dent Mater 2009;25:1205–1212.

34. Xu C, Wang Y. Cross-linked demineralized dentin maintains its mechanical stability when challenged by bacterial collagenase. J Biomed Mater Res B Appl Biomater 2011;96:242–248.
35. Zhang Y, Wang Y. Distinct photopolymerization efficacy on dentin of self-etch adhesives. J Dent Res 2012; 91:795-799.
36. Xu C, Wang Y. Collagen cross linking increases its biodegradation resistance in wet dentin bonding. J Adhes Dent 2012;14:11–18.
37. Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding. J Dent Res 2003;82:141–145.
38. Shin TP, Yao X, Huenergardt R, Walker MP, Wang, Y. Morphological and chemical characterization of bonding hydrophobic adhesive to dentin using ethanol wet bonding technique. Dent Mater 2009;25:1050-1057.
39. de Oliveira Ferraz LC, Ubaldini AL, de Oliveira BM, Neto AM, Sato F, Baesso ML et al. Analytical method to estimate resin cement diffusion into dentin. J Biomed Opt 2016; 21:55003.
40. Balooch M, Habelitz S, Kinney J, Marshall S, Marshall G: Mechanical properties of mineralized collagen fibrils as influenced by demineralization. J Struct Biol 2008;162:404–410.
41. Hara AT, Queiroz CS, Giannini M, Cury JA, Serra MC. Influence of the mineral content and morphological pattern of artificial root caries lesion on composite resin bond strength. Eur J Oral Sci 2004;112:67-72.
42. Toledano M, Aguilera FS, Osorio E, Cabello I, Toledano-Osorio M, Osorio R. Bond strength and bioactivity of Zn-doped dental adhesives promoted by load cycling. Microsc Microanal 2015;21:214-230.
43. Fawzy AS. Variations in collagen fibrils network structure and surface dehydration of acid demineralized intertubular dentin: Effect of dentin depth and air-exposure time. Dent Mater

2010;26: 35-43.

44. Greish YE, Brown PW. Phase evolution during the formation of stoichiometric hydroxyapatite at 37.4 degrees C. J Biomed Mater Res B Appl Biomater 2003;67:632-637.
45. Nudelman F, Pieterse K, George A, Bomans PH, Friedrich H, Brylka LJ, Hilbers PA, De With G, Sommerdijk NA. The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors. Nat Mater 2010;9:1004–1009.
46. Karan K, Yao X, Xu C, Wang Y. Chemical profile of the dentin substrate in non-carious cervical lesions. Dent Mater 2009;25:1205–1212
47. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. Dent Mater 1990;6:282-287.
48. Navarra CO, Breschi L, Turco G, Diolosà M, Fontanive L, Manzoli L, Di Lenarda R, Cadenaro M. Degree of conversion of two-step etch-and-rinse adhesives: In situ micro-Raman analysis. J Dent 2012;40:711-717.
49. Van Meerbeek B, Conn LJ Jr, Duke ES, Eick JD, Robinson SJ, Guerrero D. Correlative transmission electron microscopy examination of nondemineralized and demineralized resin-dentin interfaces formed by two dentin adhesive systems. J Dent Res 1996;75:879-888.

4. ARTIGO 3:

Dentin mineralization kinetics after internal bleaching

4.1 ABSTRACT

Objectives: This study evaluated the ability of two bioactive agents, Bioglass® 45S5 (BG) and Biosilicate® (BS), to stimulate new mineral formation at the resin-dentin interface created with a two-step adhesive system after internal bleaching. **Methods:** Micro-Raman Spectroscopy (MRS) was used to quantitatively analyze the mineralization kinetics of control dentin (CD) and bleached dentin (BD) (n=5) through a 15-day incubation in artificial saliva (AS). CD and BD discs (n=10) with and without remineralization treatment with BG and BS were prepared to micro-tensile bond strength test (μTBS) and tested after 1 week. Dentin surface of one fractured beam per tooth was submitted to MRS to characterize interface physic-chemical composition (n=10). Data were analyzed with 1-way ANOVA and Tukey-Kramer post-hoc test (P<.05). **Results:** MRS bioactive analysis revealed that BG and BS promoted an increase of the mineral matrix ratio in CD and BD. Significantly high μTBS values were found after CD treatment with BG (CD: 58.9 MPa ± 11,8; CD-BG: 78,5 MPa ± 15,2), and when BG and BS were applied on BD (BD: 46,2 MPa ± 8,1; BD-BG: 72,2 MPa ± 14,7; BD-BS: 64,9 MPa ± 11,4) (P<.05). MRS physic-chemical analysis showed that dentin remineralization treatment significantly increased relative mineral concentration and promoted the appearance of new interface peaks, indicating a chemical interaction (P<.05). **Significance:** Remineralization of bleached dentin is an effective therapy to restore damages caused by internal bleaching and acid conditioning step since it increases not only dentin mineral compounds but also improves its ability to chemically interact with adhesive system.

Keywords: Biosilicate, Bioactive glass, Internal bleaching, Dentin remineralization, Resin-dentin interface, Bond strength, Raman spectroscopy.

4.2 INTRODUCTION

Restorative procedures of endodontically treated teeth (ETT) are still a challenge in clinical restorative dentistry due to their high risk of biomechanical failure [1,2]. Endodontic access and root canal preparation promote excessive loss of dental tissue, modifying dental restorations retention [3]. Internal bleaching is considered a conservative option to improve the esthetics of discolored ETT, however, bleaching agents oxidative action decreases tooth fracture resistance and increases the failure rate of restorative procedures of ETT. Internal bleaching agents can chemically interact with dentin compounds [4], promoting changes in its organic composition by denaturation of the collagen proteins [5-7], causing demineralization of its mineral composition [8], thus reducing its adhesion to restorative materials [9].

Dentin bonding is a complex process in which the mineral phase of dentin is removed by acid conditioning treatment, without altering the collagen matrix, and filled with adhesive monomers forming the hybrid layer [10]. The hybrid layer is characterized as a three-dimensional polymer/collagen network with a continuous and stable bond between adhesive monomers and dentin. Literature indicates that this ideal is not reached due to a discrepancy between the depth of dentin demineralization and adhesive infiltration, resulting in unprotected collagen fibers being exposed to deterioration [11,12]. Hybrid layer loss of integrity may occur because of water-sorption-induced hydrolysis of adhesive monomers and also as a result of acidic conditioning that activate collagen fibrils degradation through endogenous matrix metalloproteinases (MMPs) [13-15].

The use of agents that promote dentin remineralization, such as sodium phosphosilicate

biomaterial (Bioglass® 45S5: 45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_2 – wt.%) [16], has been shown to be an effective alternative for preserving adhesive interface integrity after phosphoric acid conditioning [17,18]. This mechanism of remineralization has also been identified with the use of a new bioactive material based on a fully crystallized ceramic glass (Biosilicate®: 23.75% Na_2O, 23.75% CaO, 48.5% $SiO_2.4(P_2O_5)$ –wt.%) [19] by deposition of hydroxyapatite in dentin tubules in dentin hypersensitivity treatment [20] and also prior to creation of resin-dentin interfaces in sound and caries-affected dentin [21]. These bioactive compounds provide a progressive dehydration mechanism and replace the extra and intrafibrillar water, restoring the protective function of collagen apatites [22,23] and promoting the formation of a hydroxyapatite layer on dentin surface [20,24]. Remineralization process allows electrostatic, ionic and/or hydrogen bonding between demineralized collagen and silanol compounds [15,25].

Although bioactive glasses solutions have been used for dentin remineralization in demineralized, sound and caries-affected dentin, there is no information about the use of remineralization agents on bleached dentin during restorative bonding steps. Therefore, this study aimed to investigate the remineralization ability of two bioactive agents and their potential therapeutic effects on resin-dentin bond when they are used as dentin remineralizing agents during the total-etching adhesive step after internal bleaching. The null hypothesis to be tested are 1) there is no difference in mineral components of control and bleached dentin in the presence or absence of remineralization treatment, 2) dentin bond strength is not altered after internal bleaching and/or remineralization treatment, and 3) dentin remineralization does not change dentin-adhesive interface composition.

4.3 MATERIALS AND METHODS

4.3.1 Specimen preparation, bleaching and remineralization treatments

Seventy extracted, healthy and unerupted human third molars were used following the guidelines approved by the Research Ethics Committee of State University of Maringa (research protocol: 50615715.1.0000.0104). Using a low-speed diamond saw (Diamond Wheel 012"x fine, South Bay Technology Inc, CA, USA) under water-cooling a flat mid coronal dentin disc of 5 mm, without any enamel remnants, was prepared from each tooth. The exposed dentin surfaces were wet polished using 600-grit SiC paper (Carborundum Abrasives, Saint-Gobain, MA, USA) for 1 min to create a homogeneous and standard smear layer. From those seventy dentin discs, sixty were set apart for microtensile bond test (μTBS) and posterior physic-chemical analysis (PA) using micro-Raman spectroscopy (MRS), while ten were cut into four small pieces (2 mm x 2 mm x 5 mm) and were used for bioactive test (BT). The specimens were randomly divided into 6 groups based on the treatment protocol (Figure 1); control group (CD), control dentin remineralized with Bioglass® 45S5 (CD-BG), control dentin remineralized with Biosilicate® (CD-BS), bleached dentin (BD), bleached dentin remineralized with Bioglass® 45S5 (BD-BG), bleached dentin remineralized with Biosilicate® (BD-BS). Sample size was different for the three methods used; BT (n=5), μTBS (n=10) and PA (n=5).

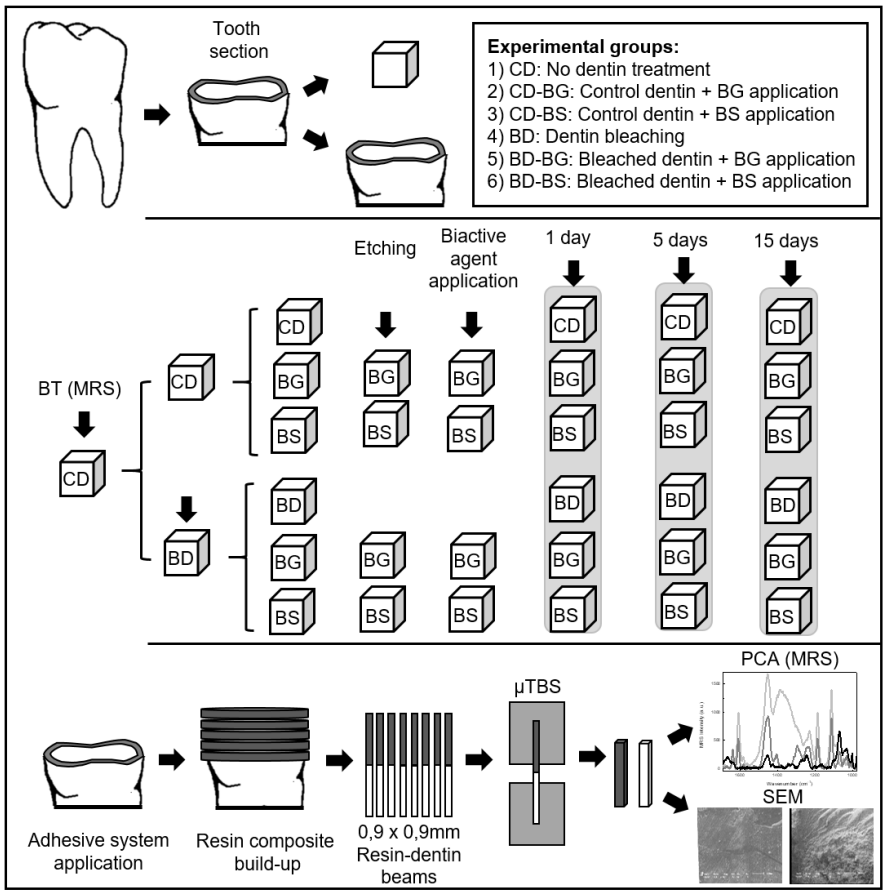

Figure 1. Schematic drawing illustrating the study setup and specimen preparation.

Bleaching treatment was done simulating the walking bleach technique. Dentin surface was treated with a paste made with 0.01 g of sodium perborate (Whiteness Perborato - FGM Produtos Odontológicos, Joenville, Brazil) and 0.5 ml of 3% hydrogen peroxide (Rioquímica, Sao Jose do Rio Preto, SP, Brazil). During the 14 days of bleaching treatment, the specimens were stored in an incubator (ProLab, São Paulo, SP, Brazil) with 95% ± 5% relative humidity at 37 °C, and the bleaching agent was replaced after the 7th day. When bleaching treatment was done, dentin surface was washed with distilled water and air-dried for 2 s to remove the excess of water.

According to experimental groups division, the remineralization treatment was performed with two different agents, the standard remineralizing agent, Bioglass® 45S5 (BG) and the experimental

remineralizing agent, Biosilicate® (BS). Control and bleached dentin surface were etched with 37% phosphoric acid (Condac37- FGM Produtos Odontológicos, Joenville, Brazil) for 10 s followed by copious water rinse for 1 min. After acid conditioning, the remineralization treatment was carried out to induce new mineral formation before the adhesive application. Remineralization solutions were done diluting 0.1 mg of the remineralizing agent powder with 1 ml of deionized water immediately before the application [21]. A micropipette (Monocanal VVCS-10, Digipet, Sao Paulo, SP, Brazil) was used to apply 10 μl of this solution in the moist dentin surface and using a microbrush the solution was slightly rubbed in dentin for 30 s. The surface was then washed with deionized water for 15 s and gently dried with filter paper to remove the excess of water.

4.3.2 Bioactive test (BT) (micro-Raman Spectroscopy)

BT analysis (n=5) was performed to investigate BG and BS ability to induce dentin remineralization in the presence or absence of bleaching treatment. MRS initial spectra were measured before treatment (as a control analysis), and after the procedures of bleaching (for bleached groups), acid conditioning and remineralization agent application. In order to enable the MRS analysis by time storage, after remineralization treatment, the specimens were adapted in a plastic device and immersed in 25 ml of artificial saliva [12] in a 37 °C water bath. Then, long term analyzes were carried out after 1 day, 5 days and 15 days. Before spectra were acquired, the specimens were rinsed with distilled water for 30 s and completely air dried.

Raman spectra were performed with a confocal Raman microscope (Senterra Bruker Optik GmbH, Ettingen, Germany). The spectra were excited by a 785 nm laser source and recorded in the spectral range of 450 to 1800 cm^{-1}. The laser power (100 mW) was focused on the specimen with a 20× magnification lens. Spatial resolution was 3 to 5 cm^{-1}, and integration time of the detector was 3 s. Each

final curve resulted from the mean of 60 spectra. All spectra were systematically collected under the same experimental conditions and reference points were determined on specimen area to enable Raman spectra to be done at the same position before and after treatment, as well as during the long term analyzes. Four spectra were obtained at each measurement and the final result for each specimen was calculated by the average of these four spectra.

Spectra were baseline corrected and the integrated areas of dentin organic (amide I: 1.650 cm^{-1}) and mineral compounds (phosphate ν1: 961 cm^{-1}) were calculated. Then, dentin structural modifications were characterized through: mineral matrix ratio (961 cm^{-1} /1.650 cm^{-1}) [24]. In order to standardize graphic visual analyzes, all ratios values were normalized by the value of the control spectra. Additionally, Raman spectra from BG and BS were obtained to characterize their chemical structures.

4.3.3 Dentin bonding and restorative procedures

An adhesive interface was performed to test interface bond strength (μTBS) and chemical modifications (MRS) of both untreated, bleached and remineralized dentin surfaces. For the groups in which dentin was tested as a control surface the restorative treatment was done right after specimens' preparation. Nevertheless, for the groups in which dentin tissue was bleached, the restorations were carried out only 14 days after dentin bleaching treatment was completed to eliminate any residual byproducts of the bleaching agent. During those 14 days the bleached dentin specimens were stored in an incubator (ProLab, São Paulo, SP, Brazil) with 95% ± 5% relative humidity at 37 °C. Bonding procedures were done with a 2-step etch-and-rinse adhesive (Optibond S, Kerr, Orange, CA, EUA) used according to the manufacturer's instructions and light-cured for 20 s at 1.200 mW/cm^2 with a LED unit (Radii – Cal - SDI, Bayswater, Australia). The specimens were immediately restored using 5 layers

of 1mm-think composite resin (Filtek Z250 - 3MESPE, AG Dental Products, Seefeld, Germany). Each layer was light-cured for 20 s at 1.200 mW/cm^2 and then a final curing of 40 s was done. Considering that bioactive agents need time to react with mineral tissue and create a hydroxyapatite layer [26], all restored specimens were stored in artificial saliva [12] for 7 days at 37 °C before testing.

4.3.4 Microtensile bond strength (μTBS) test and scanning electron microscopy (SEM) analysis of the failure modes

Restored dentin blocks (n=10) were longitudinal sectioned across their interface in both mesio-distal and buccal-lingual directions to obtain bonded sticks with cross-sectional areas of approximately 0.9 mm^2. Peripheral beams with residual enamel were excluded from the study, therefore, 9 bonded beams were obtained per tooth. The exact width of each beam was measured with a digital caliper (Zaas Precision; Amatools, SP, Brazil).

Before μTBS test, resin-dentin beams were placed in the grips of the testing jig with cyanoacrylate glue (Superbonder Gel® Henkel/Loctite, Sao Paulo, SP, Brazil). Beams position was equidistant between the jig claws and perpendicular to the tensile force. Restorations interface were tested in a 0.5 mm/min traction in a universal test machine (EZ Test, Shimadzu, Kyoto, Japan) until the moment of specimen fracture. Tensile forces were obtained in Newton (N) and divided by the cross-sectional area in mm^2 to express the bond strength value in Mega Pascal (MPa). Beams with premature failure were discarded. μTBS values were computed for each tooth by averaging the values of the 9 resin-dentin beams from that tooth.

Failure modes were evaluated using a SEM (Superscan SS-550, Shimadzu Corporation, Kyoto, Japan) with a 100× magnification. Beam fractured surfaces were placed onto aluminum discs, sputter-coating with gold-palladium alloy (IC-50, Shimadzu Corporation, Kyoto, Japan). Failure patterns were

classified as (1) adhesive fracture, when the fracture site was located between the adhesive and dentin; (2) mixed, if the fracture involved dentin-adhesive interface, including cohesive failure of dentin and composite resin; (3) cohesive dentin fracture, and (4) cohesive fracture in the composite resin.

4.3.5 Physic-chemical analysis of dentin fractured interface (PA) (micro-Raman Spectroscopy)

PA was performed in the dentin fractured interface of the five tested beams that had the highest μTBS values per experimental group (n = 5). Resin-dentin interface modifications due to bleaching and/or remineralized treatment were analyzed through a chemical mapping with a confocal Raman microscope (Senterra Bruker Optik GmbH, Ettingen, Germany). The spectra were excited by a 785 nm laser source and recorded in the spectral range of 450 to 1800 cm^{-1}. The laser power (100 mW) was focused on the specimen with a 20× magnification lens. Spatial resolution was 3 to 5 cm^{-1}, and integration time of the detector was 3 s. Each final curve resulted from the mean of 60 spectra. A scan of the entire area of the fractured dentin (0.9 mm^2) was performed and MRS mapping was carried out by the selection of 10 points on both X and Y axis with a distance of 0.09 mm between them. All spectra were systematically collected under the same conditions.

MRS spectra were submitted to baseline correction and an average spectrum was obtained per line in the X-axis of spectral map. Dentin physic-chemical modifications were investigated through the analysis of mineral matrix ratio (961 cm^{-1} /1.650 cm^{-1}). Additionally, Raman spectra of control dentin, bleached dentin and adhesive system were visually compared to the average spectra obtained from fractured interfaces evaluated. This spectra comparison evidenced the appearance of three new MRS bands at dentin interface spectra, bands that were neither identified in the control and bleached dentin spectra nor in the adhesive spectra. These new MRS bands were centered in 1.295 cm^{-1} (C-H bonds)

[27], 1.405 cm^{-1} (methylene CH_2) [28] and 1.637cm^{-1} (methacrylate monomer) [13]. The area of these new bands were measured and normalized by the area of the amide I band of each respective spectrum.

4.3.6 Statistical analysis

Statistical analyzes were performed using R i386 3.0.2 software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria). Mineral matrix ratios obtained through BT were submitted to descriptive statistics and were presented in graphics with its means and standard deviations. μTBS data were statistically analyzed with Shapiro-Wilk normality test, 1-way ANOVA and Tukey-Kramer post-hoc test ($P<.05$). The ratios obtained by dentin fractured physic-chemical investigation, were also submitted to descriptive statistical analysis and presented in graphics with their means and standard deviations.

4.4 RESULTS

4.4.1 Bioactive test (BT) (micro-Raman Spectroscopy)

MRS ratios obtained from BT analysis are illustrated in Figures 2A and 2B. Mineral matrix ratio (961 cm^{-1} / 1650 cm^{-1}) demonstrated that remineralization treatment with both agents improved mineral content of control and bleached dentin after the acid conditioning step. The relative mineral composition of CD remained constant during the 15-day of incubation (Fig. 2A), showing that immersion in artificial saliva did not promote mineral formation on control dentin surface. Bleaching treatment promoted a reduction of dentin relative mineral content, that was not restored to its initial values after the 15 days of artificial saliva immersion (Fig. 2B). The acid conditioning step reduced the mineral composition of control dentin (Fig. 2A) and intensified the loss of the mineral composition of

the bleached dentin (Fig. 2B). Nevertheless, remineralization with BG and BS restored this mineral loss. Mineral deposition of control dentin occurred immediately after remineralization treatment with BG (CD-BG), took one day of incubation when BS solution was used (CD-BS) and was stable for both groups after 15 days of artificial saliva incubation (Fig. 2A). Bleached dentin mineral restoration was more intense instantly after BG and BS remineralization treatment (BD-BG and BD-BS) and presented a continuous behavior after the 15 days of incubation. Although remineralization approach improved the mineral content of bleached dentin, this treatment was not capable of restoring dentin mineral components to their initial values (Fig. 2B).

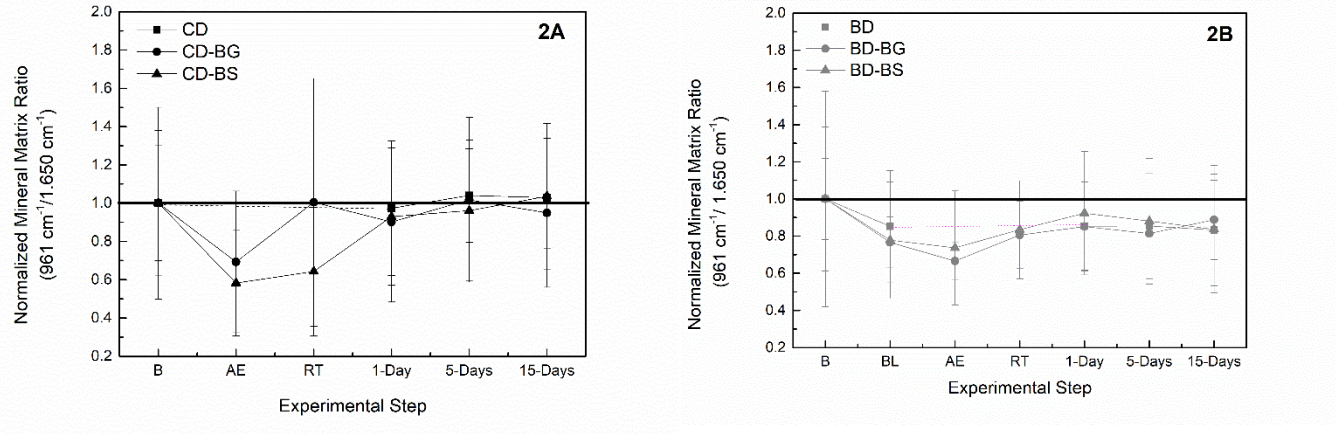

Figure 2. Mineral Matrix Ratio (961 cm^{-1} / 1.650 cm^{-1}) obtained from Bioactive Test under different experimental conditions: B = before any treatment, initial measurement; BL = after bleaching treatment; AE = after etching; RT = right after remineralization treatment; 1-Day = after 1 day of AS incubation; 5-Days = after 5 days of AS incubation; 15-Days = after 15 days of AS incubation. [A]: remineralization using Bioglass® 45S5. [B]: remineralization using Biosilicate®.

4.4.2 Microtensile bond strength (μTBS) test and scanning electron microscopy (SEM) analysis of the failure modes

μTBS results are shown in Table 1. Statistical analysis indicated that bleaching treatment significantly decreased dentin μTBS values (CD: 58.9 ± 11.8 MPa, BD: 46.2 ± 8.1 MPa) (P<.05). Remineralization treatment with BG promoted a significant increase in CD μTBS values (CD: 58.9 ± 11.8 MPa; CD-BG: 78.5 ± 15.2 MPa) (P<.05) and both BG and BS treatment resulted in significantly higher values of BD μTBS values (BD: 46.2 ± 8.1 MPa, BD-BG: 72.2 ± 14.7 MPa, BD-BS: 64.9 ± 11.4 MPa) (P<.05).

Table 1 – Mean and standard deviation (SD) of dentin μTBS (MPa).

Group	**CD**	**CD-BG**	**CD-BS**	**BD**	**BD-BG**	**BD-BS**
μTBS	58.9 ± 11.7 AB	78.5 ± 15.2 C	73.7 ± 14.7 BC	46.2 ± 8.1 A	72.1 ± 14.7 BC	64.8 ± 11.4 BC
N° of Beams	(5/85)	(0/90)	(0/90)	(10/80)	(3/87)	(5/85)
Failure mode	[37/39/8/6]	[46/40/2/2]	[44/40/4/2]	[46/38/4/2]	[32/48/6/4]	[40/36/9/5]

First row indicates the mean ± SD in MPa, different capital letters indicate significant statistical difference (*P*<.05). Second row reports number of premature failures and number of tested beams. Third row shows the percentage of failure modes [adhesive/mixed/cohesive in dentin/cohesive in composite resin].

SEM analysis of the fractured surfaces revealed that most failures in groups CD, CD-BG and CD-BS were at adhesive interface, while failure patterns were mostly mixed for groups BD, BD-BG and BD-BS. The types of failures occurring in all experimental groups are illustrated in figures 3A-3D.

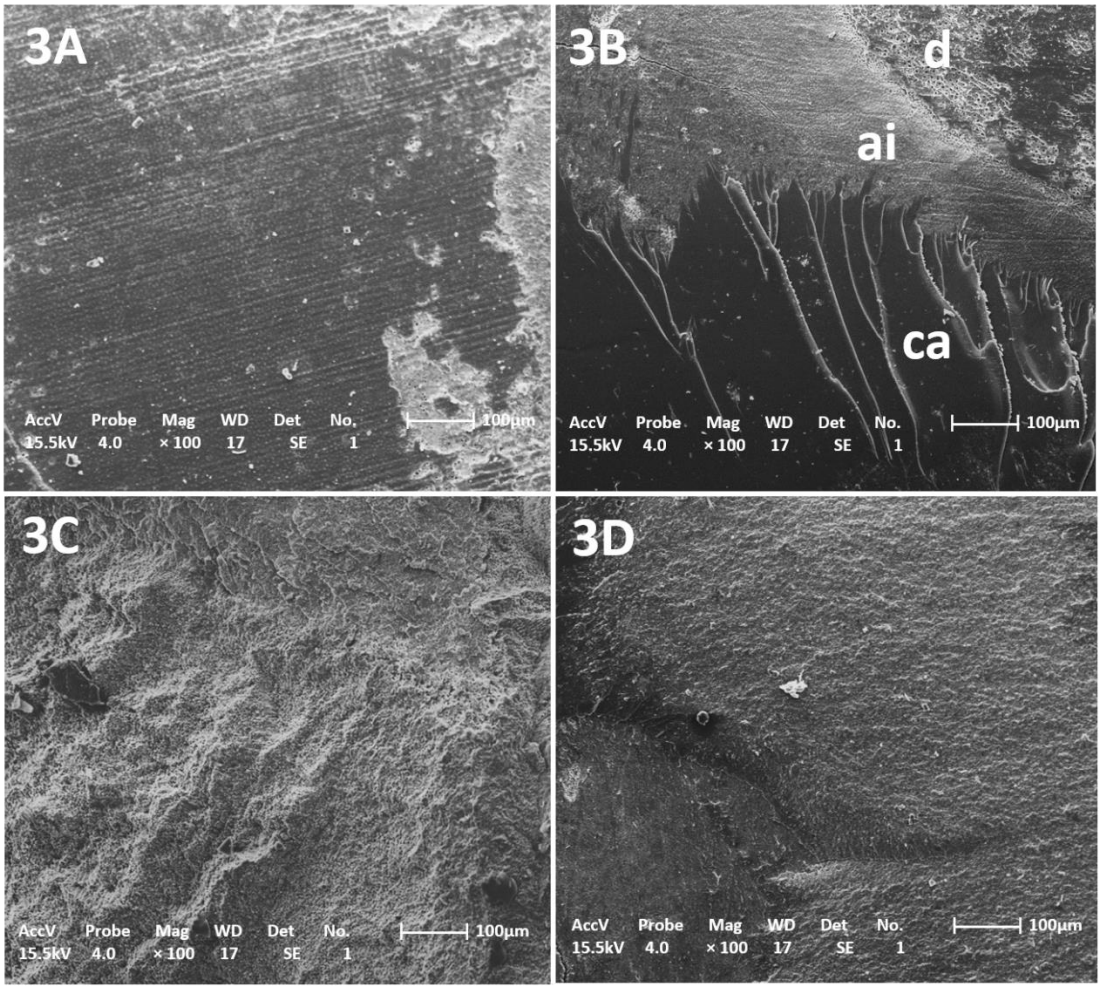

Figure 3. SEM failure analysis of fractured specimens (×100). [A] Adhesive failure along the dentin surface for CD-BG. [B] Mixed failure for CD displaying fracture at dentin surface (d), in the adhesive interface (ai), and cohesive failure in the adhesive layer (ca). [C] Cohesive failure within dentin for CD. [D] Cohesive failure within composite resin for BD-BS.

4.4.3 Physic-chemical analysis of dentin fractured interface (PA) (micro-Raman Spectroscopy)

Spectra qualitative comparison of dentin surface, adhesive system and fractured interface revealed the presence of new micro-Raman bands that can be attributed to C-H (1.295 cm^{-1}) [27], CH_2 of methylene (1.405 cm^{-1}) [28] and C=C of methacrylate bonds (1.637 cm^{-1}) [13] (Fig. 4). PA analysis of the fractured dentin beam showed an increase in mineral matrix ratio (961 cm^{-1} / 1650 cm^{-1}) on the interface of CD-BG and BD-BS (Fig. 5A). MRS new peaks ratio analyzes revealed that C-H band (1.295 cm^{-1}) was the most evident among them and presented a higher value in the groups treated with remineralizing agents, CD-BG, BD-BG and BD-BS (Fig. 5B)

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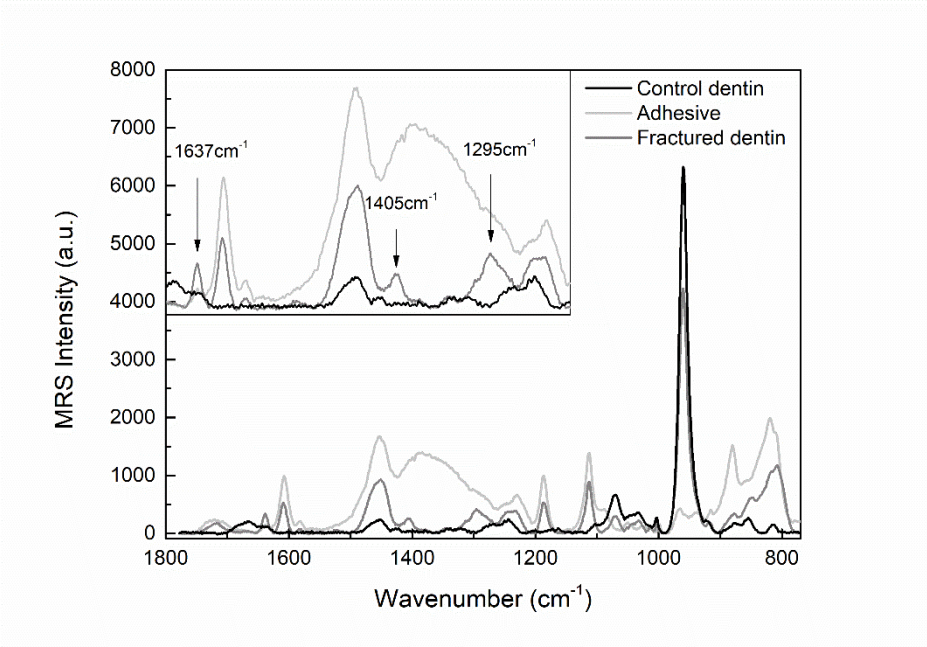

Figure 4. Comparison of MRS spectra of adhesive system, control dentin and fractured dentin (interface average spectrum). The new MRS bands are evidenced with the black arrows, C-H (1.295 cm^{-1}), CH_2 of methylene (1.405 cm^{-1}) and C=C of methacrylate bonds (1.637 cm^{-1}).

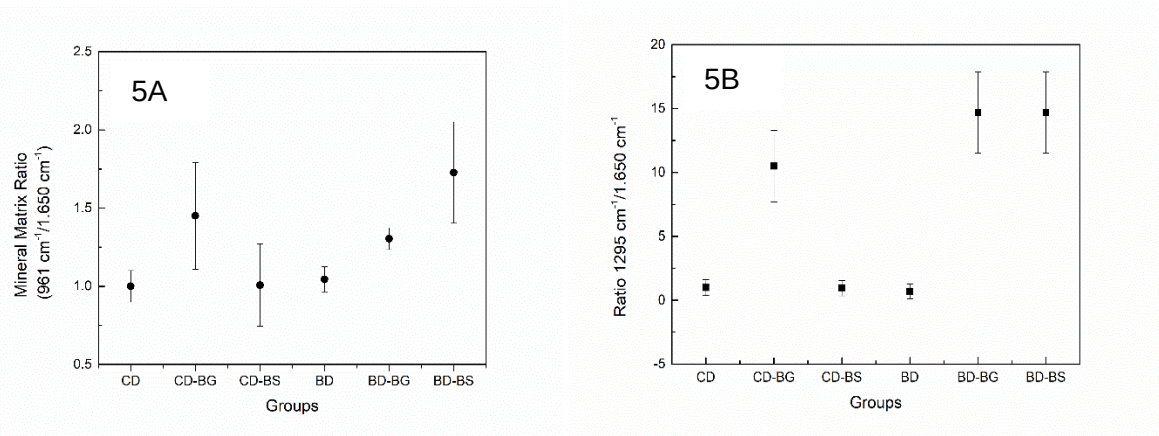

Figure 5. Mean values (DP) of fractured dentin MRS physic-chemical composition. The vertical axis represents the normalized intensity of the assessed rates and the horizontal axis presents the experimental groups. [A]: Mineral Matrix ratio (961 cm^{-1}/1.650 cm^{-1}). [B]: New MRS peak ratio (1295 cm^{-1}/1650 cm^{-1}).

4.5 DISCUSSION

This study showed higher dentin mineral matrix ratio when control and bleached dentin tissue were treated with both bioactive agents tested. Dental bleaching treatment significantly decreased dentin bond strength, however, the mineral formation induced by remineralization treatment increased not only bleached dentin but also control dentin bond strengths. Dentin fractured surface analysis proved that mineral deposition was improved due to remineralization agents' application and demonstrated that dentin tissue presented more chemical affinity with adhesive monomers after the remineralization approach. Although both biomaterials were efficient to promote dentin mineral precipitation, remineralization treatment with BG demonstrated better results since it increased both control and bleached dentin bond strength and improved those tissue chemical interactions with adhesive monomers. This would be the first study to test bleached dentin remineralization effects.

When compared to other bioactive materials, BG presents the highest bioactivity index (IB =

12.5) and is still considered the gold standard [16,26]. BS was developed aiming to combine the high bioactivity of BG with the good mechanical strength and toughness of glass-ceramics [26]. From the results of this study, it can be speculated that both BG and BS can be used to restore dentin mineral compounds after internal bleaching. The two tested agents were able to promote mineral deposition on demineralized and bleached dentin, increased bleached dentin bond strength and improved chemical interactions between this mineral tissue and the adhesive monomers. Nevertheless, only BG showed significant results for control dentin bond strength and physic-chemical interactions with the adhesive system.

Bioactive glasses and glass-ceramics have been used for dental tissue replacement and repair due to their ability to effectively bond to mineral tissues depositing hydroxycarbone apatite (HAp) within dentin demineralized collagen fibrils [20,25]. Remineralization process involves exchange of ions ($Si^{4+,}$ OH^{-}, Na^{+}, Ca^{2+}, PO_4^{3-}) from the glass silicate network with the surrounding body fluid. This process induces calcium phosphate (Ca/P) precipitation and its subsequent crystallization into HAp in the mineral tissue surface [16, 24, 29]. Glass dissolution during remineralization reaction depends on the presence of an aqueous medium [20,26]. Considering that fluoride is not a functional compound for biomineralization [14], artificial saliva was selected as aqueous medium to simulate the oral environment condition because its ionic composition is comparable to plasma's [30].

Remineralization of enamel tissue is well established in literature and is considered a simple process when compared to dentin remineralization [25]. Dentin consists of a heterogeneous and humid tissue, and its remineralization is known as a challenge process not only due to its mineral and organic composition, but also because of its tissue design, intrafibrillar orientation of minerals in the collagen network [17]. Differently from enamel remineralization process, the increase of mineral content at dentin surface after bioactive glass treatment is not enough to remineralize this tissue [31].

Improvement of dentin mechanical proprieties is dependent on deposition of extrafibrillar and particularly intrafibrillar mineral as consequence of biomimetic remineralization [31]. Mineral deposition surrounding the denuded collagen fibrils protects resin-dentin interface from water and enzymatic degradation, restores dentin mineral compounds and consequently improves this tissue dynamic behavior [32].

BT results led to rejection of the first null hypothesis of the study since control and bleached dentin remineralization treatment with BG and BS demonstrated an increase in dentin mineral contents. Control dentin specimens had their mineral content reduced after etching step, however, this loss was restored 1-day after remineralization treatments were done. The improvement of mineral matrix area ratio corroborates with the finds of an Attenuated Total Reflection Fourier Transform Infrared Spectroscopy study that proved BG remineralization effect on a partially demineralized dentin during the 7-day treatment [24]. This long period of time necessary to evidence mineral deposition might be explained by the use of a 0.5 ETDA acid solution instead of a phosphoric acid gel during demineralization step. Additionally, BS ability to induce HAp precipitation on dentin surface was also evaluated by a Fourier Transform Infrared Spectroscopy study and proved to occur 1 day after remineralization treatment [20].

It has been suggested that the application of bleaching agents in dentin tissue promotes changes in its surface morphology and structure [8,33]. The oxidative characteristic of bleaching products causes the denaturation of dentin proteins [5-7] and results in the dissolution of mineral dentin compounds [8]. Additionally, an acid-etch effect on this tissue is produced due to the acid pH of bleaching agents [34]. Modifications on dentin structure not only change the ratio between its organic and inorganic components [35] but also increase dentin collagen degradation by the activation of the MMPs [7] decreasing then dentin bonding efficacy [8,33]. Although remineralization treatment of

bleached and demineralized dentin with bioactive agents was never tested before, different studies [36,37] evaluated the use of BG and BS into desensitizing agents after enamel bleaching and concluded that the remineralization treatment reduced enamel mineral loss and preserved its surface integrity.

Dentin bleaching treatment resulted in a decrease of its mineral matrix ratio that was accentuated by the acid conditioning step. The application of both bioactive agents produced remineralization effect 1 day after the treatment was done, restoring dentin structural components that were lost because of acid etching. Nevertheless, this mineral formation was not enough to reestablish the components presented on dentin before the bleaching treatment. When nonremineralized bleached dentin was stored in AS, the mineral matrix ratio did not change and was kept constant during the 15 days of the bioactive analysis. It is known that remineralization reaction occurs only when the bioactive agents are in contact with body fluids [26]. Several liquids have been used to induce this reaction, such as water, simulated body fluid or AS [38]. In the present research AS was selected to simulate the oral environment, however, taking into account that saliva compounds are readily available to remineralize dental tissue, the use of AS can be listed as a limitation in a remineralization study. Although fluoride participates on the conventional tooth remineralization via the top-down approach, its action on biomineralizaiton was not proved [14], fact that corroborates with this research results since bioactive analysis showed that the remineralization effect was observed only in remineralized specimens.

The second null hypothesis of this study was rejected because dentin bond strength was reduced after internal bleaching and was improved as a consequence of remineralization treatment. These data are consistent with the findings of other studies that reported that internal bleaching significantly decreases dentin bond strength [39,40]. This reduction of dentin μTBS has been related to adhesive polymerization inhibition caused by oxygen and free-radicals resulted from peroxide degradation. Therefore, some studies reported that μTBS values were restored when those components are

eliminated and that was achieved by postponing the restorative procedures to 7-14 days post-bleaching treatment [41]. Nevertheless, the reduction of adhesive bonding with bleached dentin has also been associated with the oxidizing effect of peroxide [42] that promotes dissolution of dentin mineral compounds, dentin collagen denaturation and activates its organic enzymatic degradation by the MMPs [7]. BT results revealed that dentin mineral matrix decreases were not reversible even after 14 days of specimens' immersion in AS, corroborating with the μTBS findings that only remineralization treatments were able to improve bleached dentin bond strength values.

Dentin mechanical properties are directly affected by the degree and quality of its mineral content. Mineral deposition resulted from remineralization treatment preserves resin-dentin interfacial integrity from adhesive hydrolysis, through its dehydration mechanism, and protect denuded collagen of MPPs degradation [17]. Although the treatment with both bioactive agents increased μTBS values of CD and BD, this difference was not significant when BS was applied on CD. Microtensile studies revealed that dentin treatment with experimental primer or adhesives containing BG did not change μTBS values in the 24 h analysis but provided durable resin-dentin bonds when long term analyzes were done [23, 29, 43]. In the present study, a significant improvement on μTBS values were obtained 7 days after remineralization treatment, differently from when BG was introduced in the adhesive compounds. This fact can be an indicative that bioactive components are more reactive when available in a separate solution and not associated with other chemical molecules. Until the present moment, the use of BS to improve resin-dentin bonds was tested only by one study [21], that found higher μTBS values after 6 months when BS was used as a dentin remineralization solution after acid treatment approach of self-etching and etch-and-rinse adhesives.

Dentin remineralization not only changed the mineral composition (961 cm^{-1} / 1650 cm^{-1}) of dentin-adhesive interface, but also induced new physic-chemical interactions of dentin tissue with

adhesive monomers (1295 cm^{-1}/1650 cm^{-1}), thus, leading to the rejection of the third hypothesis of this study. PA of the fractured dentin beam proved the findings of BT since both analyses demonstrated the same mineral-matrix ratios patterns in control and bleached dentin before and after remineralization treatment. Micromechanical retention of etch-and-rinse adhesives is improved when methacrylate monomers and/or carboxylic esters chemically interact with etched inorganic dentin compounds [44]. Considering that MRS bands are fingerprints of specific specimen molecules, they provide chemical information, expressed by changes and/or appearance of new peaks [45]. Therefore, the new peaks observed in the remineralized groups of the present study (CD-BG, BD-BG and BD-BS) demonstrated that adhesive methacrylate monomers are more susceptible to interact with dentin when its mineral compounds are present in larger quantity. Additionally, the presence of those new peaks was more evident on the dentin beams of the groups that had higher μTBS values, proving their association with the improvement of dentin-adhesive bond.

4.6 CONCLUSION

Within the limitations of this study, it may be concluded that the two bioactive agents tested present high remineralization ability on bleached dentin surface. Remineralization treatment of bleached dentin promoted mineral deposition on its surface, improved its ability to chemically interact with adhesive monomers and consequently increased resin-dentin bond strength.

REFERENCES

1. Dietschi D, Duc O, Krejci I, Sadan A. Biomechanical considerations for the restoration of endodontically treated teeth: a systematic review of the literature, Part II (Evaluation of fatigue behavior, interfaces, and in vivo studies). Quintessence Int, 2008; 39:117-129.

2. Samran A, Al-Afandi M, Kadour JA, Kern M. Effect of ferrule location on the fracture

resistance of crowned mandibular premolars: An in vitro study. J Prosthet Dent, 2015; 114:86-91.

3. Santos-Filho PC, Veríssimo C, Raposo LH, Noritomi MecEng PY, Marcondes Martins LR. Influence of ferrule, post system, and length on stress distribution of weakened root-filled teeth. J Endod, 2014; 40:1874-1878.

4. Azevedo RA, Silva-Sousa YT, Souza-Gabriel AE, Messias DC, Alfredo E, Silva RG. Fracture resistance of teeth subjected to internal bleaching and restored with different procedures. Braz Dent J, 2011; 22:117-121.

5. Lado EA, Stanley HR, Weismann MI. Cervical resorption in bleached teeth. Oral Surg Oral Med Oral Pathol,1983; 55:78–80.

6. Ho S, Goerig AC. An in vitro comparison of different bleaching agents in the discoloured tooth. J Endod, 1989;15:106–111.

7. Toledano M, Yamauti M, Osorio E, Osorio R. Bleaching agents increase metalloproteinases-mediated collagen degradation in dentin. J Endod. 2011; 37:1668-1672.

8. Jiang T, Ma X, Wang Y, et al. Effects of hydrogen peroxide on human dentin structure. J Dent Res, 2007; 86:1040–1045.

9. Swift Jr. Effects of bleaching on tooth structure and restorations, part III: effects on dentin. J Esthet Restor Dent, 2008; 20:1-7.

10. Burrow MF, Satoh M, Tagami J. Dentin durability after three years using a dentin bonding agent with and without priming. Dent Mater, 1996;12:302-307.

11. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int, 1999; 30:501-507.

12. Hashimoto M. A review-micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions. J Biomed Mater Res B Appl Biomater, 2010; 92:268-280.

13. Wang Y, Spencer P. Hybridization efficiency of the adhesive/ dentin interface with wet bonding. J Dent Res, 2003; 82:141–145.

14. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res, 2011; 90:953-968.

15. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil- Mutluay A. State of the art etch-and-rinse adhesives. Dent Mater, 2011; 27:1-16.

16. Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J Bio Mater Research, 1971; 5: 117-141.

17. Kim J, Gu L, Breschi L, Tjäderhane L, Choi KK, Pashley DH, Tay FR. Implication of ethanol wet-bonding in hybrid layer remineralization. J Dent Res, 2010;89:575-580.

18. Ryou H, Niu LN, Dai L, Pucci CR, Arola DD, Pashley DH, Tay FR. Effect of biomimetic remineralization on the dynamic nanomechanical properties of dentin hybrid layers. J Dent Res, 2011; 90:1122-1128.

19. Zannoto ED, Ravagnani C, Peitl O, Panzeri H, Lara EH. Process and compositions for preparing particulate, bioactive or resorbable Biosilicate for use in the treatment of oral ailments. Patent: WO 2004/074199. 2004.

20. Tirapelli C, Panzeri H, Soares RG, Peitl O, Zanotto ED. A novel bioactive glass-ceramic for treating dentin hypersensitivity. Braz Oral Res, 2010; 24:381-387.

21. de Morais RC, Silveira RE, Chinelatti M, Geraldeli S, de Carvalho Panzeri Pires-de-Souza F. Bond strength of adhesive systems to sound and demineralized dentin treated with bioactive glass ceramic suspension. Clin Oral Investig. 2017 Nov 28. doi: 10.1007/s00784-017-2283-z.

22. Brackett MG, Li N, Brackett WW, Sword RJ, Qi YP, Niu LN, Pucci CR, Dib A, Pashley DH,

Tay FR. The critical barrier to progress in dentine bonding with the etch-and-rinse technique. J Dent, 2011; 39:238-248.

23. Profeta AC, Mannocci F, Foxton R, Watson TF, Feitosa VP, De Carlo B, Mongiorgi R, Valdré G, Sauro S. Experimental etch-and-rinse adhesives doped with bioactive calcium silicate-based micro-fillers to generate therapeutic resin-dentin interfaces. Dent Mater, 2013; 29:729-741.

24. Wang Z, Jiang T, Sauro S, Wang Y, Thompson I, Watson TF, Sa Y, Xing W, Shen Y, Haapasalo M. Dentine remineralization induced by two bioactive glasses developed for air abrasion purposes. J Dent, 2011; 39:746-756.

25. Niu L, Zhang W, Pashley DH, Breschi L, Mao J, Chen J, Tay FR. Biomimetic remineralization of dentin. Dent Mater, 2014; 31:77-96.

26. Crovace MC, Souza MT, Chinaglia CR, Peitl O, Zanotto ED. Biosilicate® — A multipurpose, highly bioactive glass-ceramic. In vitro, in vivo and clinical trials. J Non Cryst Solids, 2016:90-110.

27. Colthup NB, Daly LH, Wiberley SE. Introduction to Infrared and Raman Spectroscopy (Third Edition). 1990. ISBN: 978-0-12-182554-6.

28. Xu J, Stangel I, Butler IS, Gilson DFR. An FT-Raman Spectroscopic Investigation of Dentin and Collagen Surfaces Modified by 2-Hydroxyethylmethacrylate. J Dent Res,1997; 76: 596-601.

29. Sauro S, Osorio R, Watson TF, Toledano M. Therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface. J Mater Sci Mater Med, 2012; 23:1521-1532.

30. Ten Cate's oral histology: development, structure, and function. Nanci, Antonio; Ten Cate, A R (Arnold Richard) Oral histology. 8th ed. / Antonio Nanci. St. Louis, Mo.: Elsevier, c2013.

31. Bertassoni LE, Habelitz S, Kinney JH, Marshall SJ, Marshall GW, Biomechanical perspective on the remineralization of dentin. Caries Res; 2009, 43:70–77.

32. Tay FR, Pashley DH. Biomimetic remineralization of resin-bonded acid-etched dentin. J Dent Res, 2009; 88:719-724.

33. Uysal T, Er O, Sagsen B, Ustdal A, Akdogan G. Can intracoronally bleached teeth be bonded safely? Am J Orthod Dentofacial Orthop, 2009; 136:689-694.

34. Price RBT, Sedarous M, Hiltz GS. The pH of tooth whitening products. J Can Dent Assoc 2000; 66:421–6.

35. Plotino G, Buono L, Grande NM, Pameijer CH, Somma F. Nonvital tooth bleaching: a review of the literature and clinical procedures. J Endod, 2008; 34:394-407.

36. Deng M, Wen HL, Dong XL, Li F, Xu X, Li H, Li JY, Zhou XD. Effects of 45S5 bioglass on surface properties of dental enamel subjected to 35% hydrogen peroxide. Int J Oral Sci, 2013; 5:103-10.

37. Pintado-Palomino K, Tirapelli C. The effect of home-use and in-office bleaching treatments combined with experimental desensitizing agents on enamel and dentin. Eur J Dent, 2015; 9:66-73.

38. Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials, 2006; 27:2907–2915.

39. Demarco FF, Turbino M L, Jorge AG, Matson E. Influence of bleaching on dentin bond strength. Am J of Dent, 1998;11:78–82.

40. Elkhatib H, Nakajima M, Hiraishi N, Kitasako Y, Tagami J, Nomura S. Surface pH and bond strength of a self-etching primer/adhesive system to intracoronal dentin after application of hydrogen peroxide bleach with sodium perborate. Oper Dent. 2003; 28:591-597

41. Cavalli V, Sebold M, Shinohara MS, Pereira PNR, Giannini M. Dentin bond strength and nanoleakage of the adhesive interface after intracoronal bleaching. Microsc Res Tech. 2018; 2018 Jan 18. doi: 10.1002/jemt.22995.

42. Tam LE, Noroozi A. Effects of direct and indirect bleach on dentin fracture toughness. J Dent Res, 2007;86:1193-1197.

43. Profeta AC. Preparation and properties of calcium-silicate filled resins for dental restoration. Part II: Micro-mechanical behaviour to primed mineral-depleted dentine. Acta Odontol Scand, 2014;72:607-617.

44. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond-strength tests and clinical outcomes. Dent Mater, 2010; 26:e100-121.

45. Zhang Y, Wang Y. Effect of application mode on interfacial morphology and chemistry between dentine and self-etch adhesives. J Dent, 2013; 41:231-40.

5. ARTIGO 4:

Accelerated fatigue of endodontically-treated internally-bleached maxillary incisors restored with and without post

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5.1 ABSTRACT

Purpose: To assess and compare the fatigue resistance, load-to-failure, and failure mode of endodontically-treated incisors (ETIs) submitted to the walking bleach technique and restored with three different techniques. **Methods:** Maxillary incisors were endodontically-treated, then a glass-ionomer cement was placed over the orifice. The bleaching procedure was performed with 3% hydrogen-peroxide mixed with sodium-perborate for three consecutive 1-week application. After, all the teeth were restored according to the specific procedures for three groups: 1) glass-ionomer cement base replacing dentin and covered with a composite resin restoration (GI), 2) short-fiber reinforced composite resin replacing dentin and covered with a composite resin restoration and (EX), 3) composite-resin restoration over a luted fiberglass post (FP). Masticatory forces were simulated in an artificial mouth using a closed-loop electro-dynamic system. The force was applied at a palatal angle of 30º with the flat surface of the disc contacting minimum three-fourths of the incisal edge. Samples were loaded until fracture or to a maximum of 76,500 cycles. **Results:** Only one specimen survived 76,500 load cycles, from group GI. All three groups presented with a major amount of nonrestorable failures. The GI-group seemed to present a more favorable outcome with only 69% of nonrestorable failures and the highest amount of repairable fractures compared to EX (79%) and FP (86%). **Conclusions:** Keeping the glass-ionomer base and simply replacing the surface of the defect with composite-resin will not affect the mechanical behavior of the restored tooth when compared to the more complicated alternatives such as the placement of a post.

Clinical significance: Considering the limitations of this in vitro study, when restoring intact internally-bleached ETIs, it can be concluded that there is no difference in accelerated fatigue strength between the three conservative restorative approaches. The simple use of a glass ionomer base as a dentin replacement to be covered with 2 mm of restorative composite resin seems to be the best choice compared to the use of fiber posts or other reinforced materials.

Keywords: Fatigue resistance, Incisors, Walking bleach technique, Fiber post, Glass ionomer cement, Fiber-reinforced composite resin.

5.2 INTRODUCTION

Loss of tooth vitality due to dental trauma is the main reason for the endodontic treatment of intact incisors [1]. As a result of the traumatic injury, pulpal hemorrhage and/or pulp necrosis can promote tooth intrinsic discoloration. [2]. In view of the biomechanical integrity of nonvital traumatized teeth, dental bleaching is the most conservative alternative to assure the functional, mechanical and esthetic recovery of the tooth. [1, 3, 4] Although nonvital bleaching could be done with different techniques, such as the walking bleach technique, inside/outside bleaching and in-office bleaching, literature shows that the greatest bleaching effect is obtained when the bleaching agent is applied for a long period of time. [5] Therefore, the walking bleach technique is considered the best option for the esthetic treatment of traumatized discolored nonvital teeth. [1] In this technique, the mixture of sodium perborate and 3% to 30% hydrogen peroxide creates an oxidant paste that is placed and sealed in the pulp chamber for repeated periods of one week to 10 days. The bleaching effect is weekly monitored, and this process is repeated until the desired color has been obtained.

Restorative procedures of endodontically treated teeth including endodontically-treated incisors

(ETIs) have been recognized as one of the greatest challenge of clinical dentistry because of the increased risk of biomechanical failure. [6,7] Endodontic access and preparation can result in significant loss of dental tissue, structural weakness, and consequently, loss of restoration retention. [8] In addition, intracoronal bleaching can contribute to restoration failures, in turn making the tooth itself more susceptible to fracture. [9] Bleaching agents can interact chemically with the morphological structure of dentin, [10] increasing it porosity, modifying its demineralization pattern [11,12] and also reducing its adhesion to restorative materials. [13,14] Therefore, preserving intact coronal and radicular tooth structure and maintaining cervical tissue are considered crucial to optimize the biomechanical behavior of the ETIs. [15,16]

Because discolored ETIs often present minimal loss of tooth structure, composite resin restoration with or without fiber post reinforcement are the usual approaches that have been used and tested in order to restore them after the bleaching treatment. [1, 9, 10] The improvement of composite resin mechanical properties associated with the development of adhesive systems has allowed the direct bonded restoration of ETIs to reach values of fracture resistance and stiffness similar to that of unaltered teeth. [15,17] The use of fiber post to restore relatively intact ETIs is questionable. Existing literature proved that they did not improve the fracture resistance of teeth, presenting similar failure mode and performance as ETI restored only with composite resin. [9, 10,] The only benefit of a fiber post has been related to the retention of the filling material within the remaining tooth structure. [18] However, it is still a controversial topic whether sound dentin should be removed to prepare the root canal to receive a post in order to increase stability of the restoration foundation. In more recent studies, fiber posts have demonstrated a systematic association to catastrophic failure (root fracture). [19,20]

The most conservative restorative approach should be used for the preservation of enamel and dentin tissues and to improve the long-term success of the restoration [5]. Therefore, the aim of this

study is to assess and compare the fatigue resistance, load-to-failure, failure mode of ETIs submitted to the walking bleach technique and restored with three different techniques. The ETI restorative protocols to be tested are 1) glass ionomer cement base replacing dentin and covered with a composite resin restoration, 2) short-fiber reinforced composite resin replacing dentin and covered with a composite resin restoration and 3) composite resin restoration over a luted fiberglass post. The null-hypotheses considered are that 1) no significant difference would be found in accelerated fatigue resistance of the ETIs among the restorative techniques and materials used; 2) there would be no difference in the fracture patterns of the ETIs restored with the three techniques used in this study.

5.3 MATERIALS AND METHODS

5.3.1 Specimens preparation

Forty-two extracted intact human maxillary incisors were carefully selected and stored in thymol-saturated solution (Thymol, Aqua Solutions Inc., Deer Park, Texas, USA) after the approval from the Ethical Committee of the University of Southern California Review Board.

A standard lingual access (using slow speed round and GK269 burs to smoothen the internal walls) was prepared to simulate root canal treatment in each tooth. Patency was achieved using #10k files (Dentsply Tulsa Dental, Johnson City, TN, USA). Coronal flare was created using Gates #2 (Dentsply Tulsa Dental, Johnson City, TN, USA) and canals were chemo-mechanically debrided using 04 rotary files (Protaper Niti Rotary, Dentsply, Tulsa Dental, Johnson City, TN, USA) and irrigated with 5,25% sodium hypochlorite (Clorox, Oakland, CA, USA) to within 3-mm of the apex. A final rinse with H_2O_2 was be performed and canals were dried using paper points (Kerr Endodontics Absorbent Points; Kerr, Orange, CA, USA). Then, warm vertical obturation of the canals was

performed using gutta-percha to the orifice level and condensed.

A glass ionomer (GI) cement 2-mm-thick cervical barrier was placed over the orifice (at the level of the cementoenamel junction – Ketac Molar; 3M ESPE, St.Paul, MN, USA). The bleaching procedure was then performed with 3% hydrogen peroxide (Essential Oxygen; San Rafael, CA, USA) mixed with sodium perborate (Sultan Healthcare, New York City, NY, USA) for three consecutive 1-week application. During that time, the teeth were temporary sealed with a cotton pellet and with GI cement (Ketac Molar; 3M ESPE, St.Paul, MN, USA) and stored in distilled water at room temperature. After the three bleaching sessions, the bleaching paste was removed by aspiration and irrigation with 1% sodium hypochlorite (Clorox, Oakland, CA, USA), rinsed with water and the pulp chamber was completely filled with GI cement (Ketac Molar; 3M-ESPE, St.Paul, MN, USA) and stored for 4 weeks in distilled water at room temperature. After this delay, the teeth were restored according to the specific procedures for each group and inserted in a special positioning device with acrylic resin (Palapress vario; Heraeus Kulzer, Armonk, NY, USA) embedding the root up to 2mm below the cementoenamel junction (CEJ).

5.3.2 Restorative procedures

In order to evenly distribute the teeth according to their size, all specimens (N=42) were separated in “small” and “large” sizes and subsequently re-assigned randomly to one of the three experimental groups (n=14).

In group GI (glass ionomer), a spherical diamond bur (Brasseler, Savannah, GA, USA) was used to remove the GI base on a depth of 2-mm and the enamel margin was beveled. The entire lingual enamel was etched with 35% phosphoric acid (Ultra-Etch, Ultradent, UT, USA) for 30 seconds followed abundant rising and drying. A three-step etch-and-rinse bonding agent (Optibond FL; Kerr,

Orange, CA, USA) was applied and polymerized for 20 s at 1,000mW/cm^2 (VALO Curing Light, Ultradent Products, Inc., South Jordan, UT, USA). Composite resin (Inspiro Direct, shade SW, Edelweiss DR, Zug, Switzerland) was then used to fill the residual space and polymerized for 20 s (VALO Curing Light) followed by an additional 20 s light polymerization under an air-blocking barrier (K-Y Jelly; Personal Products Company, Skillman, NJ, USA).

In group EX, a short fiber-reinforced composite resin (EverX Posterior, GC Europe, Leuven, Belgium) was used to replace the GI base material (but the GI barrier was maintained). After removal of the GI base, exposed dentin and enamel were etched with 35% phosphoric acid (Ultra-Etch, 10 seconds for dentin and 30 seconds for enamel), abundantly rinsed and dried. The same three-step etch-and-rinse bonding agent (Optibond FL) was applied and polymerized. The pulp chamber was filled with a single increment of short fiber-reinforced composite resin (EverX Posterior). Composite resin (Inspiro Direct, shade SW, Edelweiss DR, Zug, Switzerland) was used for the last 1mm-thick increment and polymerized for 20 s (VALO Curing Light) followed by an additional 20 s light polymerization under an air-blocking barrier (K-Y Jelly).

In group FP (fiber post), all GI material was removed and an intra-radicular fiberglass posts (ParaPost Fiber Lux, no. 5, 1.25-mm diameter, Coltène Whaledent, Altstätten, Switzerland) was placed. The post space WAS prepared by retaining at least 5 mm of root filling at the apical level using ParaPost drills specifically designed for the ParaPost Fiber Lux (no. 5, 1.25-mm diameter, Coltène Whaledent, Altstätten, Switzerland). Using a cutting disc, the head of the post was removed to fit the pulp chamber space and terminate 2mm above the interdental level of the cementoenamel junction. Prior to the luting procedure, the posts were cleaned with alcohol and air-dried. First, the enamel was etched with 35% phosphoric acid for 30 seconds, rinsed and dried. All dentin walls were dried (including canal with paper points) and treated with a non-rinse conditioner (ParaBond Non-Rinse Conditioner, Coltène

Whaledent, Altstätten, Switzerland) for 30 seconds followed by drying with paper points and brief air-drying. Adhesive resin (ParaBond Adhesive A & B, Coltène Whaledent, Altstätten, Switzerland) was then applied for 30 seconds followed by drying with paper points, brief air-drying and filling with cement (ParaCore Dentin, Coltène Whaledent, Altstätten, Switzerland). The post was then inserted and cement excesses were cleaned, leaving a uniform space around the coronal portion of the post. The cement system was light cured for 40 seconds (VALO Curing Light, Ultradent Products, Inc, South Jordan, UT, USA). Optibond FL adhesive resin was applied to the enamel and polymerized and composite resin (Inspiro Direct, shade SW, Edelweiss DR, Zug, Switzerland) was used to fill the residual space and polymerized for 20 s (VALO Curing Light, Ultradent Products, Inc, South Jordan, UT, USA) followed by an additional 20 s light polymerization under an air-blocking barrier (K-Y Jelly, Johnson & Johnson, USA).

5.3.3 Accelerated fatigue test

Masticatory forces were simulated in an artificial mouth using a closed-loop electro-dynamic system (Acumen III; MTS Systems, Eden Prairie, MN, USA). The chewing cycle was simulated by an isometric load applied through a flat antagonist 10mm-diameter composite resin disc (MZ100; 3M-ESPE, St. Paul, MN, USA). The force was applied at a palatal angle of 30º with the flat surface of the disc contacting minimum three-fourths of the incisal edge (Figure 1). The load chamber was filled with distilled water to submerge the sample during testing. A cyclic load (sine wave) was applied at a frequency of 5 Hz, starting with a load of 100 N (warm-up of 5000 cycles), followed incremental increase of 25N each 1,700 cycles until a load of 1,200N was reached (maximum 45 increments). Samples were loaded until fracture or to a maximum of 76,500 cycles.

Figure 1. Specimen in the load device. Cyclic isometric loading was applied to the incisal edge at an angle of 30°.

All fatigue tests were monitored using a macro video camera and recorded continuously in order to determine the crack propagation mode. The numbers of endured cycles, load to failure, and failure mode of each specimen were recorded. After the test, each sample was evaluated by transillumination (Microlux, Addent, Danbury, CT, USA) and an optical microscope (Leica MZ 125, Leica Microsystems, Wetzlar, Germany) at 10:1 magnification. A visual distinction was made among three fracture modes, considering the reparability of the tooth: catastrophic, that is, cohesive root fracture that would require tooth extraction (below level of the mounting resin); possibly reparable, that is, cohesive/adhesive failure involving the root but no deeper that the level of the mounting resin; or reparable fracture, that is, cohesive or adhesive failure of coronal structure only.

The fatigue resistance of the groups was compared using the Kaplan-Meier survival table (for number of cycles survived) using the Log-rank test at a significance level of 0.05.

5.4 RESULTS

Only one specimen survived 76,500 load cycles, from group GI. The mean fracture cycles was calculated from the fractured specimens: GI 40.653X ± 12.562, EX 41.621X ± 13,701 and FP 35,967X ± 10,448 (Figure 2), and corresponding loads of 675N, 700N and 625N respectively. The Kaplan-Meier survival graphs for all groups are displayed in Figure 3 and the analysis did not yield significant outcomes (*P*=.35).

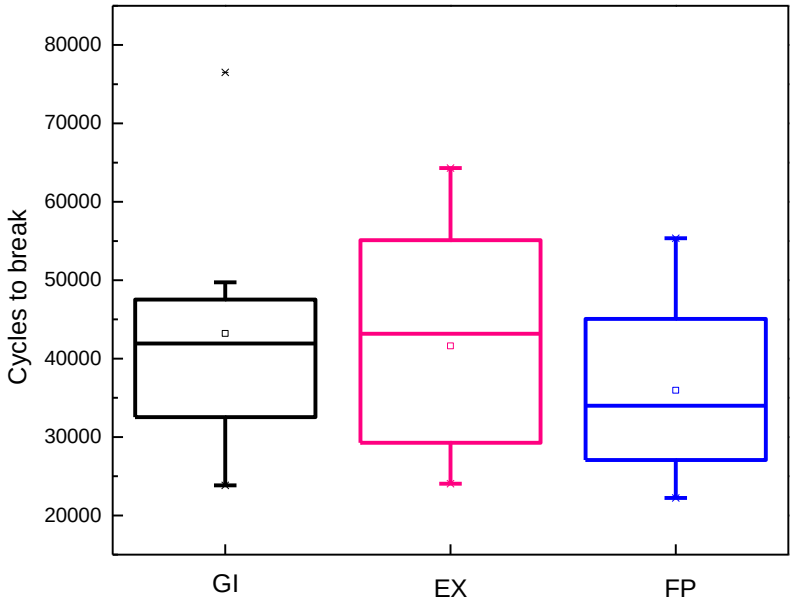

Figure 2. Box plot of number of survived load cycles for groups GI, EX and FP.

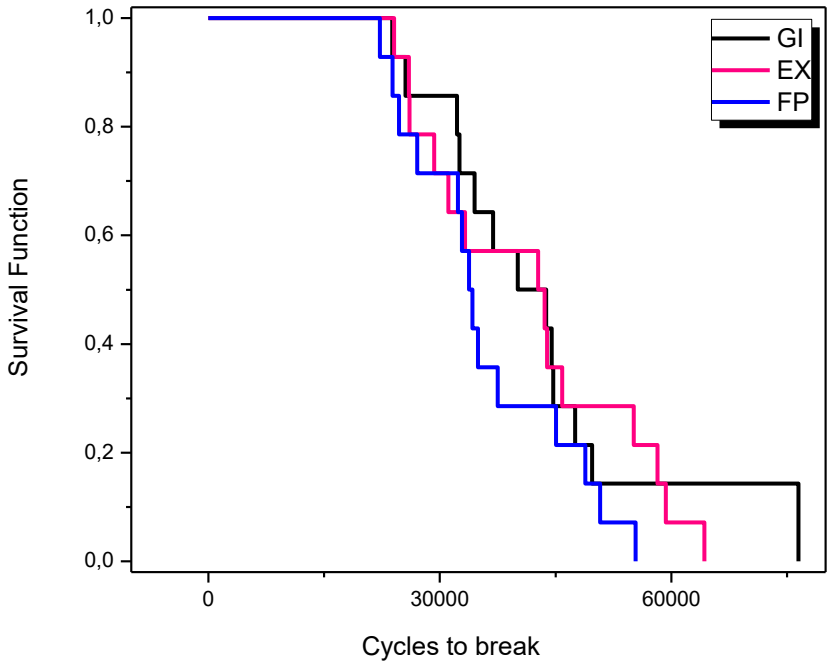

Figure 3. Kaplan-Meier survival graphs for groups GI, EX and FP.

Failure type is illustrated in Figure 4 and its distribution is presented in Figure 5. All three groups presented with a major amount of nonrestorable failures. The GI group seemed to present a more favorable outcome with only 69% of nonrestorable failures and the highest amount of repairable fractures compared to EX (79%) and FP (86%).

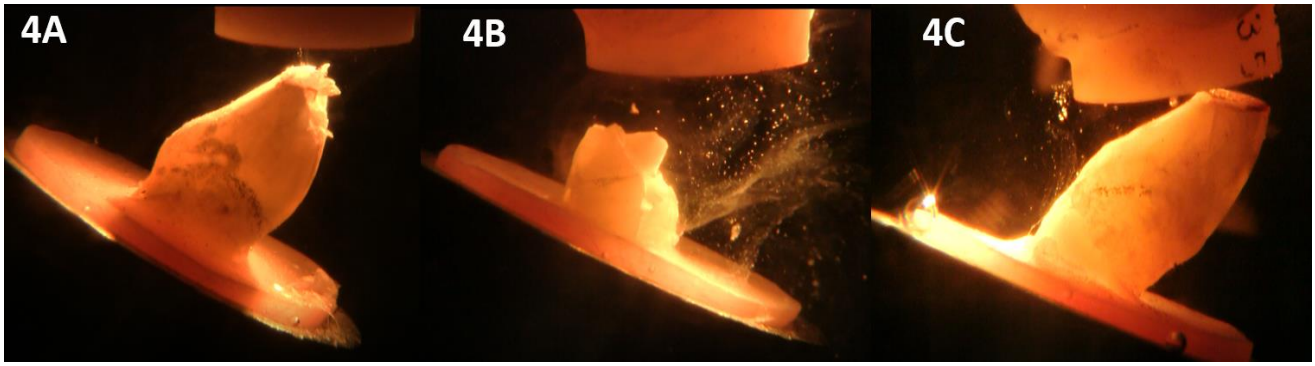

Figure 4. Specimens failure mode classification: (4A) ‘‘reparable’’ fracture (cohesive or cohesive/ adhesive fracture of tooth structure or restoration), (4B) ‘‘possibly reparable’’ (cohesive/adhesive failure with fragment and minor damage, chip, or crack, of underlying tooth structure) or (4C) ‘‘catastrophic’’ (tooth/root fracture that would require tooth extraction).

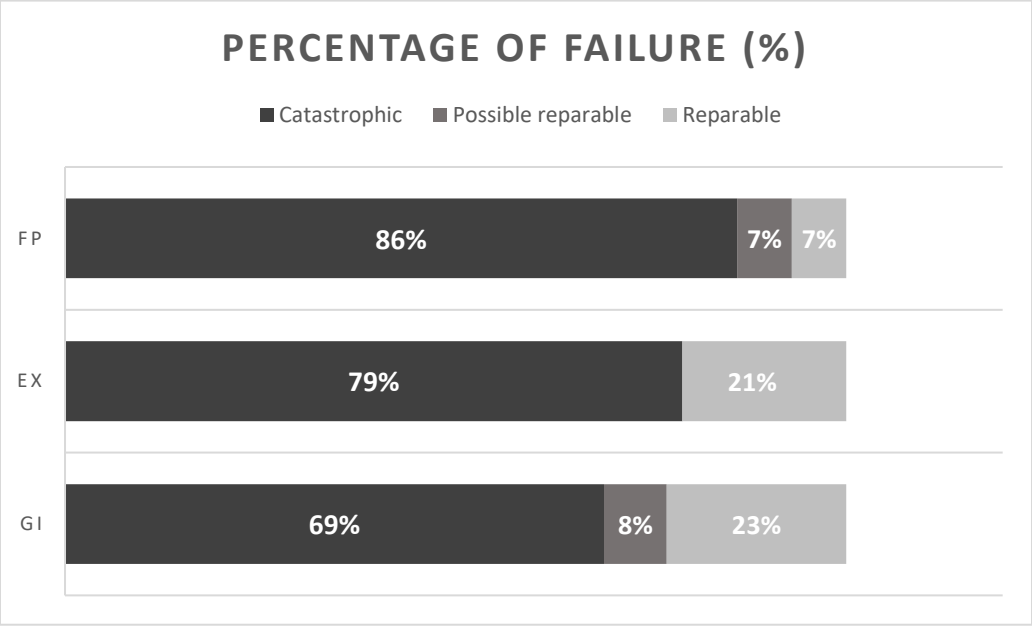

Figure 5. Percentage of specimens per group for each fracture mode.

5.5 DISCUSSION

This study evaluated the effect various restorative approaches on the survival of relatively intact internally-bleached ETIs. The first null hypothesis can be accepted because there was no significant difference on survival rates when comparing the three treatment modalities. The second hypothesis can be partially rejected because more favorable outcome was found with group GI compared to EX and FP.

One of the major challenge of this in vitro study is the limited availability and extreme variability of extracted human teeth (age, size, and shape). Using standardized specimens is of paramount importance and allows minimizing confounding variables and gaining sensitivity in testing. Nevertheless, many confounding variables could be eliminated by having absolute control not only on the restorative steps (absence of existing restorations, standardized size of endodontic preparation etc.), but also by normalizing the load configuration and load protocol. The accelerated fatigue test represents a compromise between the conventional load-to-failure protocol and the time-consuming low-load fatigue test. The cyclic loads were applied in increments, increasing the load in 25N steps up to 1200 N at a frequency of 5 Hz. In order to prevent localized and intense point loads and unrealistic surface damage, a flat composite resin surface was used as an antagonist. [19,20] In the anterior region, the isometric bite force ranges between 243 N (women) and 287 N (men), [21] even though higher forces may be encountered in bruxism, trauma (high extrinsic loads), or intrinsic masticatory accidents, higher forces were required to break specimens in the present study. The first failures occurred at 425N. On the other hand, one specimen in group GI survived until 1,200N. Average failure loads ranged between 625 and 700N.

In view of the present results, the use of glass-fiber posts can be questioned. No significant advantage could be observed. Fiber posts have already proven their lack of influence according to

previous studies on severely broken-down ETI with or without ferrule. [19,20]. The results of this investigation confirms that this conclusion can be extended to relatively intact ETIs. This is a significant finding because the presence of intact coronal structure usually renders post insertion more difficult and may lead some clinicians to remove coronal tissue in order to place the post. However, maintaining intact coronal tissues is paramount in the treatment of ETIs. This is proven by the general consensus and abundant data about the advantage of the ferrule effect. In this investigation, because of the moderately conservative preparation approach, a "natural" ferrule effect was obtained by the substantial amount of circumferential dentin and enamel. This in turn may have "masked" the effect of the restorative approach.

Placing a post is not without risks, in particular in the presence of limited coronal access. If the three approached presented in this worked were ranked according to simplicity, efficiency, minimal risks (including failure mode) and cost, group GI would certainly be the most favorable. It does not require removal of the existing barrier. The glass ionomer base was used as a dentin replacement and covered with a 2mm-thick composite resin over the entire lingual surface as an additive reinforcement approach. This technique was used in order to create some kind of palatal reinforcement [21] and compensate for the flexible behavior of the ETI. This is demonstrated in Figure 4A displaying a specimen with an intact composite resin lingual surface that forced the initial crack to start from a very coronal position, almost at the incisal edge.

Recently, so called bulk-fill composite resins were introduced amongst which short-fiber reinforced restorative composite resins. Physical and mechanical properties of materials show promising results. [12, 13, 14, 20] However, maybe due to the very limited amount of material used in this study and also due to the fact that EverX was located in the center of the tooth (near the neutral zone), the effect could not be detected. In fact, when anterior teeth are subjected to bending from a

lingual load, the tooth is separated in two distinct areas: the palatal half is subjected to tensile stresses and the labial half to compressive ones. The center of the tooth (near the pulp) is located at the transition between those two areas and exhibits neutrality (stresses near to “0”). [22] This does not apply only to EverX but also the other groups and might have been the main reason for the absence of effect of the restorative approach.

From a clinical perspective, a 3-to-4-week delay is generally required before applying adhesive restorative procedures to a bleached tooth, either externally or internally bleached. [23] This not only permits the recovery of the bond strength but also allows for color stabilization before the restorative treatment. Hence, the tooth need to be restored temporarily during that time. Glass ionomer is a good choice because it is easy to place, it will provide a decent seal, appropriate color and opacity. The present study also demonstrates that keeping this glass ionomer base and simply replacing the surface of the defect with composite resin will not affect the mechanical behavior of the restored tooth when compared to the more complicated alternatives such as the placement of a post. Further studies will have to determine whether the choice of the restoration material and technique will affect the color stability of the bleached tooth. Differences may be expected due to the very different optical behavior of glass ionomer compared to fiber reinforced composites.

REFERENCES

1. Magne P, Belser U. Bonded porcelain restorations in the anterior dentition: a biomimetic approach. Quintessence Publishing Company, 2002.
2. Arens D. The role of bleaching in esthetics. Dent Clin North Am. 1989 Apr; 33(2): 319-336.
3. Arı, H, & Üngör, M. In vitro comparison of different types of sodium perborate used for intracoronal bleaching of discoloured teeth. International Endodontic Journal. 2002; 35(5), 433-436.

4. Amato M, Scaravilli MS, Farella M, Riccitiello F. Bleaching teeth treated endodontically: long-term evaluation of a case series. JOE. 2006 Apr; 32(4): 376-378.

5. Dietschi, D. Nonvital bleaching: general considerations and report of two failure cases. Eur J Esthet Dent. 2006; 1(1): 52-61.

6. Juloski J, Radovic I, Goracci C, Vulicevic ZR, Ferrari M. Ferrule effect: a literature review. J Endod. 2012 Jan; 38(1):11-19.

7. Samran A, Al-Afandi M, Kadour JA, Kern M. Effect of ferrule location on the fracture resistance of crowned mandibular premolars: An in vitro study. J Prosthet Dent. 2015 Jul;114(1):86-91.

8. Santos-Filho PC, Veríssimo C, Raposo LH, Noritomi MecEng PY, Marcondes Martins LR. Influence of ferrule, post system, and length on stress distribution of weakened root-filled teeth. J Endod. 2014 Nov; 40(11): 1874-1878.

9. Roberto AR, Sousa-Neto MD, Viapiana R, Giovani AR, Souza Filho CB, Paulino SM, Silva-Sousa YT. Effect of different restorative procedures on the fracture resistance of teeth submitted to internal bleaching. Braz Oral Res. 2012 Jan-Feb; 26(1): 77-82.

10. Azevedo RA, Silva-Sousa YT, Souza-Gabriel AE, Messias DC, Alfredo E, Silva RG. Fracture resistance of teeth subjected to internal bleaching and restored with different procedures. Braz Dent J. 2011; 22(2): 117-21.

11. Rodrigues JA, Marchi GM, Ambrosano GM, Heymann HO, Pimenta LA. Microhardness evaluation of in situ vital bleaching on human dental enamel using a novel study. Dent Mat. 2005 Nov; 21(11):1059-1067.

12. Soldani P, Amaral CM, Rodrigues JA. Microhardness evaluation of in situ vital bleaching and thickening agents on human dental enamel. Int J Periodontics Restorative Dent. 2010 Apr; 30(2): 203-211.

13. Swift Jr EJ. Critical Effects of bleaching on tooth structure and restorations, part II: enamel bonding. J Esthet Restor Dent. 2008a; 20(1): 68-73.

14. Swift Jr. Effects of bleaching on tooth structure and restorations, part III: effects on dentin. J Esthet Restor Dent. 2008b; 20(2):1-7.

15. Dietschi D, Duc O, Krejci I, Sadan A. Biomechanical considerations for the restoration of endodontically treated teeth: a systematic review of the literature--Part 1. Composition and micro- and macrostructure alterations. Quintessence Int. 2007 Oct; 38(9): 733-743.

16. Dietschi D, Duc O, Krejci I, Sadan A. Biomechanical considerations for the restoration of endodontically treated teeth: a systematic review of the literature, Part II (Evaluation of fatigue behavior, interfaces, and in vivo studies). Quintessence Int. 2008 Feb; 39(2):117-129.

17. Bonfante G, Kaizer OB, Pegoraro LF, Valle AL. Fracture resistance and failure pattern of teeth submitted to internal bleaching with 37% carbamide peroxide, with application of different restorative procedures. J Appl Oral Sci. 2006 Aug; 14(4): 247-52.

18. Fokkinga WA, Kreulen CM, Le Bell-Ronnlof AM, Lassila LV, Vallittu PK, Creugers NH. In vitro fracture behavior of maxillary premolars with metal crowns and several post-and-core systems. J Oral Sci 2006; 114:250-256.

19. Magne P, Lazari PC, Carvalho MA, Johnson T, Del Bel Cury AA. Ferrule-effect dominates over use of a fiber post when restoring endodontically treated incisors: An in vitro study. Oper Dent. 2017 Jul/Aug; 42(4): 396-406.

20. Lazari PC, de Carvalho MA, Del Bel Cury AA, Magne P. Survival of extensively damaged endodontically treated incisors restored with different types of posts-and-core foundation restoration material. J Prosthet Dent. 2017 Sep 16.

21. Magne P, Douglas WH. Additive contour of porcelain veneers: a key element in enamel preservation, adhesion, and esthetics for aging dentition. J Adhes Dent. 1999 Spring; 1(1): 81-92.

22. Magne P, Versluis A, Douglas WH. Rationalization of incisor shape: experimental-numerical analysis. J Prosthet Dent. 1999 Mar; 81(3): 345-55.

23. Cavalli V, Reis AF, Giannini M, Ambrosano GM. The effect of elapsed time following bleaching on enamel bond strength of resin composite. Oper Dent. 2001 Nov-Dec; 26(6): 597-602.

6. CONCLUSÕES

A utilização do sistema adesivo pré-aquecido aumentou a resistência de união dentinária no tempo inicial, resultado que se manteve constante após seis meses de armazenamento. O tratamento remineralizador demonstrou ser eficaz para promover o aumento da composição mineral do tecido dentinário, resultou em modificações estruturais da interface adesiva proporcionando melhora na resistência de união entre o sistema adesivo e os tecidos dentinários sadios e clareados. Além disto, a substituição do tecido dentinário pelo cimento de ionômero de vidro na restauração de incisivos com tratamento endodôntico submetidos ao clareamento interno demonstrou ser uma alternativa conservadora e satisfatória do ponto de vista mecânico. Desta forma, considerando os achados dos presentes estudos laboratoriais, é possível concluir que o uso de estratégias biomiméticas durante o procedimento restaurador melhora significativamente o desempenho das restaurações adesivas diretas.