UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

DAYLANA PACHECO DA SILVA

CONCENTRAÇÃO INTRAPULPAR DE PERÓXIDO DE HIDROGÊNIO EM DENTES RESTAURADOS COM COMPÓSITOS BIOATIVOS CONVENCIONAL E BULK-FILL

INTRAPULPAL CONCENTRATION OF HYDROGEN PEROXIDE OF TEETH RESTORED WITH CONVENTIONAL AND BULK-FILL BIOACTIVE COMPOSITES

PIRACICABA

2019

.

DAYLANA PACHECO DA SILVA

CONCENTRAÇÃO INTRAPULPAR DE PERÓXIDO DE HIDROGÊNIO EM DENTES RESTAURADOS COM COMPÓSITOS BIOATIVOS CONVENCIONAL E BULK-FILL

INTRAPULPAL CONCENTRATION OF HYDROGEN PEROXIDE OF TEETH RESTORED WITH CONVENTIONAL AND BULK-FILL BIOACTIVE COMPOSITES

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Clínica Odontológica, na Área de concentração em Dentística.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Clinical Dentistry, in the Restorative Dentistry Area.

Orientadora: Prof.ª Dr.ª Vanessa Cavalli Gobbo

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA DAYLANA PACHECO DA SILVA E ORIENTADA PELA PROF. DRA. VANESSA CAVALLI GOBBO.

PIRACICABA

2019

.

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

Si38c

Silva, Daylana Pacheco da, 1992-
Concentração intrapulpar de peróxido de hidrogênio em dentes restaurados com compósitos bioativos convencional e bulk-fill / Daylana Pacheco da Silva. – Piracicaba, SP : [s.n.], 2019.

Orientador: Vanessa Cavalli Gobbo.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Dentes - Clareamento. 2. Peróxido de hidrogênio. 3. Restauração dentária permanente. I. Cavalli, Vanessa, 1977-. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Intrapulpal concentration of hydrogen peroxide of teeth restored with conventional and bulk-fill bioactive composites
Palavras-chave em inglês:
Teeth - Bleaching
Hydrogen peroxide
Dental restoration, permanent
Área de concentração: Dentística
Titulação: Doutora em Clínica Odontológica
Banca examinadora:
Vanessa Cavalli Gobbo [Orientador]
Flávia Lucisano Botelho do Amaral
Fabiana Mantovani Gomes França
Flavio Henrique Baggio Aguiar
Valentim Adelino Ricardo Barão
Data de defesa: 28-05-2019
Programa de Pós-Graduação: Clínica Odontológica

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-1690-6451
- Currículo Lattes do autor: http://lattes.cnpq.br/8713331142682810

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 28 de Maio de 2019, considerou a candidata DAYLANA PACHECO DA SILVA aprovada.

PROFª. DRª. VANESSA CAVALLI GOBBO

PROFª. DRª. FLÁVIA LUCISANO BOTELHO DO AMARAL

PROFª. DRª. FABIANA MANTOVANI GOMES FRANÇA

PROF. DR. FLAVIO HENRIQUE BAGGIO AGUIAR

PROF. DR. VALENTIM ADELINO RICARDO BARÃO

A Ata da defesa, assinada pelos membros da Comissão Examinadora, consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

DEDICATÓRIA

À **Deus**, por me conceder força e coragem durante os momentos mais difíceis desta jornada, iluminando meu caminho e decisões.

Aos meus amados pais, **Maria da Graça Pacheco e Alcenor José da Silva,** pelo incentivo, amor e compreensão.

À minha irmã e mãe de coração, **Danila Pacheco e Adelaide Carmina**, por serem minha inspiração diária de amor, honestidade, humildade e paciência. Obrigada por enfrentarem este sonho junto comigo!

À minha querida avó, **Maria de Lourdes Pacheco (in memoriam),** por ser minha eterna fortaleza e exemplo de amor e dignidade. Mesmo de longe, obrigada por guiar meus passos.

À minha **família**, que sempre me apoiou e me ajudaram a seguir sempre em frente.

Ao meu namorado, **Alex Fricelli**, por ser meu maior incentivador e melhor companheiro, durante estes anos. Obrigada por tanto amor, carinho, apoio e amizade. A você terei eterna gratidão!

Aos meus pais paulistas, **Ana Cristina e Eduardo Fricelli**, por me acolherem e tornarem meus dias mais alegres, aliviando a saudade de casa. Obrigada por tanto carinho!

.

AGRADECIMENTOS ESPECIAIS

À **Profa. Dra. Vanessa Cavalli Gobbo,** pela orientação durante estes anos. Sou eternamente grata por toda a paciência, compreensão, sabedoria, experiência, e oportunidades concedidas a mim. Sua calma, conselhos e ensinamentos foram fundamentais para tornar essa caminhada mais leve. Você sempre será minha inspiração como mãe e profissional dedicada! Obrigada por tudo!

Aos companheiros de orientação, **Matheus Kury e Bruna Resende**, pela fraternidade e companheirismo durante os momentos vividos em laboratórios e clinicas. Sou grata por toda dedicação, amizade e carinho dados à mim.

Aos amigos e irmãos nortestinos, **Paulo Campos, Bárbara Emanuelle, Carol Veloso, Ygor Telles e Mayara Noronha,** por me acolherem durante o período de adaptação em Piracicaba, e dividirem tantos momentos inesquecíveis vivenciados no cotidiano. Obrigada por todos os sorrisos bobos, amor e amizade leve!

À **Profa. Dra. Carmem Dolores Vilarinho Soares de Moura,** da Universidade Federal do Piauí, por todo incentivo e dedicação durante o mestrado, para despertar em mim o interesse em seguir o caminho da pesquisa.

.

AGRADECIMENTOS

À Universidade Estadual de Campinas (UNICAMP), na pessoa do Magnífico Reitor, **Prof. Dr. Marcelo Knobel**.

À direção da Faculdade de Odontologia de Piracicaba (FOP) da Universidade Estadual de Campinas, na pessoa do Diretor **Prof. Dr. Francisco Haiter Neto** e do Diretor Associado **Prof. Dr. Flávio Henrique Baggio Aguiar.**

O presente trabalho foi realizado com apoio da **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior** – Brasil (CAPES) - Código de Financiamento 001.

À **Profa. Dra. Karina Gonzales Silvério Ruiz**, coordenadora dos cursos de Pós-Graduação e ao **Prof. Dr. Valentin Aderino Ricardo Barão,** coordenador do Programa de Pós-Graduação em Clínica Odontológica.

Aos professores **Prof. Dr. Valentin Aderino Ricardo Barão, Prof. Dr. Flávio Henrique Baggio Aguiar, Profa. Dra. Flávia Lucisano Botelho do Amaral, Profa. Dra. Fabiana Mantovani Gomes França**, pelo aceite em participar como banca de tese, e tempo dedicado a este trabalho.

Ao Laboratório de Bioquímica da FOP-Unicamp, em nome da Prof(a). Dr(a). **Cinthia Pereira Machado Tabchoury**, por todo o empenho em nos ajudar durante a execução das análises químicas desta tese.

Ao Centro de Microscopia e Imagens da FOP-Unicamp, em nome do Msc. **Adriano Lima Martins**, pelo auxílio na obtenção das imagens apresentadas nesse trabalho.

Aos técnicos de laboratórios, **Alfredo e Marcelo**, por serem tão prestativos e dispostos à ajudar.

.

Aos amigos da Bioquímica, **Aline, Luziana Adele, Luís Fernando, Robson Ferreira, Alejandra Brenes,** por toda a ajuda e apoio durante as dificuldades metodológicas.

Aos Professores da Área de Dentística, **Profa. Dra. Giselle Maria Marchi Baron, Profa. Dra. Débora Alves Nunes Leite Lima, Profa. Dra. Vanessa Cavalli Gobbo, Prof. Dr. Flávio H. Baggio Aguiar, Prof. Dr. Luís Roberto M. Martins e Prof. Dr. Marcelo Giannini**, por todos os conhecimentos transmitidos durante o doutorado.

Às alunas de iniciação científica **Beatriz Medeiros** e **Roberta Soares Kobayashi** pela dedicação e trabalho realizado durante o período de vigência da bolsa PIBIC.

À **Mayara Zagui Dal Piccolo**, por ser tão companheira e prestativa com toda a equipe! Sem dúvidas, deixou nossos dias mais felizes!

Aos colegas de Mestrado **(Priscila Regis**, **Janaína Damasceno**, **Danielle Ferreira**, **Jorge Soto**, **Marcela Ferreti**, **Joyce Figueiredo**, **Marco Túlio)** e Doutorado **(Bruna Guerra, Caroline Mathias, Laura Ferraz, Maicon Sebold, Mariana Flor, Mayara Noronha, Mayara Zaghi, Renata Pereira, Rodrigo Lins)** por sempre deixarem a rotina de laboratório e estudos mais leve e alegre. A amizade de vocês foi essencial!

.

RESUMO

Apesar do sucesso da técnica de clareamento dental, moléculas excedentes de peróxido de hidrogênio (PH) podem alcançar a câmara pulpar de dentes restaurados, e causar respostas inflamatórias e citotóxicas ao tecido pulpar. Portanto, o objetivo deste estudo foi avaliar a concentração intrapulpar e penetração do PH (9,5% ou 35%) na interface de dentes restaurados com compósitos bioativos, utilizando técnica convencional ou de incremento único (bulk-fill). Cavidades foram preparadas (4 mm diâmetro x 3 mm de profundidade) na superfície vestibular das coroas dos incisivos bovinos e restauradas com: compósito bioativo convencional (BII, Beautifil II), compósito bioativo bulk-fill (AC, Activa Bio-ACTIVE), ionômero de vidro modificado por resina (RMGI, Riva Light Cure), compósito convencional (FZ, Filtek Z350) e composito bulk-fill (FB, Filtek Bulk). Os corpos de prova foram termociclados (5000 ciclos) e após 24 horas, as restaurações foram expostas ao clareamento com PH de alta (35%; n=10) ou baixa (9,5%; n=10) concentrações. O clareamento com PH 35% foi realizado em 4 sessões (4 aplicações de 8 min/sessão) e PH 9,5%, aplicado por 14 dias (30 min/dia). Na última aplicação de clareador, solução tampão de acetato foi inserida na câmara pulpar, e após o protocolo de clareamento, esta solução foi recolhida e transferida para o tubo de ensaio contendo leucocristais violeta e enzima peroxidase. A densidade óptica foi analisada por espectrofotometria e convertida em µg/mL, equivalente à concentração de PH. Os dentes foram pigmentados com solução de rodamina B, e a penetração de PH ao redor das restaurações adesivas foi observada em microscopia confocal de varredura por fluorescência a laser (MCVFL). Os dados de concentração de PH foram submetidos ao teste de ANOVA dois fatores e teste de Tukey ($\alpha = 0,05$), e as imagens de MCVFL foram submetidas à análise qualitativa. Dentes restaurados submetidos ao agente clareador de alta concentração apresentaram maior quantidade de PH na câmara pulpar. Não houve diferença significativa na concentração intrapulpar de peróxido de dentes restaurados e submetidos ao PH 9,5%. Foi observada menor difusão do peróxido em dentes restaurados com RMGI, comparados aos compósitos convencional (FZ; p=0,004) e bulk-fill (FB; p=0,01), submetidos ao PH 35%. Imagens de MCVFL mostraram que PH 35% promoveu maior degradação da rodamina B na interface de dentes restaurados. A concentração intrapulpar e a penetração de HP na interface adesiva

.

dos dentes restaurados com compósitos bioativos, convencional ou bulk-fill, foram maiores quando expostos ao clareamento de alta concentração (PH 35%).

Palavras-chaves: Clareamento dental. Peróxido de hidrogênio. Restauração dentária permanente.

.

ABSTRACT

Despite the success of the bleaching technique, excess hydrogen peroxide (HP) molecules can reach the pulp chamber of restored teeth, and cause inflammatory and cytotoxic responses to pulp tissue. Therefore, the objective of this study was to evaluate the intrapulpal concentration and PH penetration (9.5% or 35%) at the interface of teeth restored with bioactive composites, using conventional or bulk-fill technique. Cavites were prepared (4 mm diameter x 3 mm deep) on the buccal surface of the bovine incisor crowns and restored with: bioactive conventional composite (BII, Beautifil II), bioactive bulk-fill composite (Activa Bio- ACTIVE), resin modified glass-ionomer (RMGI, Riva Light Cure), conventional composite (FZ, Filtek Z350) and bulk-fill composite (FB, Filtek Bulk). Samples were thermocycled (5000 cycles) and after 24 hours, the restorations were exposed to high HP (35%; n = 10) or low (9.5%; n = 10) bleaching concentrations. Bleaching with 35% HP was perfomerd in four sessions (4 applications of 8 min/session) and 9.5% HP was applied for 14 days (30 min/day). In the last bleaching application, the acetate buffer solution was inserted into the pulp chamber, and after the bleaching protocol, this solution was collected and transferred to test tube containing leucocrystal violet and peroxidase. The optical density was analyzed by spectrophotometry and converted to µg / mL equivalent to HP concentration. The teeth were pigmented with rhodamine B solution, and HP penetration around adhesive restorations was observed under laser scanning confocal fluorescence microscopy (LSCFM). The HP concentration data were submitted to two-way ANOVA and Tukey's test (α = 0.05) and the LSCFM images were qualitatively analysed. Restored teeth submitted to high-concentrated agent presented higher HP intrapulpal concentration. No significant difference in HP intrapulpal concentration was observed among groups (p> 0.05) when exposed to 9.5% HP. Lower diffusion of peroxide was observed for teeth restored with RMGI, compared to conventional (FZ; p=0,004) and bulk-fill composites (FB; p=0,01), submitted to HP 35%. LSCFM images showed that 35% HP promoted greater degradation of rhodamine B at the restored teeth interface. The intrapulpal concentration and penetration of HP at the adhesive interface of teeth restored with bioactive composites, conventional or bulk-fill, were higher when exposed to high concentration bleaching (35% HP).

.

Key words: Tooth bleaching. Hydrogen Peroxide. Permanent Dental Restoration.

.

SUMÁRIO

.

1 INTRODUÇÃO

O clareamento dental tem sido cada vez mais indicado nos planejamentos odontológicos, devido sua capacidade de remover pigmentos de origem exógena e exibir propriedades conservadoras e de baixo custo (Ubaldini et al., 2013). O excelente resultado estético proporcionado pelos géis clareadores está baseado na rápida difusão trans-dentinária e transformação do peróxido de hidrogênio (PH) em radicais livres (Mena-Serrano et al., 2015). Apesar do sucesso da técnica, moléculas excedentes de PH podem alcançar a câmara pulpar, causando respostas inflamatórias e alterações morfológicas ao tecido pulpar (Hanks et al., 1993; Gokay et al. 2000; Benetti et al., 2004; Marson et al., 2015).

A indicação do clareamento dental deve ser realizada de forma cautelosa, uma vez que estudos in vitro apontam que, o uso de altas concentrações de PH tem exibido danos pós-operatórios severos e efeitos citotóxicos à polpa (Bowles et al., 1987; Costa et al., 2010). Por outro lado, estudos clínicos observaram reações esporádicas ou insignificantes, após o tratamento com altas e baixas concentrações (Matis et al., 2000). Portanto, ainda não há consenso sobre a relação da concentração inicial do gel e a quantidade de PH presente na câmara pulpar.

A literatura mostra que além da concentração, o tempo de aplicação pode influenciar diretamente a difusão do peróxido (Ubaldini et al., 2013; Marson et al., 2015; Matis et al., 2000). Assim, diversos protocolos e composições dos géis clareadores foram investigados, a fim de evitar uma exposição desnecessária à estrutura dental (Marson et al., 2015; Costa et al., 2010; Briso et al., 2016). Nesse contexto, Marson et al. (2015) e Soares et al. (2014) observaram que a difusão do PH é dependente da concentração e do protocolo de aplicação do gel clareador.

Além disso, estudos relatam alterações na superfície do esmalte e variações nas propriedades físicas, mecânicas e estéticas dos materiais restauradores submetidos a agentes clareadores (Polydorou et al., 2007; Wang et al., 2011; White et al., 2008). Relatos anteriores mostraram que dentes restaurados submetidos ao clareamento dental, apresentaram maior quantidade de peróxido de hidrogênio na câmera pulpar do que em dentes hígidos (Cavalli et al., 2017; Benetti et al., 2004). De acordo com os autores, tal fato se deve à interface adesiva estabelecer um caminho para a microinfiltração do PH (Ubaldini et al., 2013).

.

Portanto, estudos têm sido conduzidos para desenvolver materiais restauradores que ofereçam uma adequada vedação marginal e boas propriedades mecânicas. Embora nenhum material restaurador bloqueie completamente a penetração do PH, sua difusão pode ser afetada pela falha adesiva promovida na interface dos compósitos convencionais (Ubaldini et al., 2013; Gokay et al., 2000; Owens et al., 1998). Portanto, na tentativa de reduzir o tempo clínico e a tensão de polimerização, compósitos de incremento único ou *bulk-fill* foram desenvolvidos, permitindo incrementos de até 4 mm de espessura (Fronza et al., 2015).

Compósitos bioativos contendo partículas de carga de ionômero de vidro pré-reagido (S-PRG - Beautifil II®, Shofu) foram criados para permitir a interação com o tecido adjacente pela liberação de flúor por um mecanismo de troca iônica no hidrogel pré-reagido (Ikemura et al., 2018). Estudos demonstraram que estes materiais oferecem propriedades biológicas satisfatórias, inibição bacteriana, biocompatibilidade e bom selamento marginal (Wiegand et al., 2007; Sadr et al., 2009; Braga et al., 2005). Assim, para tornar os materiais bioativos ainda mais aplicáveis, um novo compósito bioativo tipo bulk-fill (Activa BioACTIVE, Pulpdent®) foi formulado com uma matriz resinosa iônica, que além de poder ser inserido em incremento único, promove a liberação de fluoreto, cálcio e fosfato, conforme indicado pelo fabricante.

Diante disto, acreditamos que os compósitos bioativos, convencional e bulk-fill, podem apresentar maior capacidade de selamento marginal e influenciar na difusão do PH para câmara pulpar. Assim, a literatura mostra poucas evidências sobre a penetração do PH em dentes restaurados com diferentes materiais e técnicas restauradoras. Portanto, o objetivo deste estudo foi avaliar a concentração intrapulpar e penetração do PH (9,5% ou 35%) na interface de dentes restaurados com compósitos bioativos, utilizando técnica conventional incremental ou de incremento único (bulk-fill).

.

2 ARTIGO: Intrapulpal concentration of hydrogen peroxide of teeth restored with bulk-fill and conventional bioactive composites

Running Title: Concentration of hydrogen peroxide in the pulp chamber of restored teeth

*Manuscrito formatado de acordo com normas do periódico internacional *Operative Dentistry*.

Authors:

Daylana Pacheco da Silva, DDS, PhD student, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil.

Bruna Almeida Resende, DDS, MS student, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil.

Matheus Kury, DDS, MS, PhD student, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil.

Carolina Bosso André, DDS, MS, PhD, Department of Restorative Dentistry, Piracicaba Dental School University of Campinas, Piracicaba, Brazil.

Cínthia Pereira Machado Tabchoury, DDS, MSc, PhD, Department of Physiological Sciences, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil

Marcelo Giannini, DDS, MS, PhD, Associate professor, Department of Restorative Dentistry Piracicaba Dental School, University of Campinas, Piracicaba, Brazil.

Vanessa Cavalli, DDS, MS, PhD, Associate professor, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil*.

.

Corresponding author: Dr. Vanessa Cavalli

Piracicaba Dental School, University of Campinas, 901 Limeira Avenue, Areão, 13414-903, Piracicaba, SP, Brazil.

+551921065338 / vcavalli@yahoo.com

.

SUMMARY

This study evaluated intrapulpal concentration and peroxide hydrogen (PH) penetration at the interface of teeth restored with bioactive composites, using conventional or bulk-fill technique. Cavites were prepared (4 mm diameter x 3 mm deep) on the buccal surface of the bovine incisor crowns and restored with: bioactive conventional composite (BII, Beautifil II), bioactive bulk-fill composite (AC, Activa Bio-ACTIVE), resin modified glass-ionomer (RMGI, Riva Light Cure), conventional composite (FZ, Filtek Z350) and bulk-fill composite (FB, Filtek Bulk). The samples were thermocycled (5000 cycles) and after 24 hours, the restorations were exposed to high HP (35%; n = 10) and low (9.5%; n = 10) bleaching concentrations. Bleaching with 35% HP was perfomerd in four sessions (4 applications of 8 min/session) and 9.5% HP was applied for 14 days (30 min/day). In the last bleaching application, the acetate buffer solution was inserted into the pulp chamber, and after the bleaching protocol, this solution was collected and transferred to test tubes containing leucocrystal violet and peroxidase. The optical density was analyzed by spectrophotometry and converted to µg / mL equivalent to the HP concentration. The teeth were pigmented with rhodamine B solution, and HP penetration around adhesive restorations was observed under laser scanning confocal fluorescence microscopy (LSCFM). The HP concentration data were submitted to two-way ANOVA and Tukey's test (α = 0.05), and the LSCFM images were qualitatively analysed. Restored teeth submitted to high-concentrated agent presented higher HP intrapulpal concentration. No significant difference in HP intrapulpal concentration was observed among groups (p> 0.05) when exposed to 9.5% HP. Lower diffusion of peroxide was observed for teeth restored with RMGI, compared to conventional (FZ; p=0,004) and bulk-fill composites (FB; p=0,01), submitted to HP 35%. LSCFM images showed that 35% HP promoted greater degradation of rhodamine B at the restored teeth interface. The intrapulpal concentration and penetration of hydrogen HP at the adhesive interface of teeth restored with bioactive composites, conventional or bulk-fill, were higher when exposed to high concentration bleaching (35% HP).

.

Clinical Relevance: The concentration of the bleaching agent and the type of restorative material can influence the concentration of hydrogen peroxide into the pulp chamber.

.

INTRODUCTION

The excellent aesthetic results provided by the bleaching agents are based on the trans-dentin diffusion and decomposition of hydrogen peroxide (HP) into free radicals[1]. The interaction of bleaching agents occurs mainly in dentin, due to the absence of organic chromophores in the enamel structure[1]. Thus, the excess of HP molecules potentially reaches the pulp chamber, causing inflammatory responses, morphological changes to pulp tissue and postoperative sensitivity[2,3,4,5.]

Tooth bleaching should be performed with caution, as the use of high concentrations of HP has shown to promote severe post-operative damage and cytotoxic effects[6,7,8]. However, studies indicate that even at low concentrations, bleaching agents could reduce pulp cell viability[9,10]. On the other hand, clinical studies have observed sporadic or insignificant reactions after treatment with high and low concentrations of hydrogen peroxide[11,12].

Clinically, pulp damage manifests as dental sensitivity, ofently leading the patient to cease bleaching[13]. Studies report that, in addition to the concentration, the time of application directly influences the diffusion of the peroxide[3,4,5,14]. Thus, different protocols and concentrations of HP have been investigated in order to avoid unnecessary exposure to the dental structure and to decrease the intra-pulp concentration of hydrogen peroxide. [4,15]. In this context, Marson et al. (2015) have observed that HP diffusion is dependent on the concentration and bleaching gel application protocol.

Literature shows that restored teeth submitted to tooth whitening present greater amount of HP in the pulp chamber than sound, non-restored teeth. This occurs because the restorative interface becomes a path for peroxide microleakage[16]. Therefore, studies have been conducted to develop restorative materials that promote both adequate marginal sealing and mechanical properties. Although no restorative material completely blocks HP penetration, the diffusion of HP can be affected by polymerization shrinkage and the stress of polymerization promoted at the adhesive interface of conventional composites [17,3]. Therefore, in an attempt to obtain greater marginal integrity, reduce clinical time and polymerization stress, bulk-fill composites were developed, allowing increments up to 4 mm thick.

Bioactive composites containing pre-reacted glass ionomer (S-PRG - Beautifil II®, Shofu) charge particles were created to allow interaction with the adjacent tissue

.

by the release of fluoride by an ion exchange mechanism in the pre-reacted hydrogel[18]. Studies have shown that the S-PRG-containing bioactive materials offer satisfactory biological properties, bacterial inhibition, biocompatibility and good marginal sealing [19,20]. To make bioactive materials even more applicable, a new bulk-fill composite (Activa BioACTIVE, Pulpdent®) was formulated with an ionic resin matrix, which allowed the release of not only fluoride but also the release of calcium and phosphate.

Given this, we believe that bioactive composites, conventional or bulk-fill, may present greater marginal sealing ability and influence the diffusion of the PH to the pulp chamber. Considering that these materials are used in posterior teeth (premolars with involvement of the buccal surface), and may be subjected to dental bleaching, the diffusion and concentration of HP into the pulp chamber must be evaluated. Therefore, this work evaluated diffusion and concentration of HP into the pulp chamber of teeth restored with conventional bioactive composites (S-PRG-based material - Beautifil II), or bulk-fill bioactive composite (Activa BioACTIVE). The null hypotheses tested were: (1) the concentration of the bleaching agent would not influence the intrapulpal concentration of HP of restored teeth; (2) The type of restorative material (conventional or bulk/fill bioactive composites) would not influence the intrapulpal concentration of HP.

.

METHODS AND MATERIALS

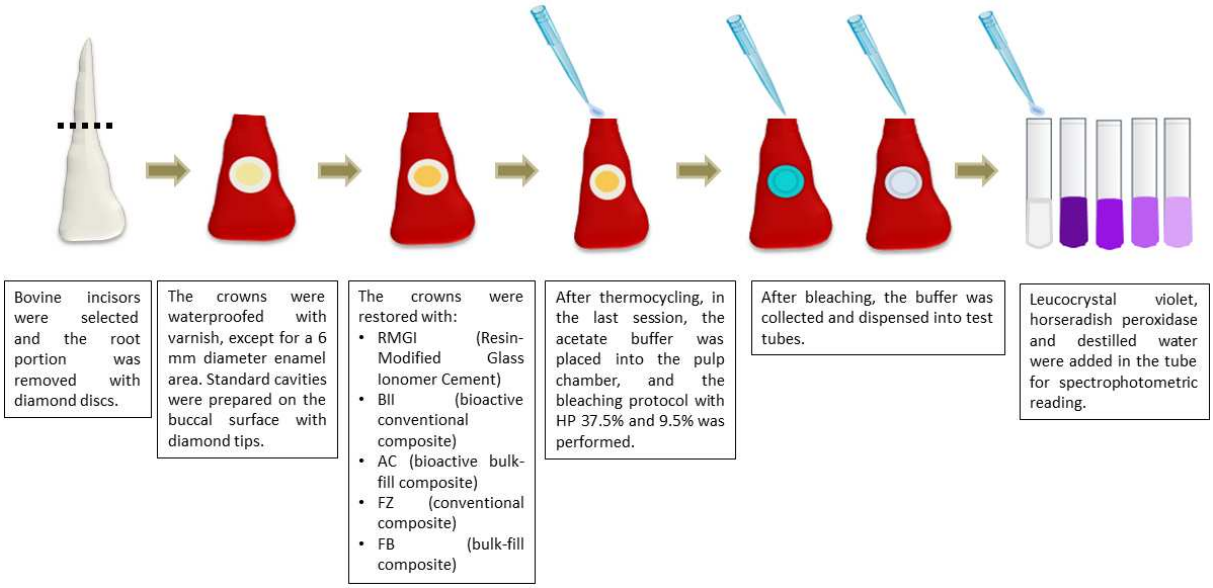
Sample Preparation

Table 01. Experimental design of the study.

Experimental Units	Crowns of bovine incisors (n = 10).	
Factors under study	Bleaching Gels	35%HP (Pola Office, SDI, Austrália); 9,5% HP (Pola Day, SDI, Austrália).
	Restorative Materials	1. Bioactive Composites (Beautifill II, Shofu, Kyoto, Japão); 2. Bulk-fill Bioactive Composite (Activa BioActive Restorative, Pulpdent Corporation, MA, EUA); 3. Conventional Composite (Filtek Z350 XT, 3M Oral Care, St. Paul, MN, EUA); 4. Bulk-fill Composite (Filtek Bulk-fill, 3M Oral Care); 5. Resin-modified Glass Ionomer Cement (Riva Light Cure, SDI).
Variable response	- Intrapulp concentration of bleaching agents (µg / ML) applied to adhesive restorations; - Adhesive interface of restorations submitted to bleaching agents, observed in confocal microscopy of fluorescence by laser scanning.	

.

Figure 1. Description of the samples to measure the concentration of hydrogen peroxide in the pulp chamber.



Bovine incisors were collected, cleaned and stored in a 0.1% thymol solution at 4^0C for 30 days. All crowns were examined under a stereomicroscope in order to discard teeth with surface defects. One hundred crowns with standard dimensions (4.0 ± 0.1 mm) of the buccal surface (enamel and dentin) were selected. After cleaning, teeth were stored in a 0.1% thymol solution at 4 ° C for 30 days. The roots were cut with diamond discs (KG Sorensen, Barueri, Brazil) up to 2 mm below the cementoenamel junction. The pulp tissue was removed using files (Hedstrom files, Maillefer Dentsply, Ballaigues, Switzerland), and the pulp chamber was throughly rinsed with distilled water. The cervical pulp orifice was enlarged with spherical diamond tip bur (#1016, KG Sorensen), allowing the acetate buffer to be placed in the pulp chamber[16].

Cavities (4 mm diameter x 3mm depth) were prepared in the buccal surface of the specimens with diamond burs (n^o. 3053 and n^o. 3017, KG Sorensen). The application of the adhesive system and restorative materials followed the manufacturers' instructions (Table 2). The adhesive was previously applied only in cavites restored with the composite resins and light cured for 20 s with a LED device (1200 mW / cm^2, Valo, Ultradent Products Inc., South Jordan, USA). For resin-modified glass ionomer restorations, Riva Conditioner was applied, followed by manipulation, insertion of the cement and light cured for 20 s. The finishing and

.

polishing of the restorations were performed with extra-thin tips (3118 FF, KG Sorensen), sandpaper discs (Sof-Lex, 3M Oral Care) and each granulation was used for 15 s (medium, fine and superfine).

Table 1. Composition and manufacturer's instructions of the restorative material.

Materials (Abbreviation)	Commercial Names and Manufacturer	Composition	Manufacturer's instructions
Dental Adhesive (SBU)	Single Bond Universal (3M Oral Care, St. Paul, MN, USA)	10-MDP,phosphate monomer, dimetacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, waterwater, initiators, silane.	1) Selective acid etching of enamel for 15 seconds. 2) Apply adhesive with a microbrush to the surface (20 s), followed by a gentle air-spray for 5 s and light cure for 10 s.
Conventional Bioactive Composite (BII)	Beautifil II (Shofu, Kyoto, Japão)	Bisphenol-A glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), particles pre-reacted glass ionomer (S-PRG).	Apply composite in increments of 2 mm and light cure (20 s).
Conventional Composite (FZ)	Filtek Z350 XT (3M Oral Care)	Bis-GMA, Bis-EMA, UDMA, TEGDMA. Nanoparticles of non-agglomerated silica, zirconia/silica nano-agglomerates, free-bound agglomerates.	Apply composite in increments of 2 mm and light cure (20 s).
Bulk-fill Bioactive Composites (AC)	Activa BioACTIVE (Pulpdent, MA, USA)	Mixture of diurethane and other methacrylates with modified polyacrylic acid (44.6%) Amorphosine (6.7%) Sodium fluoride (0.75%).	Apply composite in increments of 4 mm and light cure (20 s).
Bulk-fill Composite (FB)	Filtek Bulk Fill Posterior (3M Oral Care)	AUDMA, UDMA, 1,12-dodecane-DMA.	Apply composite in increments of 4 mm and light cure (20 s).
Resin-modified glass ionomer (RMGI)	Riva Light Cure (SDI Limited, Victoria, Australia)	Powder: Fluoroaluminosilicate glass. Liquid: Polycyclic acid, tartaric acid, polyacrylic	Enamel ecthing for 10 s, followed by abundant rinsing and air-drying; Apply the material

.

		acid.	and light-cure for 10 s.

Bis-GMA-bisphenol A-glycidyl methacrylate; HEMA-2-hydroxyethyl methacrylate; TEGDMA -triethylene glycol dimethacrylate; UDMA - urethane dimethacrylate; BisEMA-ethoxylate bisphenol-A-glycol dimethacrylate.

Thermal Cycling and Bleaching Procedure

In order to age the adhesive interface, the specimens were submitted to 5.000 thermal cycles [16,21] (MCT2 – AMM, São Paulo, SP, Brazil) in deionized water baths at 5° to 55°C ± 1°C, corresponding to 6 months of aging. Twenty-four hours after thermal cycling, specimens received two layers of enamel polish (Revlon Inc., New York, NY, USA), covering all exposed enamel except 1 mm around the restorations.

After 24 h of thermal cycling, the restored specimens were submitted to high (35% hydrogen peroxide) or low-concentrated (9.5% hydrogen peroxide) bleaching agents. Therefore, each restorative material would be exposed to high or low-bleaching therapy (n = 10). The composition and application protocols of bleaching agentes are decribed in table 2. Among bleaching applications, teeth were stored at 37ºC and immersed in artificial saliva (20 mM Tris buffer, pH 7.0, 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F / mL)[22].

Table 2: Bleaching agentes composition and manufacturer's instructions.

Bleaching Agents	Commercial Name (Manufacturer, Address)	Specification/Composition	Manufacturer's Instruction
35% Hydrogen Peroxide	Pola Office* (SDI Limited, Victoria, Australia)	Liquid: 35% Hydrogen peroxide, 65% Water. Powder: 73.26% Thickeners, 26.2% Catalysts, 0.04% Dye, 0.5% Desensitizing agents (Potassium Nitrate). **pH=3,7	Indicated for in-office bleaching therapy, in 4 sessions. Apply a mixture to the enamel for 8 minutes, 4 times.
9.5% Hydrogen Peroxide	Poladay* (SDI Limited)	Bleaching gel: Hydrogen peroxide (9.5%). Activator: Additives (47%), Glycerol (30%), Water (20%), Flavorings (0.1%). **pH=5,7	Indicated for at-home bleaching therapy, for 14 days. Apply the whitening gel to the tray 1 time for 30 minutes.

.

** According to manufacturer's directions, SDI, Australia.*

*** Preliminary pH results obtained by the authors*

Concentration of Hydrogen Peroxide Into the Pulp Chamber

High concentration bleaching (35% HP) was conducted in 4 sessions with intervals of 7 days, and in each session 4 applications of 8 min were performed. The low-concentrated agents (9.5% HP) were applied for 14 days and 30 min/day. In the last bleaching application, the pulp chamber was dried and 150 µL of acetate buffer solution 2M (pH 4.5) was placed into the coronary chamber in order to stabilize the HP that penetrates into the pulp throughout bleaching. Subsequently, 0.01 g of the high-concentrated bleaching agent was applied on the buccal surface for 8 min in four consecutive applications. The low-concentrated agent (0,01 g) remained in contact with the enamel surface for 30 min.

After the bleaching procedure, the solution was removed and transferred to a glass test tube. The pulp chamber of each tooth was filled for a second time with 150 µl of the acetate buffer for 1 min, and this solution was placed in the same glass tube. In addition, deionized water (2650 µL), leuco crystal violet (100 µL of 0.5 mg / mL, Sigma-Aldrich, St. Louis, MO, USA), and horseradish peroxidase (50 µL of 1 mg / mL; Sigma-Aldrich) were added to each tube[23]. These procedures are illustrated in Figure 1.

As a result, a blue solution was obtained, allowing the optical density measurement in a spectrophotometer (DU 800, Beckman Coulter Inc, Brea, CA, USA) at wavelength of 596 nm. For this purpose, a standard curve with known HP concentrations were used to convert the optical density values into microgram (µg) of HP/mL of solution. The values were then converted into micrograms per milliliter (Figure 1).

Laser Scanning Confocal Fluorescence Microscopy (LSCFM)

To observe the HP diffusion at the adhesive interface, additional specimens of each group (n=3) were prepared and submitted to either high or low concentrations bleaching protocols. The crowns were fully immersed in Rhodamine B solution (Aldrich Chem. Co) for 7 days at a concentration of 0.1 mM in 30 ml. Subsequently,

.

the in-office or at-home bleaching protocols were performed, with the exception of the control group, which remained not bleached. The center of the restorations were cross-sectionally cut with diamond disc (Isomet 1000, Buehler, Lake Bluff, IL, USA).

The inner face was polished (EcoMet 3000, Buehler, Lake Bluff, Illinois, USA) with abrasive papers (nº 400, 600 and 1200 granulations) and specimens were immersed in vegetable oil and immersed in ultrasonic chamber for 15 min for the complete removal of the polishing residues. The interface was analyzed by an argon laser scanning microscope (TCS SP5AOBS, Leica Microsystems CMS GmbH, Germany) with a wavelength of 543 µm, with the 20x objective, in scan mode over the entire length of the sample.

Statistical Analyses

Statistical calculations were conducted by the SPSS 23 program (SPSS Inc., Chicago, IL, USA). Data were transformed in Log10, and submitted to Shapiro-Wilk and Levene tests exploratory analysis to determine normal distribution, responding to the parametric and homoscedasticity assumptions. The results were analyzed to two-way ANOVA and Tukey's test, with significance level of 5%.

RESULT

Table 3 indicates the concentration of HP (µg / mL) in the pulp chamber of teeth restored with the different materials. The interaction between factors "concentration" and "restorative materials" ($p = 0.046$) significantly influenced the results of intrapulpal peroxide concentration, according to the two-way ANOVA and Tukey test.

The HP intrapulpal concentration was significantly higher when the high-concentrated bleaching gel was applied, regardless the restorative material used ($p<0.05$). No significant difference was detected among groups ($p> 0.05$) when exposed to the low-concentrated bleaching (9.5% HP). When submitted to the high-concentrated agent (35% HP), no statistical difference ($p>0.05$) was observed between the bioactive composites (BII and AC), and the other materials. However, the RMGI promoted lower HP intrapulpal concentration than the conventional (FZ; $p=0,004$) and bulk-fill composites (FB; $p=0,01$).

.

Table 3. Mean (standard deviation) of hydrogen peroxide concentration (HP) in the pulp chamber (µg/mL) of restored teeth, submitted to low- and high-concentrated bleaching agents.

Restorative Materials	9.5% HP	35% HP
Resin-modified glass ionomer (Riva Light Cure)	0.09 (0.06) Ba	0.27 (0.13) Ab
Bulk-fill Composite (Filtek Bulk-fill)	0.09 (0.03) Ba	0.51 (0.38) Aa
Conventional Composite (Filtek Z350 XT)	0.07 (0.04) Ba	0.62 (0.55) Aa
Bulk-fill Bioactive Composites (Activa BioACTIVE)	0.09 (0.04) Ba	0.42 (0.29) Aab
Conventional Bioactive Composite (Beautifil II)	0.07 (0.02) Ba	0.43 (0.26) Aab

Means followed by distinct letters differ statistically at 5%, according to two-way Analysis of Variance and Tukey test. Uppercase letters compare bleaching agents (lines) and lowercases letters compare restorative materials (columns).

Representative images of LSCFM of each group (without bleaching, high and low concentration of HP) exhibited degradation of dye by HP, indicating the diffusion ability of the bleaching agents. High-concentrated bleaching agents (Fig. 2B) promoted greater degradation of rhodamine B at the restorative interface and enamel than the low-concentrated agents (Fig. 1C) and the control group (without bleaching) (Fig. 1A). No differences were observed between teeth restored with different composites, exposed to high- or low- concentrated bleaching agents.

In addition, darkened areas present in dentin (Fig. 2B) and enamel prisms (Fig. 2C) were noted. These areas could indicate incomplete degradation of HP, since only one part oh HP reaches the pulp chamber.

.

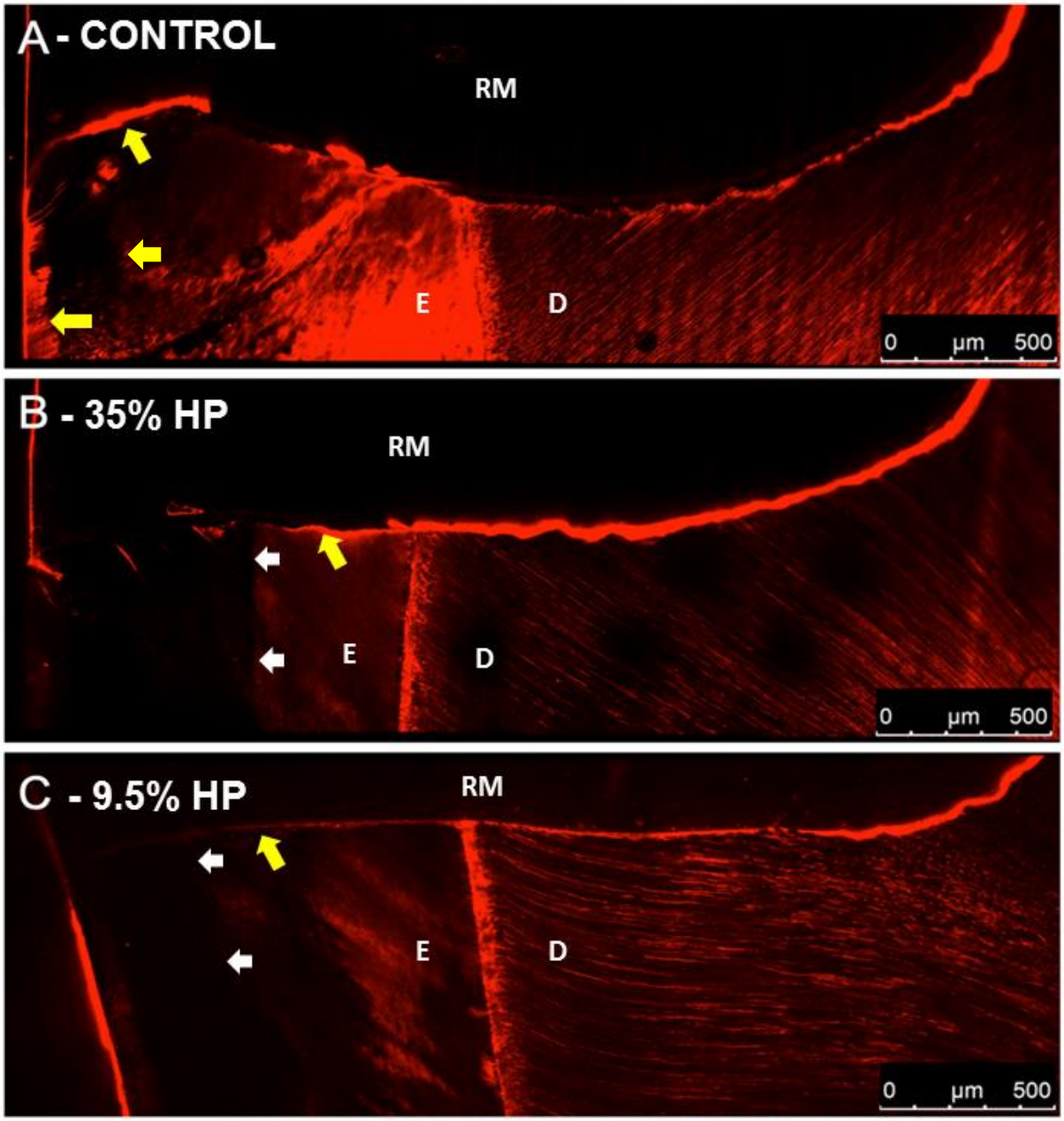


Figure 2. Laser scanning confocal fluorescence microscopy of penetration of hydrogen peroxide at the interface of restored teeth. (A): Control group (enamel restored unbleached) exhibits infiltration of rhodamine B dye (red) at the adhesive interface and enamel. (B): Bleaching with 35% HP shows decomposition of rhodamine B dye at the adhesive interface and enamel (white arrows) indicating dye degradation by the peroxide and the black spaces, as the result of this interaction. In addition, dentin shows black round spaces where decomposition of dye by HP could have ocurred. (C): Bleaching with 9.5% HP shows penetration of HP and decomposition of rhodamine B dye at the adhesive interface and enamel and scattered areas with black lines indicating rhodamine decomposition. (RM:

.

Restorative Material; E: Enamel; D: Dentin; Yellow arrows: Infiltration of rhodamine B dye; White arrows: absence of rodhamine B, indicating the degradation and diffusion of hydrogen peroxide).

DISCUSSION

This study showed that high concentrations of bleaching agents influenced the amount of HP in the pulp chamber. Therefore, the first hypothesis was rejected. The results suggested that intrapulpal concentration of peroxide was proportional to the concentration of the bleaching agent, as observed previously [3,14,24]. In fact, the LSCFM images demonstrated greater removal of rhodamine B by the high-concentrated bleaching agent (35% HP), both in enamel and in the adhesive interface, which possibly displays the paths of HP penetration.

According to Kwon et al. (2012)[25], the immersion of the specimens in rhodamine B for 7 days allowed adequate enamel, dentin and amelodentinal junction staining. Therefore, the removed “black” area possibly displays the paths of HP penetration. Although the spectrophotometer analysis showed the presence of HP in the pulp chamber, the images indicated degradation of rhodamine concentrated in enamel, although signs of degradation could be observed in dentin. This could be a limitation of this methodology, since bleaching agent was not able to degrade the whole pigment in dentin, but HP was able to reach the pulp chamber.

Penetration of bleaching agents in the pulp chamber can be modulated according to peroxide concentration[3,6,13,23], composition and pH of bleaching gels[26], presence of restorations[24], enamel thickness or quality[27]. Soares et al. (2014)[14] and Cintra et al. (2016)[28] emphasized that both the concentration and the time of application are able to influence the diffusion of HP. These same results were observed by Marson et al. (2015)[5] and Camargo et al. (2007)[29], showing that the longer the contact of the bleaching agent with enamel, the greater was the penetration into dental tissues.

Regarding pH, the literature reports that an alkaline bleaching gel increases the dissociation of HP in free radicals, reducing the risk of penetration of peroxide molecules into the pulp tissues[30]. Previous studies indicated that the contact time of the bleaching agent with an acidic pH with the substrate, may result in changes in

.

enamel microhardness, roughness, sensitivity and wear [30,31]. The pH of the bleaching agents used in the current study exhibit acid values for both concentrations (35% HP = 3.7 and 9.5% HP = 5.7). Therefore, these values may increase the intrapulpal concentrations of HP.

The rheological properties of the peroxide influences its behavior. Kwon et al. (2018)[32] suggested that bleaching agents with higher viscosity promoted lower concentration of peroxide in the pulp chamber than low and medium viscosity gels. The greater viscosity exhibited by the low-concentrated agent (9.5% HP) could have contributed to the more favorable results for this agent. Therefore, the concentration of HP in the agents may not be the only reason for the higher intrapulpal concentration.

Another factor that seems to influence the diffusion of HP is the presence of desensitizing agents in the composition of bleaching gels. In this study, the high-concentrated gel (Pola Office®) presents potassium nitrate, which is able to reduce the risk and intensity of postoperative sensitivity[33]. Thus, we believe that this gel can promote lower diffusion of the HP, when compared to other high-concentrated bleaching gels. However, there are no reports in literature about the intrapulpal concentration of HP in restored teeth exposed to bleaching agents with different desensitizers.

In this study, the type of restorative material directly influenced the intrapulpal HP concentration, since the RMGI showed lower penetration than the FB and FZ composites, when exposed to the high-concentrated agent. Based on this fact, the second null hypothesis was rejected. Several studies investigated the penetration of bleaching agents into the interface of restored teeth [17,3,24,34]. Gokay et al. (2000)[3] and Benetti et al. (2004)[24] compared the penetration of 30% HP and 10% or 35% CP in sound and restored enamel. The authors suggested that the presence of restorations and the use of high-concentrated bleaching agents allow the diffusion and greater amount of peroxide in the pulp chamber. Although no restorative material has been able to completely prevent the penetration of HP, the final concentration can be affected by the type, shape, and volume of the material, that influence the polymerization stress of the material[24]. Previous studies by Klein et al. (2018)[35] and Piemjai et al. (2018)[36] showed the presence of marginal microleakage in enamel restored with composite resin exposed to bleaching agents of different

.

concentrations, which may favor the diffusion of the agent into the pulp. However, the literature still shows controversial results[37].

In our study, restored teeth with RMGI submitted to 35% of HP promoted a lower concentration of HP intrapulpar when compared with non-bioactive materials (FB and FZ). This fact can be explained by the interaction of the metal ions present in the RMGI (Al^{3+},SiO_3^{2-},Ca^{2+}) with HP, resulting in the formation of oxides and hydroxyl radicals (OH-), and peroxide degradation[38]. In addition, previous studies have shown that this material (Riva Self Cure®), in particular, presents good physical and mechanical properties due to the arrangement of its particles inside the matrix, promoting a good marginal integrity and lower risk of peroxide diffusion at the interface[39]. Although bioactive composites also exhibit release of metal ions (Ca^{2+},BO_3^{3-},Sr^{2+},Si_3^{2-},Na^{+},B^{3-}), these are trapped in charge particles, and possibly their release mechanism is impaired. Thus, this is the reason why there is no statistical difference between bioactive and non-bioactive composites.

In addition it should be kept in mind that HP is able to alter the physical, mechanical and aesthetic properties of restorative materials. Therefore, the contact of HP may influence the surface roughness and porosity of the composites[15], cracks formation, microhardness reduction[15] and interface integraty[40]. Kimyai et al. (2017)[40] evaluated the effect of high and low concentration of bleaching agents on the properties of S-PRG (BII)-based bioactive composites and observed an undesirable effect on microhardness and marginal sealing of this material. On the other hand, Garoushi et al. (2018)[41] observed stable mechanical properties of BII, and attributed the results to the presence of greater amount of different types of fillers, including (besides S-PRG fillers) large pre-polymerized fillers. According to these authors, the presence of an ionic resin matrix and bioactive fillers offer physical-chemical properties similar to human teeth.

In the present study, the restorations were thermocycled prior to the application of HP in order to age interface and simulate a restoration in function. Due to the temperature variation throughout the 5,000 cycles, differences in the coefficient of thermal expansion (CTE) of the materials may result in marginal misadaptation and microleakage[42]. Therefore, the use of materials with a CTE close to the dental structure could potentially reduce the HP penetration. In this context, Sidhu et al. (2004)[43] observed that RMGI showed low dimensional changes due to a compensatory effect. At high temperatures, there is a thermal contraction due to

.

dehydration, however, in the cooling, there is an expansion and rehydration of the material. In composite resins, the authors showed significant thermal expansions only in heating[43]. These results can be influenced by the amount of inorganic particles, matrix composition and degree of polymerization[42]. Therefore, possibly the RMGI provided lower stress at the interface and, consequently, lower penetration of HP compared to other materials.

A self-etching multimode adhesive system (Single Bond Universal - SBU) was used in combination with all the resin composites, with the objective of not influencing the material behavior. Studies have shown that SU provides adequate bond strength results due to the chemical interaction between the 10-MDP monomer and the dental substrate[44]. Possibly, the formed adhesive layer prevents the peroxide from reach the pulp chamber, especially when exposed to the low concentration gels.

The results of this in vitro study must be carefully examed and concluded[45], since in clinical situations there are various physiological conditions affecting the HP intrapulpal concentration such as, intrapulpal pressure of the dentinal fluid, the presence of peroxidase and catalase enzymes, cytoplasmic extensions of odontoblasts and other intratubular componentes [8,46]. Still, within the limitations of this in vitro study, we recommend the use of low-concentrated agents for bleaching purposes in order to prevent high HP-intrapulpal concentrations. Yet, although restored teeth with resin-modified glass ionomer cement presented satisfactory results, its clinical applicability is still restricted. Therefore, bulk-fill or conventional bioactive composites could be the esthetic adhesive material of choice.

CONCLUSION

The intrapulpal concentration and penetration of hydrogen peroxide at the adhesive interface of teeth restored with bioactive composites, conventional or bulk-fill, were higher when exposed to high concentration bleaching (35% HP).

.

REFERENCES

1. Ubaldini AL, Baesso ML, Medina Neto A, Sato F, Bento AC, Pascotto RC (2013) Hydrogen peroxide diffusion dynamics in dental tissues *Journal of Dental Research* **92(7)** 661-5.
2. Hanks CT, Fat JC, Wataha JC, Corcoran JF (1993) Cytotoxicity and dentin permeability ofcarbamide peroxide and hydrogen peroxide vital bleaching materials in vitro *Journal of Dentistry Research* **72** 931– 8.
3. Gokay O, Yilmaz F, Akin S, Tuncbilek M, Ertan R (2000) Penetration of the pulp chamber bybleaching agents in teeth restored with various restorative materials *Journal of Endodontics* **26** 92– 4.
4. Benetti AR, Valera MC, Mancini MN, Miranda CB, Balducci I (2004) In vitro penetration ofbleaching agents into the pulp chamber *International Endodontics Journal* **37** 120–4.
5. Marson FC, Gonçalves RS, Silva CO, Cintra LT, Pascotto RC, Santos PH, Briso AL (2015) Penetration of hydrogen peroxide and degradation rate of different bleaching products *Operative Dentistry* **40** 72-79.
6. Bowles WH, & Ugwuneri Z (1987) Pulp chambre penetration by hydrogen peroxide following vital bleaching procedures *Journal of Endodontics* **13(8)** 375-377.
7. Costa CA, Riehl H, Kina JF, Sacono NT, & Hebling J (2010) Human pulp responses to in-office tooth bleaching *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* **109(4)** e59-e64.
8. Hanks CT, Fat JC, Wataha JC, & Corcoran JF (1993) Cytotoxicity and dentin permeability of carbamide peroxide and hydrogen peroxide vital bleaching materials, in vitro *Journal of Dental Research* 72(5) 931-938.
9. Soares DG, Ribeiro AP, Sacono NT, Coldebella CR, Hebling J, Costa CA (2011) Transenamel and transdentinal cytotoxicity of carbamide peroxide bleaching gels on odontoblast-like MDPC-23 cells *International of Endodontics Journal* **44(2)** 116-25.
10. Lima AF, Ribeiro AP, Soares DG, Sacono NT, Hebling J, de Souza Costa CA (2013) Toxic effects of daily applications of 10% carbamide peroxide on odontoblast-like MDPC-23 cells *Acta Odontol Scand* **71(5)** 1319-25.
11. Robertson WD, Melfi RC Pulpal response to vital bleaching procedures (1980*)* *Journal of Endodontics* **6(7)** 645-9.

.

12. Matis BA, Mousa HN, Cochran MA, Eckert GJ (2000) Clinical evaluation of bleaching agents of different concentrations *Quintessence International* **31(5)** 303-10.
13. Briso AL, Fagundes TC, Gallinari MO, Moreira J, de Almeida L, Rahal V Gonçalves RS, Santos PD (2016) An in situ study of the influence of staining beverages on color alteration of bleached teeth *Operative Dentistry* **41(6)** 627-633.
14. Soares DG, Basso FG, Pontes EC, Garcia Lda F, Hebling J, de Souza Costa CA (2014) Effective tooth-bleaching protocols capable of reducing H(2)O(2) diffusion through enamel and dentine Journal of Dentistry **42(3)** 351-8.
15. Cintra LT, Benetti F, da Silva Facundo AC, Ferreira LL, Gomes-Filho JE, Ervolino E, Rahal V, Briso AL (2013) The number of bleaching sessions influences pulp tissue damage in rat teeth *Journal of Endodontics* **39(12)** 1576-80.
16. Cavalli V, Silva BG, Berger SB, Abuna G, Marson FC, Tabchoury C, Giannini M (2017) Effect of Adhesive Restoration and Bleaching Technique on the Concentration of Hydrogen Peroxide In the Pulp Chamber *Operative Dentistry* **42(2)** e44-E54.
17. Owens BM, Rowland CC, Brown DM, Covington JS (1998) Postoperative dental bleaching: effect of microleakage on Class V tooth colored restorative materials *Journal of the Tennessee Dental Association* 78(4) 36-40.
18. Ikemura K, Tay FR, Endo T, Pashley DH (2008) A review of chemical-approach and ultramorphological studies on the development of fluoride-releasing dental adhesives comprising new pre-reacted glass ionomer (PRG) fillers *Dental Materials Journal* **27(3)** 315-39.
19. Wiegand A, Buchalla H, Attin T (2007) Review on fluoridereleasing restorative materials-Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation *Dental Materials* **23** 343-362.
20. Sadr A, Shimada H, Lu H, Tagami J (2009) The viscoelastic behavior of dental adhesives A nanoindentation study *Dental Materials* **25** 13–9.
21. Gale MS, Darvell BW (1999) Thermal cycling procedures for laboratory testing of dental restorations. *J Dent* Bristol **27** 89-99.
22. Queiroz CS, Hara AT, Paes Leme AF, Cury JA (2008) pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization *Braz Dent J* **19** 21-27.

.

23. Berger SB, Tabchoury CP, Ambrosano GM, Giannini M (2013) Hydrogen peroxide penetration into the pulp chamber and dental permeability after bleaching *General Dentistry* **61(3)** e21-e25.
24. Benetti AR, Valera MC, Mancini MN, Miranda CB, & Balducci I (2004) In vitro penetration of bleaching agents into the pulp chamber *International Endodontics Journal* **37(2)** 120-124.
25. Kwon SR, Wertz PW, Li Y, Chan DC (2012) Penetration pattern of rhodamine dyes into enamel and dentin: confocal laser microscopy observation *Int J Cosmet Sci* **34(1)** 97-101.
26. Costa CA, Riehl H, Kina JF, Sacono NT, & Hebling J (2010) Human pulp responses to in-office tooth bleaching *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology Endodontics* **109(4)** e59-e64.
27. Moncada G, Sepulveda D, Elphick K, Contente M, Estay J, Bahamondes V, Fernandez E, Oliveira O, & Martin J (2013) Effects of light activation, agente concentration, and tooth thickness on dental sensitivity after bleaching *Operative Dentistry* **38(5)** 467-476.
28. Cintra LT, Benetti F, Ferreira LL, Gomes-Filho JE, Ervolino E, Gallinari Mde O, Rahal V, Briso AL (2016) Penetration Capacity, Color Alteration and Biological Response of Two In-office Bleaching Protocols *Brazilian Dental Journal* **27(2)** 169-75.
29. Camargo SE, Valera MC, Camargo CH, Gasparoto Mancini MN, Menezes MM (2007) Penetration of 38% hydrogen peroxide into the pulp chamber in bovine and human teeth submitted to office bleaching technique *Journal of Endodontics* **33(9)** 1074-7.
30. Llena C, Martínez-Galdón O, Forner L, Gimeno-Mallench L, Rodríguez-Lozano FJ, Gambini J (2018) Hydrogen Peroxide Diffusion through Enamel and Dentin *Materials (Basel)* 12;**11(9)** pii: E1694.
31. Cadenaro M, Breschi L, Nucci C, Antoniolli F, Visintini E, Prati C, Matis BA, Di Lenarda R (2008) Effect of two in office whitening agents on the enamel surface in vivo a morphological and non-contact profilometric study *Operative Dentistry* **33(2)** 127-134.
32. Kwon SR, Pallavi F, Shi Y, Oyoyo U, Mohraz A, Li Y (2018). Effect of bleaching gel viscosity on tooth whitening efficacy and pulp chamber penetration: an in vitro study *Operative Dentistry* **43(3)** 326-334.

.

33. Ayres APA, Berger SB, Carvalho AO, Giannini M (2016) Efeito do peróxido de hidrogênio na permeabilidade dental *Revista Brasileira de Odontologia* **73(2)**: 96-100.
34. Gökay O, Tunçbilek M, Ertan R (2000) Penetration of the pulp chamber by carbamide peroxide bleaching agents on teeth restored with a composite resin *Journal Oral Rehabilitation* **7(5)** 428-31.
35. Klein CA Jr, da Silva D, Reston EG, Borghetti DL, Zimmer R (2018) Effect of At-home and In-office Bleaching on Marginal Microleakage in Composite Resin Restorations using Two Adhesive Systems *Journal of Contemporary Dental Practice* **19(3)**:248-252.
36. Piemjai M, Lenglerdphol S (2018) Effect of strong tooth-bleaching with 38% hydrogen peroxide on marginal seal of dental restorations using self-etch and total-etch adhesives *Journal of Esthetic and Restorative Dentistry* **30(2)**:153-159.
37. Hashemikamangar SS, Ghavam M, Mahinfar N, & Kharazi Fard MJ (2014) Effect of 30% hydrogen peroxide on marginal integrity of silorane-based versus methacrylate- based composite restorations *Journal of Dentistry of Tehran University of Medical Sciences* **11(5)** 545-553.
38. Lin SS, Gurol MD (1998) Catalytic decomposition of hydrogen peroxide on iron oxides: kinetics, mechanism and implication *Environmental Science and Technologic* **32** 1417-1423.
39. Barata TJE, Bresciani E, Adachi A, Fagundes TC, Carvalho CAR, Navarro MFL (2008) Influence of ultrasonic setting on compressive and diametral tensile strengths of glass ionomer cements *Mater Res* **11(1)**:57-61.
40. Kimyai S, Bahari M, Naser-Alavi F, Behboodi S (2017) Effect of two different tooth bleaching techniques on microhardness of giomer *Journal of Clinical and Experimental Dentistry* **9(2)** e249-e253.
41. Garoushi S, Vallittu PK, Lassila L (2018) Characterization of fluoride releasing restorative dental materials *Dental Materials* **37(2)** 293-300.
42. Yamaguchi R, Powers JM, Dennison JB (1989). Thermal expansion of visible-light-cured composite resins *Operative Dentistry* **(14)** 64-7.
43. Sidhu SK, Carrick TE, McCabe JF (2004) Temperature mediated coefficient of dimensional change of dental tooth-colored restorative materials *Dental Materials* **20(5)** 435-40.

.

44. Mena-Serrano A, Kose C, De Paula EA, Tay LY, Reis A, Loguercio AD (2013) A new universal simplified adhesive: 6-month clinical evaluation *Journal of Esthetic and Restorative Dentistry* (**25)** 55–69.
45. Vongsavan K, Vongsavan N, Matthews B (2000) The permeability of the dentine and other tissues that are exposed at the tip of a rat incisor *Archives of Oral Biology* **45(11)** 927-30.
46. Kina JF, Huck C, Riehl H, Martinez TC, Sacono NT, Ribeiro AP, Costa CA (2010) Response of human pulps after professionally applied vital tooth bleaching *International Endodontics J* **43(7)**572-80.

CONFLICT OF INTEREST

The authors report that they have no financial, professional nor other interest of any nature on the divulgation of the data herein present.

.

3 CONCLUSÃO

De acordo com os resultados obtidos no estudo in vitro, foi possível concluir que:

- A concentração intrapulpar e a penetração de HP na interface adesiva dos dentes restaurados com compósitos bioativos, convencional ou bulk-fill, foram maiores quando expostos ao clareamento de alta concentração (PH 35%).

.

REFERÊNCIAS*

Benetti AR, Valera MC, Mancini MN, Miranda CB, Balducci I. In vitro penetration ofbleaching agents into the pulp chamber. Int Endod J 2004;37:120–4.

Bowles WH, & Ugwuneri Z. Pulp chambre penetration by hydrogen peroxide following vital bleaching procedures. J Endod. 1987; 13(8) 375-377.

Braga RR, Ballester RY, Ferracane JL. Factors involved in the development of polymerization shrinkage stress in resin-composites: a systematic review. Dent Mater. 2005; 21;(10):962-70.

Briso AL, Fagundes TC, Gallinari MO, Moreira J, de Almeida L, Rahal V Gonçalves RS, Santos PD. An In Situ Study of the Influence of Staining Beverages on Color Alteration of Bleached Teeth. Oper Dent. 2016; 41(6) 627-633.

Cavalli V, Silva BG, Berger SB, Abuna G, Marson FC, Tabchoury C, Giannini M. Effect of Adhesive Restoration and Bleaching Technique on the Concentration of Hydrogen Peroxide In the Pulp Chamber. Oper Dent . 2017; 42(2) e44-E54.

Costa CA, Riehl H, Kina JF, Sacono NT, & Hebling J. Human pulp responses to in-office tooth bleaching Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology. 2010;109(4):e59-e64.

Fronza BM, Rueggeberg FA, Braga RR, Mogilevych B, Soares LE, Martin AA, Ambrosano G, Giannini M. Monomer conversion, microhardness, internal marginal adaptation, and shrinkage stress of bulk-fill resin composites. Dent Mater. 2015;31(12):1542-51.

Gokay O, Yilmaz F, Akin S, Tuncbilek M, Ertan R. Penetration of the pulp chamber by bleaching agents in teeth restored with various restorative materials. J Endod 2000; 26:92-94.

.

Hanks CT, Fat JC, Wataha JC, Corcoran JF. Cytotoxicity and dentin permeability of carbamide peroxide and hydrogen peroxide vital bleaching materials in vitro. J Dent Res 1993;72:931– 8.

Ikemura K, Tay FR, Endo T, Pashley DH. A review of chemical-approach and ultramorphological studies on the development of fluoride-releasing dental adhesives comprising new pre-reacted glass ionomer (PRG) fillers. Dent Mater J. 2008; 27(3) 315-39.

Marson FC, Gonçalves RS, Silva CO, Cintra LT, Pascotto RC, Santos PH, Briso AL. Penetration of hydrogen peroxide and degradation rate of different bleaching products. Oper Dent. 2015;40(1):72-9.

Matis BA, Mousa HN, Cochran MA, Eckert GJ. Clinical evaluation of bleaching agents of different concentrations. Quintessence Int. 2000; 31(5): 303-10.

Mena-Serrano AP, Parreiras SO, do Nascimento EM, Borges CP, Berger SB, Loguercio AD, Reis A. Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber. Oper Dent. 2015;40(2):E76-82.

Owens BM, Halter TK, Brown DM. Microleakage of tooth-colored restorations with a beveled gingival margin Quintessence Int. 1998; 29(6) 356-61.

Polydorou O, Hellwig E, Auschill TM. The effect of at-home bleaching on the microhardness of six esthetic restorative materials. J Am Dent Assoc . 2007;(138): 978-984.

Sadr A, Shimada H, Lu H, Tagami J. The viscoelastic behavior of dental adhesives A nanoindentation study. Dent Mater. 2009; (25): 13–9.

Soares DG, Basso FG, Pontes EC, Garcia Lda F, Hebling J, de Souza Costa CA. Effective tooth-bleaching protocols capable of reducing H(2)O(2) diffusion through enamel and dentine. J Dent. 2014; 42(3): 351-8.

.

Ubaldini AL, Baesso ML, Medina Neto A, Sato F, Bento AC, Pascotto RC. Hydrogen peroxide diffusion dynamics in dental tissues. J Dent Res. 2013; 92(7) 661-5.

Wang L, Francisconi LF, Atta MT, Dos Santos JR, Del Padre NC, Gonini A Jr, Fernandes KB. Effect of bleaching gels on surface roughness of nanofilled composite resins. Eur J Dent 2011; (5):173-179.

White DJ, Duschner H, Pioch T. Effect of bleaching treatments on microleakage of Class I restorations J Clin Dent. 2008;19(1):33-36.

Wiegand A, Buchalla H, Attin T. Review on fluoridereleasing restorative materials-Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. Dent Mater. 2007; (23): 343-362.

.

APENDICE 1 – Delineamento Experimental e metodologia ilustrada

DELINEAMENTO EXPERIMENTAL:

Tabela 01. Delineamento experimental do estudo.

Unidades experimentais	Coroas de incisivos bovinos (n=10)	
Fatores em estudo	Géis clareadores	PH 35% (Pola Office, SDI, Austrália) PH 9,5% (Pola Day, SDI, Austrália)
	Materiais Restauradores	6. Compósito bioativo convencional (Beautifill II, Shofu, Kyoto, Japão) 7. Compósito bioativo bulk-fill (Activa BioActive Restorative, Pulpdent Corporation, MA, EUA) 8. Compósito convencional (Filtek Z350 XT, 3M Oral Care, St. Paul, MN, EUA) 9. Compósito bulk-fill (Filtek Bulk-fill, 3M Oral Care) 10. Cimento de ionômero de vidro modificado por resina (Riva Light Cure, SDI).
Variável resposta	- Concentração intrapulpar de agentes clareadores (µg/ML) aplicado às restaurações adesivas - Morfologia da interface adesiva das restaurações adesivas submetidas aos agentes clareadores, observado em microscopia confocal de fluorescência por varredura a laser.	

.

METODOLOGIA ILUSTRADA

Preparo das amostras

Incisivos bovinos foram coletados, limpos e armazenados em solução de timol 0,1% a 30ºC por 30 dias. Cem coroas foram selecionadas com dimensões padronizadas (4,0 ± 0,1 mm) da superfície vestibular (esmalte e dentina). As raízes foram cortadas com discos diamantados (KG Sorensen, Barueri, Brasil) até 2 mm abaixo da junção cemento-esmalte. O tecido pulpar foi removido utilizando limas (Hedstrom, Maillefer Dentsply, Ballaigues, Suíça), e a câmara pulpar lavada com água destilada. O orifício cervical foi ampliado com ponta diamantada esférica (nº 1016, KG Sorensen), permitindo que o tampão acetato fosse colocado na câmara pulpar.

Cavidades (4 mm de diâmetro x 3 mm de profundidade) foram preparadas na superfície vestibular das amostras com pontas diamantadas (nº 3053 e nº 3017, KG Sorensen). A aplicação do sistema adesivo e materiais restauradores seguiram as instruções do fabricante. O adesivo foi aplicado somente nas cavidades que foram restauradas com resinas compostas, seguida da fotopolimerização por 20 s com o dispositivo de LED (1200 mW / cm2, Valo, Ultradent Products Inc., Sul da Jordânia, EUA) (Figura 01).

Os compósitos bulk-fill (Activa Bio-Active e Filtek bulk-fill) foram inseridos nas cavidades em incrementos únicos, enquanto os convencionais (Beautifil II e Filtek Z350), com incrementos de 2 mm (Figure 02). Para restaurações de ionômero de vidro modificadas por resina, o Riva Conditioner foi aplicado, seguido de manipulação e inserção do cimento (Figure 03). O acabamento e polimento das restaurações foram realizados com pontas extra-finas (3118 FF, KG Sorensen) e discos de lixa (Sof-Lex, 3M Oral Care), cada granulação foi utilizada por 15 s (média, fina e superfina).

.

Figura 01. Preparo das amostras.

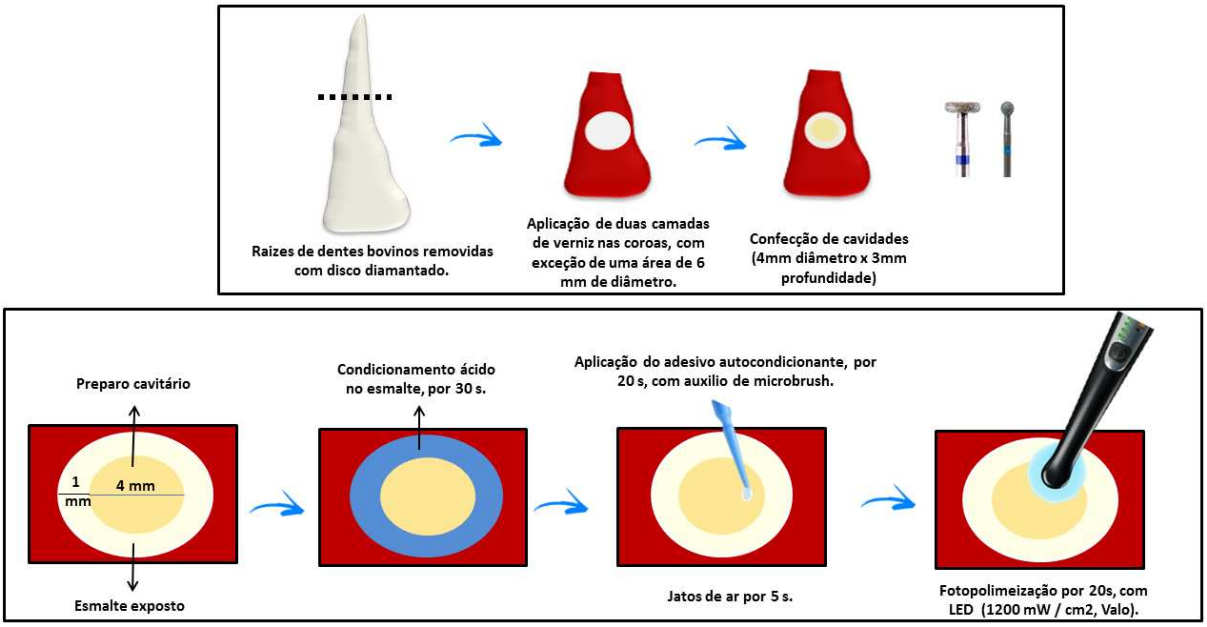


Figura 02. Restauração dos preparos cavitários com materiais restauradores convencionais.

Figura 03. Restauração dos preparos cavitários com materiais restauradores bulk-fill.

.

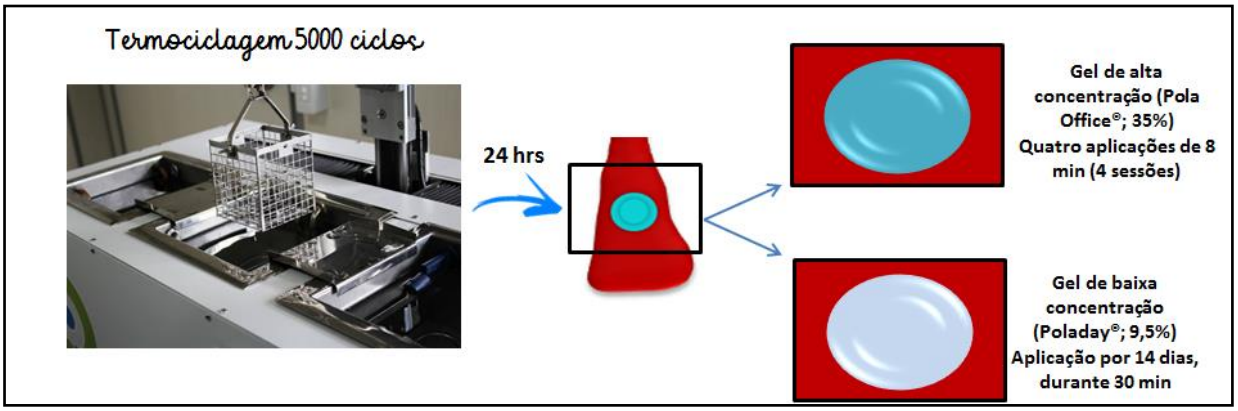
Figura 04. Restauração dos preparos cavitários com Cimento de Ionômero de Vidro Modificado por Resina.

Ciclagem Térmica e Clareamento das amostras

Para o envelhecimento da interface adesiva, os espécimes foram submetidos a 5.000 ciclos térmicos (MCT2 - AMM, São Paulo, SP, Brasil) em banhos de água desionizada a 5 ° a 55 ° C ± 1 ° C, correspondendo a 6 meses de envelhecimento.

Após 24 h de ciclos térmicos, os espécimes restaurados foram submetidos aos agentes clareadores de alta (37,5% de peróxido de hidrogênio) ou de baixa concentração (peróxido de hidrogênio a 9,5%). Portanto, cada material restaurador foi exposto ao clareamento de alta ou baixa concentração (n = 10). Entre as aplicações, os dentes foram armazenados a 37ºC, imersos em saliva artificial (tampão Tris 20 mM, pH 7,0, Ca 1,5 mM, P 0,9 mM, KCl 150 mM, 0,05 µg F / mL) (Figura 05).

Figura 05. Ciclagem térmica e protocolo do clareamento de alta e baixa concentração.



.

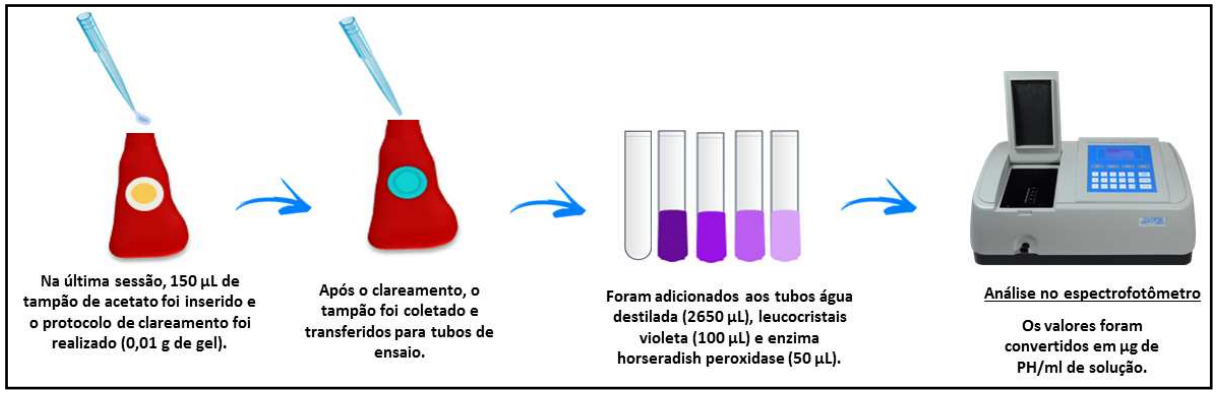
Concentração Intrapulpar do Peróxido de Hidrogênio

O clareamento de alta concentração (35% HP) foi realizado em 4 sessões de 8 min cada, com intervalos de 7 dias entre as sessões. Os agentes de baixa concentração (9,5% HP) foram aplicados por 14 dias e 30 minutos/dia. Na última sessão do clareamento, a câmara pulpar foi seca e 150 µL de solução tampão acetato 2M (pH 4,5) foram colocados dentro da câmara coronária. Posteriormente, 0,01 g do agente clareador de alta concentração foram aplicados na superfície vestibular por 8 min e quatro aplicações consecutivas. O agente de baixa concentração (0,01 g) permaneceu em contato com a superfície do esmalte por 30 min (Figura 6).

Após o procedimento de clareamento, a solução foi removida e transferida para um tubo de ensaio, e em seguida, adicionados água deionizada (2650 µL), leucocristal violeta (100 µL de 0,5 mg/mL, Sigma-Aldrich, St. Louis, MO, EUA) e enzima peroxidase (50 µL de 1 mg/mL; Sigma-Aldrich) (Figura 06).

Como resultado, uma solução azul foi obtida, permitindo a medição da densidade óptica em um espectrofotômetro (DU 800, Beckman Coulter Inc., Brea, CA, EUA) no comprimento de onda de 596 nm. Para este propósito, uma curva padrão com concentrações conhecidas de HP foi usada para converter os valores de densidade óptica em microgramas (µg) de HP/mL de solução. Os valores foram então convertidos em microgramas por mililitro (Figura 06).

Figura 06. Análise intra-pulpar da concentração do Peróxido de Hidrogênio.



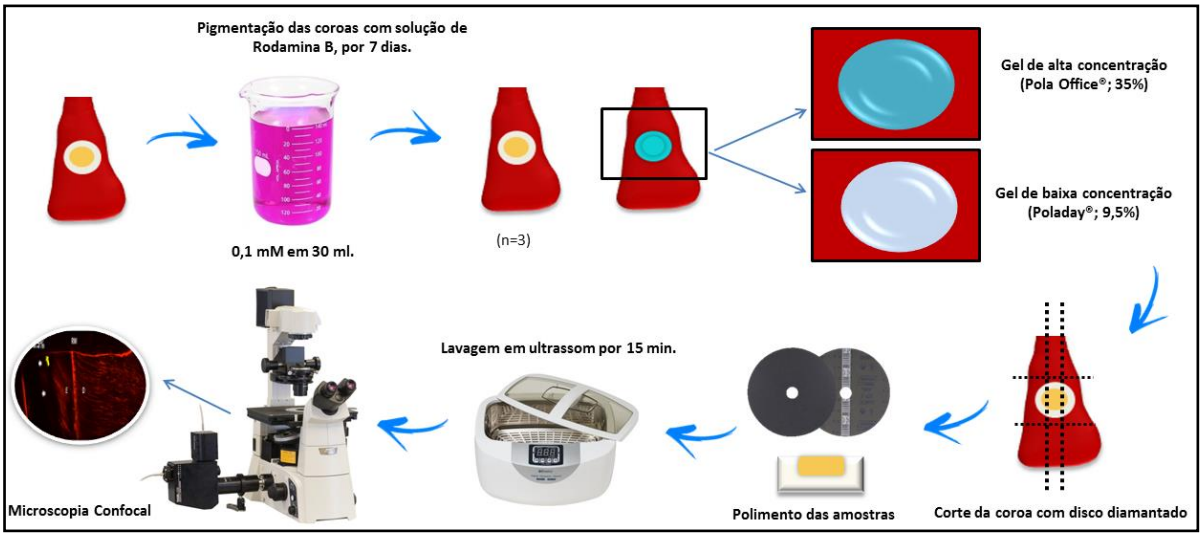
.

Microscopia Confocal de Varredura por Fluorescência a Laser (MCVFL)

Para observar a difusão do HP na interface adesiva, amostras adicionais de cada grupo (n = 3) foram preparadas e submetidas a protocolos de clareamento de alta ou baixa concentração. As coroas foram totalmente imersas em 30 ml de solução de 0,1 mM de Rodamina B (Aldrich Chem. Co) durante 7 dias. Posteriormente, foram realizados os protocolos de clareamento em consultório ou caseiro, com exceção do grupo controle, que permaneceu sem branqueamento. O centro das restaurações foram seccionadas no eixo transversal com discos diamantados (Isomet 1000, Buehler, Lake Bluff, IL, EUA).

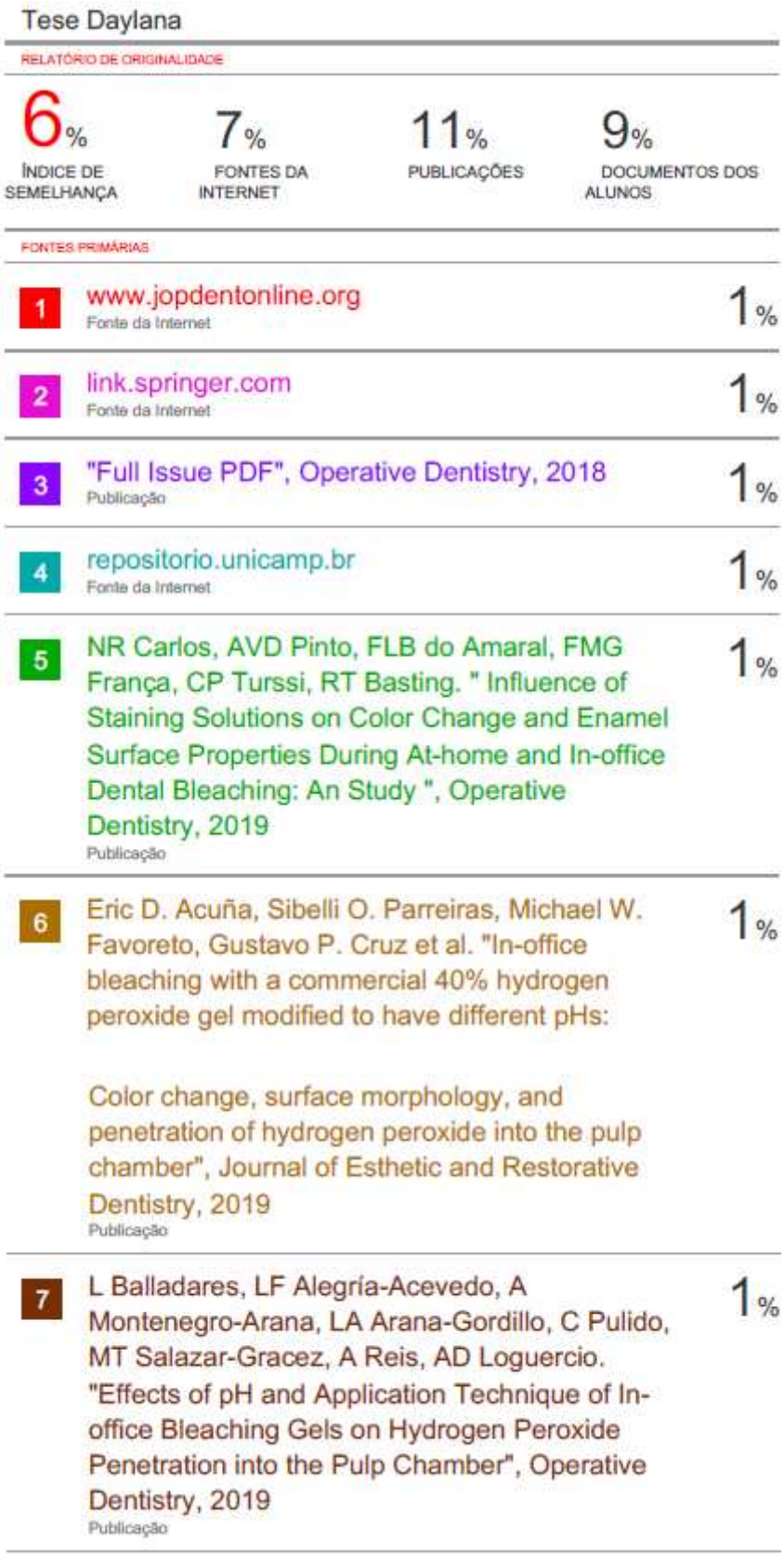
A face interna foi polida (EcoMet 3000, Buehler, Lake Bluff, Illinois, EUA) com papéis abrasivos (granulações nº 400, 600 e 1200) e os espécimes foram imersos em óleo vegetal e imersos em cuba ultrassônica por 15 min para a remoção completa os resíduos de polimento. A interface foi analisada por microscópio de varredura a laser de argônio (TCS SP5AOBS, Leica Microsystems CMS GmbH, Alemanha) com comprimento de onda de 543 µm, com a objetiva de 20x, em modo de varredura ao longo de toda a extensão da amostra.

Figura 07. Preparo das amostras para análise de microscopia de fluorescência confocal com varredura a laser.



.

ANEXO 1 - Relatório do Turnitin



Tese Daylana

RELATÓRIO DE ORIGINALIDADE

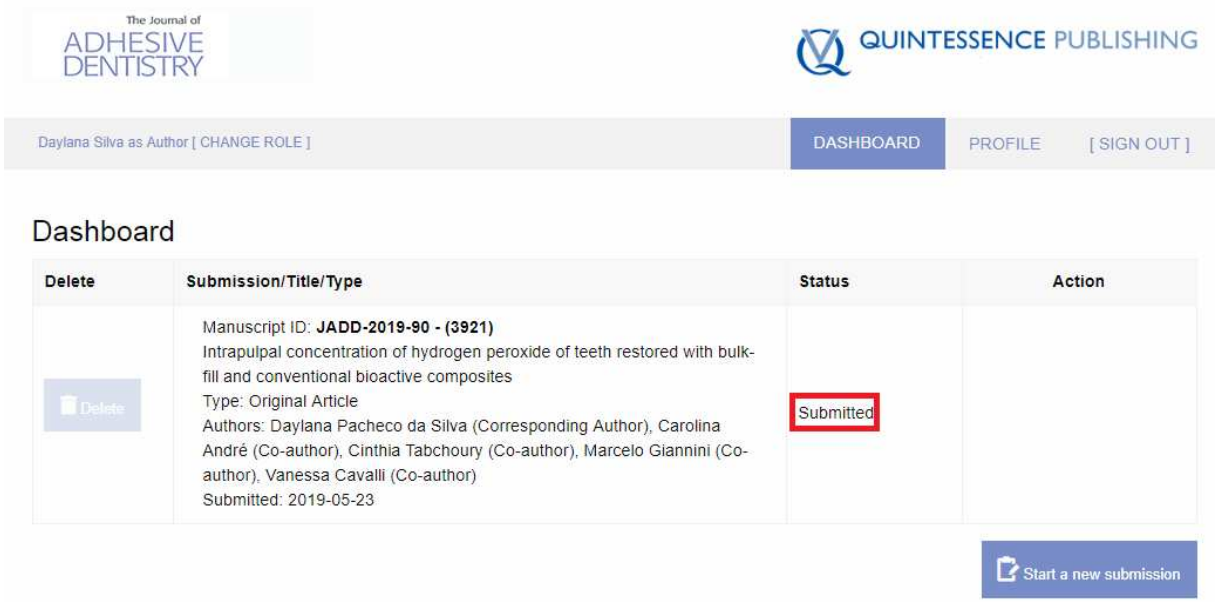
6%	7%	11%	9%
ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS

FONTES PRIMÁRIAS

1	www.jopdentonline.org Fonte da Internet	1%
2	link.springer.com Fonte da Internet	1%
3	"Full Issue PDF", Operative Dentistry, 2018 Publicação	1%
4	repositorio.unicamp.br Fonte da Internet	1%
5	NR Carlos, AVD Pinto, FLB do Amaral, FMG França, CP Turssi, RT Basting. " Influence of Staining Solutions on Color Change and Enamel Surface Properties During At-home and In-office Dental Bleaching: An Study ", Operative Dentistry, 2019 Publicação	1%
6	Eric D. Acuña, Sibelli O. Parreiras, Michael W. Favoreto, Gustavo P. Cruz et al. "In-office bleaching with a commercial 40% hydrogen peroxide gel modified to have different pHs: Color change, surface morphology, and penetration of hydrogen peroxide into the pulp chamber", Journal of Esthetic and Restorative Dentistry, 2019 Publicação	1%
7	L Balladares, LF Alegría-Acevedo, A Montenegro-Arana, LA Arana-Gordillo, C Pulido, MT Salazar-Gracez, A Reis, AD Loguercio. "Effects of pH and Application Technique of In-office Bleaching Gels on Hydrogen Peroxide Penetration into the Pulp Chamber", Operative Dentistry, 2019 Publicação	1%

.

ANEXO 2 - Comprovante de submissão do artigo



.