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Intrapulpal Concentration of Hydrogen Peroxide of Teeth Restored With Bulk Fill and Conventional Bioactive Composites

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Clinical Relevance

Using a 35% hydrogen peroxide bleaching agent in-office increases the concentration and diffusion of hydrogen peroxide into the pulp chamber compared to a low-concentration (9.5%) hydrogen peroxide gel.

SUMMARY

This study evaluated intrapulpal concentration and hydrogen peroxide (HP) penetration at the interface of teeth restored with bioactive composites, using conventional or bulk-fill composites. Cylindrical cavities were prepared on the buccal surface of bovine incisor crowns (n=20) and restored with: resin modified glass-ionomer (RMGI, Riva Light Cure, SDI), non-bioactive bulk-fill composite (FB, Filtek Bulk, 3M Oral Care), non-bioactive conventional composite (FZ, Filtek Z350, 3M Oral Care), bioactive bulk-fill composite (AC, Activa BioActive, Pulpedent), and bioactive conventional composite (BII, Beautifil II, Shofu). After 5,000 thermal cycles, restorations (n=10)

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were exposed to high (35% HP [4 applications of 8 min/session-4 sessions]) or low (9.5% HP [30 min/day-14 days]) concentration bleaching protocols. After the last bleaching application, the HP intrapulpal concentration was determined. Additional teeth were restored, pigmented with rhodamine B solution, and HP penetration around the interface was observed under laser scanning confocal fluorescence microscopy (LSCFM, n=3). The presence of gaps at the interface was observed on replicas of the cross-sectioned samples under scanning electron microscopy (SEM, n=5). Data were submitted to one-way (gap analysis) and two-way analysis of variance (ANOVA; HP intrapulpal concentration) and Tukey test (α=0.05). The LSCFM images were qualitatively analyzed. The restored teeth submitted to 35% HP presented higher HP intrapulpal concentration than teeth submitted to 9.5% HP (p<0.05). No differences in HP intrapulpal concentration was observed among groups (p>0.05) when exposed to 9.5% HP. Lower HP intrapulpal concentration was observed for teeth restored with RMGI exposed to HP 35%, when compared with teeth restored with non-bioactive conventional (FZ; p=0.004) and bulk-fill composites (FB; p=0.01). No gap formation was observed at the outer enamel adhesive interface for all restorative materials. LSCFM images showed that 35% HP promoted greater degradation of rhodamine B at the enamel, except for RMGI. In this context, RMGI promoted lower HP intrapulpal concentration than non-bioactive conventional and bulk-fill composites.

INTRODUCTION

The excellent aesthetic results provided by the bleaching agents are based on the trans-dentinal 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.[1,2] Thus, the resulting free radicals of HP potentially reach the pulp chamber, causing inflammatory responses, morphological changes to pulp tissue, and postoperative sensitivity.[2-4]

Tooth bleaching should be performed with caution, as the use of high concentrations of HP has shown to promote severe post-operative damages and cytotoxic effects.[5] However, studies indicate that even at low concentrations, bleaching agents could reduce pulp cell viability.[6,7] On the other hand, clinical studies have observed sporadic or transitory sensitivity after treatment with high and low concentrations of hydrogen peroxide.[8]

Clinically, pulp damage manifests as dental sensitivity, frequently leading the patient to cease bleaching.[9] Studies report that, in addition to the concentration, the length of time of an application directly influences the diffusion of the peroxide.[3,4,10] Thus, different protocols and concentrations of HP have been investigated in order to avoid unnecessary exposure and to decrease the intrapulpal concentration of hydrogen peroxide.[7,10,11] In this context, Marson and others[4] have previously observed that HP diffusion is dependent on the concentration and bleaching gel application protocol.[4]

Literature shows that restored teeth submitted to tooth whitening present a greater amount of HP in the pulp chamber than sound, non-restored teeth.[12] This occurs because the restorative interface becomes a path for peroxide microleakage.[12] In this regard, restorative materials have been developed to promote adequate marginal sealing and mechanical properties.[13] Although no restorative material completely blocks HP penetration, the diffusion of HP can be affected by polymerization shrinkage and the polymerization stress promoted at the adhesive interface of conventional composites.[3] Thus, in an attempt to obtain greater marginal integrity and to reduce clinical time and the polymerization stress, bulk-fill composites were developed, allowing increments of up to 4 mm thickness.[13]

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.[14] Studies have shown that the S-PRG-containing bioactive materials offer satisfactory biological properties, bacterial inhibition, biocompatibility, and good marginal sealing.[13-15] To make bioactive materials even more applicable, a new bulk-fill composite (Activa BioACTIVE, Pulpdent) was formulated with an ionic resin matrix, allowing the release of not only fluoride but also calcium and phosphate.[16]

Bioactive or bulk-fill composites hypothetically present greater marginal sealing ability that may influence the diffusion of the HP into the pulp chamber.[12] Considering that these materials are used in posterior teeth (up to premolars with involvement of the buccal surface) that are possibly subjected to dental bleaching, the diffusion around existing restorations and concentration of HP into the pulp chamber must be evaluated. Therefore, this study evaluated the concentration and diffusion

of HP into the pulp chamber of teeth restored with a resin-modified glass ionomer (Riva Light Cure), a non-bioactive bulk-fill composite (Filtek Bulk Fill), a non-bioactive conventional incremental resin composite (Filtek Z350 XT), a bioactive bulk-fill composite (Activa BioACTIVE), and a bioactive conventional composite (S-PRG-based material-Beautifil II, Shofu). The null hypotheses tested were that 1) a higher concentration of the bleaching agent (35%) would not significantly influence the intrapulpal concentration of HP of restored teeth compared to a lower concentration (9.5%), and 2) the type of restorative material would not significantly influence the intrapulpal concentration of HP for both HP concentrations.

METHODS AND MATERIALS

Sample Preparation

Bovine incisors were collected, cleaned, and stored in a 0.1% thymol solution at 4ºC for 30 days. All crowns were examined under a stereomicroscope in order to discard teeth with surface defects. 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 thoroughly rinsed with deionized water. The cervical pulp orifice was enlarged with a spherical diamond tip bur (#1016, KG Sorensen) to allow for the placement of the acetate buffer solution into the pulp chamber.[12] The buccal enamel-dentin thickness was assessed and 100 crowns with total thickness of 4.0 ± 0.1 mm were selected.

Cylindrical cavities (4 mm diameter x 3mm depth) were manually prepared in the buccal surface of the specimens with diamond burs (3053 and 3017, KG Sorensen) in a high-speed handpiece (Kavo, Joinville, SC, SP Brazil) under water-cooling. A periodontal probe and a specimeter were used to measure the depth and diameter of the cavity during preparation. Five cavities were prepared per discarded diamond bur. The application of the adhesive system and restorative materials followed the manufacturers' instructions (Table 1). The adhesive system was previously applied only in cavities restored with the resin composites and light-cured for 20 seconds with a LED device (Valo, Ultradent Products Inc, South Jordan, UT, USA). Irradiance was measured using a spectroradiometer (1200 mW/cm^2, USB 4000, Ocean Optics, Dunedin, FL, USA). For resin-modified glass ionomer restorations, Riva Conditioner was applied, followed by manipulation, insertion of the cement, and light-curing for 20 seconds. The emitting end of the light source was placed parallel and as close as possible to the cavosurface margin, without touching the restorative materials. The operator used a blue-blocker eyeglass during the restoration procedure to correctly position the light-curing tip. The finishing and polishing of the restorations were performed with extra-thin tips (3118 FF, KG Sorensen), sandpaper discs (Sof-Lex, 3M Oral Care, St Paul, MN, USA) and each granulation was used for 15 seconds (medium, fine, and superfine). The specimens were stored in distilled water at 37ºC for 48 hours prior to the thermal cycling procedure.

Thermal Cycling and Bleaching Procedure

To age the adhesive interface, the specimens were submitted to 5,000 thermal cycles (MCT2-AMM, São Paulo, SP, Brazil) in deionized water baths at 5° to 55°C ± 1°C, corresponding to six months of aging.[12] Twenty-four hours after thermal cycling, two layers of nail polish (Revlon Inc, New York, NY, USA) were applied, covering all exposed enamel except for a 1-mm space around the restorations. Then, the restored teeth were submitted to high- (35% HP) or low-concentration (9.5% HP) bleaching agents (Table 2). Therefore, each restorative material would be exposed to high- or low-bleaching therapy (n=10). The low-concentration agent (9.5% HP) was applied daily for 14 days, and 35% HP was applied in four sessions at intervals of seven days. Specimens were stored in artificial saliva in between treatments (20 mM Tris buffer, pH 7.0, 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 μg F/mL),[17] at 37ºC, renewed every two days.

Concentration of Hydrogen Peroxide Into the Pulp Chamber

The bleaching agents (35% HP or 9.5% HP) were used following the manufacturer's instructions (Table 2). At the last bleaching application, the pulp chamber was dried and 150 μL of acetate buffer solution 2M (pH 4.5) was placed into the chamber in order to stabilize the HP that penetrated into the pulp throughout bleaching. Subsequently, 0.01 g of the high-concentration bleaching agent was applied on the buccal surface for 8 minutes in four consecutive applications, and 0.01 g of the low-concentration agent remained in contact with the enamel surface for 30 minutes.

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 minute, and this solution was transferred to the same glass tube. Deionized water (2650 μL), leuco crystal violet (100 μL of 0.5 mg/mL, Sigma-Aldrich, St. Louis, MO,

Table 1: *Composition and Manufacturer's Instructions of the Restorative Materials*

Materials (Abbreviation)	Commercial Names and Manufacturer	Composition	Manufacturer's Instructions
Polyacrylic acid conditioner	Riva conditioner (SDI Limited)	26% polyacrylic acid	After tooth preparation, apply the conditioner to the prepared surfaces. Leave for 10 s and rinse thoroughly with water. Remove excess water and keep it moist.
Dental adhesive (SBU)	Single Bond Universal (3M Oral Care)	10-MDP, phosphate monomer, dimethacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, water, initiators, silane	1) Selective acid etching (37% phosphoric acid) of enamel for 15 s 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.
Resin-modified glass ionomer (RMGI)	Riva Light Cure (SDI Limited)	Powder: Fluoroaluminosilicate glass. Liquid: Polycyclic acid, tartaric acid, polyacrylic acid	After enamel etching for 10 s with polyacrylic acid, followed by abundant rinsing and air-drying, apply and light-cure for 10 s.
Non-bioactive 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).
Non-bioactive 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).
Bioactive bulk-fill composites (AC)	Activa BioACTIVE (Pulpdent)	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).
Bioactive conventional composite (BII)	Beautifil II (Shofu)	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).

Abbreviations: AUDMA, aromatic urethane dimethacrylate; Bis-GMA, bisphenol A-glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA; urethane dimethacrylate; Bis-EMA; ethoxylate bisphenol-A-glycol dimethacrylate; s, second.

USA), and horseradish peroxidase (50 μL of 1 mg/mL; Sigma-Aldrich) were added to each tube.[18]

As a result, a colored solution was obtained, allowing the optical density measurement in a spectrophotometer (DU 800, Beckman Coulter Inc, Brea, CA, USA) at a wavelength of 596 nm. For this purpose, a standard curve with known HP concentrations was used to convert the optical density values into micrograms (μg) of HP/mL of solution. The values were then converted into micrograms per milliliter.[12,18]

Laser Scanning Confocal Fluorescence Microscopy (LSCFM)

To observe the HP diffusion at the adhesive interface and through enamel and dentin, additional specimens of each group were prepared as previously described, submitted to thermal cycles, and subsequently to either high- or low-concentration bleaching protocols (n=3). An additional control group, restored with a resin-modified glass ionomer, was added to this methodology and consisted of restored samples that

Table 2: *Bleaching Agent Composition and Manufacturer's Instructions*

Bleaching Agents	Commercial Name (Manufacturer, Location)	Composition	Manufacturer's Directions
35% hydrogen peroxide	Pola Office[a] (SDI Limited)	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[b]	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[a] (SDI Limited)	Bleaching gel: hydrogen peroxide (9.5%). Activator: additives (47%), gycerol (30%), water (20%), flavorings (0.1%). pH=5.7[b]	Indicated for at-home bleaching therapy, for 14 days. Apply the whitening gel to the tray 1 time for 30 minutes.

[a]*According to manufacturer's directions.*
[b]*Preliminary pH results obtained by the authors.*

remained unbleached, in order to confirm if rhodamine B solution would be able to diffuse through enamel and dentin.

The crowns were fully immersed in rhodamine B solution (Aldrich Chem. Co) for seven days at a concentration of 0.1 mM in 30 mL of deionized water. Subsequently, the in-office or at-home bleaching protocols were performed (except the control group). The center of the restorations was cross-sectionally cut with a diamond disc under vegetable oil irrigation (Isomet 1000, Buehler, Lake Bluff, IL, USA).

The inner face was polished (EcoMet 3000, Buehler) with abrasive papers (400, 600, and 1200 granulations), specimens were immersed in vegetable oil for 30 seconds and then immersed in an ultrasonic chamber for 15 minutes 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 nm (red channel-for rhodamine B) and 488 nm (green channel-dental tissues autofluorescence), with 20x objective lenses in scan mode, over the entire length from enamel to the bottom of the cavity. The green channel was used to confirm the focus of each image.

Internal Marginal Adaptation

Impressions of the polished cross-sectioned restored surfaces, regardless of the previous bleaching procedure, were taken using a polyvinyl siloxane material with light and heavy consistency (Express XT, 3M ESPE, St. Paul, MN, USA). After 24 hours, the impressions were poured with an epoxy resin (EpoxiCure, Buehler Ltd), to obtain the restoration replicas (n=5). The polymeric replicas were sputter-coated with gold (SCD050, Bal-Tec AG, Balzers, Liechtenstein) in order to analyze the presence of gaps between the restorative material and enamel/dentin under a scanning electron microscope (SEM) (JEOL, JSM-5600LV, Tokyo, Japan). For each specimen, approximately 15 images (100 × magnification) were obtained to observe the entire length of the restored cavity. Software (ImageJ, National Institutes of Health, Bethesda, MD, USA) was used to determine the sum length of debonded segments and the entire perimeter of each restoration, which were calibrated by the SEM image scale bar. The percentage of the internal marginal gap was obtained for each sample according to the following formula:

$$\% \text{ gap} = \frac{100\% \text{ x length of gap}}{\text{total length of restoration}}$$

Statistical Analysis

The exploratory analyses of results were submitted to the SPSS software (SPSS Inc., Chicago, IL, USA). The normal distribution and homoscedasticity of the values were conducted by Shapiro-Wilk and Levene tests, and log-transformed data were required for intrapulpal concentration and square root for the percentage of internal gap values. The results were then submitted to

one-way analysis of variance (ANOVA) (gap analysis), and two-way ANOVA (intrapulpal concentration) followed by the Tukey test, with a significance level set at 5%.

RESULTS

According to the two-way ANOVA and Tukey test, the concentration of the bleaching agents and the restorative materials tested influenced the intrapulpal concentration of peroxide (p=0.046, Table 3). The HP intrapulpal concentration was higher when the high-concentration agent (35% HP) was applied compared to the low-concentration agent (9.5% HP), regardless of the restorative material used (p<0.05). No differences in the HP intrapulpal concentration were observed among restorative materials (p>0.05) when exposed to the low-concentration agent (9.5% HP). The intrapulpal peroxide concentration following bleaching with 35% HP was higher in teeth restored with non-bioactive conventional (FZ; p=0.004) and bulk-fill (FB; p=0.01) composite resins compared to RMGI. No differences were observed among groups restored with the bioactive composites (both conventional and bulk-fill) and the other materials (p>0.05).

Representative images of LSCFM of each group are presented in Figure 1. The control group, with no bleaching procedures, exhibited rhodamine B dye penetration through enamel and dentin. However, half of the top enamel (in-depth) remained without staining even after 7 days in rhodamine B solution. This same pattern was observed in all samples, although some differences between restorative materials groups and bleaching agents were found. For both bleaching procedures, the RMGI pattern of rhodamine B staining remained similar to the unbleached control group, characterized by a bright red color. The amount of rhodamine B oxidized (loss of bright red) by 35% HP was higher compared to 9.5% HP for all other restorative materials tested. No difference was observed regarding rhodamine B oxidation by bleaching agents comparing non-bioactive and bioactive conventional and bulk-fill composites. No bleaching procedure was able to remove rhodamine B from dentin, possibly due to the degree of dye saturation in each tooth. The black spots in dentin, with no presence of green (dentin autofluorescence), captured during the scanning mode used, can indicate a lack of focus in this area. Regarding the bonding interface, no differences were observed between groups, and confocal images failed to identify gap formation with the parameters used.

The percentage of internal marginal gap formation is depicted in Table 4. AC presented the highest percentage of gap formation, except when compared to RMGI, which presented intermediate values. The gap formation for AC, however, was always set at the bottom of the cavity. Figure 2 shows that for all restorative materials, no gap formation was observed between the outer enamel and the bonding interface.

DISCUSSION

This study showed that high concentrations of bleaching agents influenced the amount of HP in the

Table 3: *Mean (Standard Deviation) of Hydrogen Peroxide Concentration (HP) into the Pulp Chamber (µg/mL) of Restored Teeth, Submitted to Low- and High-concentration Bleaching Agents*

Restorative Materials (Brand Name)	**9.5% HP**	**35% HP**
Resin-modified glass ionomer (RMGI -Riva Light Cure)	0.09 (0.06) Ba	0.27 (0.13) Ab
Non-bioactive bulk-fill composite (FB - Filtek Bulk-fill)	0.09 (0.03) Ba	0.51 (0.38) Aa
Non-bioactive conventional composite (FZ - Filtek Z350 XT)	0.07 (0.04) Ba	0.62 (0.55) Aa
Bioactive bulk-fill composites (AC - Activa BioACTIVE)	0.09 (0.04) Ba	0.42 (0.29) Aab
Bioactive conventional composite (BII - Beautifil II)	0.07 (0.02) Ba	0.43 (0.26) Aab

Abbreviations: HP, hydrogen peroxide.
Mean values followed by distinct letters differ statistically by 5%, according to two-way ANOVA and Tukey test. Uppercase letters compare bleaching agents (rows) and lowercase letters compare restorative materials (columns).

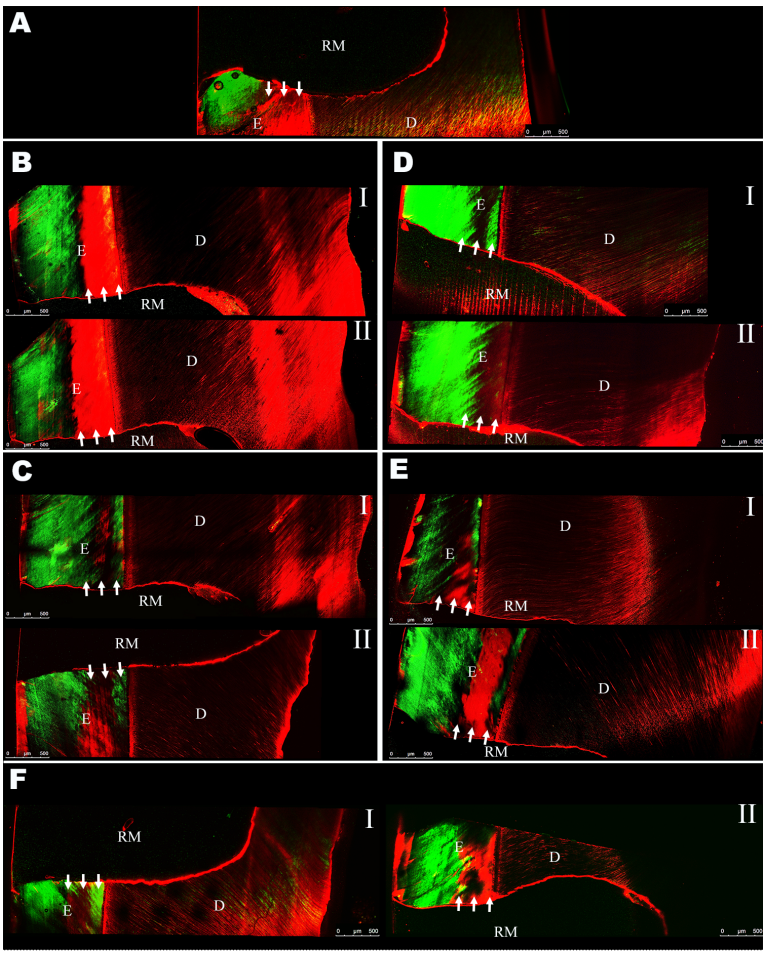

Figure 1. *Representative images of hydrogen peroxide at the interface of restored teeth under laser scanning confocal fluorescence microscopy. (A): Control group (unbleached restored teeth). (B): Resin-modified glass ionomer - Riva Light Cure. (C): Bulk-fill composite - Filtek Bulk-fill. (D): Conventional composite - Filtek Z350 XT. (E): Bulk-fill bioactive composite - Activa BioACTIVE. (F): Conventional bioactive composite - Beautifil II. (i): Bleached with 35% HP. (ii) Bleached with 9.5% HP. White arrows: indicate the oxidation, or not, of rhodamine B in the enamel area). Abbreviation: HP, hydrogen peroxide.*

Table 4: *Mean (Standard Deviation) Percentage of Internal Gap Formation*

Material (Brand Names)	**Gap (%)**
Resin-modified glass ionomer (RMGI -Riva Ligh- Cure)	16.90 (6.36) ab
Non-bioactive Bulk-fill Composite (FB - Filtek Bulk-fill)	12.52 (2.95) bc
Non-bioactive Conventional Composite (FZ - Filtek Z350 XT)	11.75 (5.54) bc
Bioactive Bulk-fill Composites (AC - Activa BioACTIVE)	27.11 (12.11) a
Bioactive Conventional Composite (BII - Beautifil II)	6.09 (3.04) c

Means followed by the same letter are not statistically different (p>0.05). n = 5 specimens/ group.

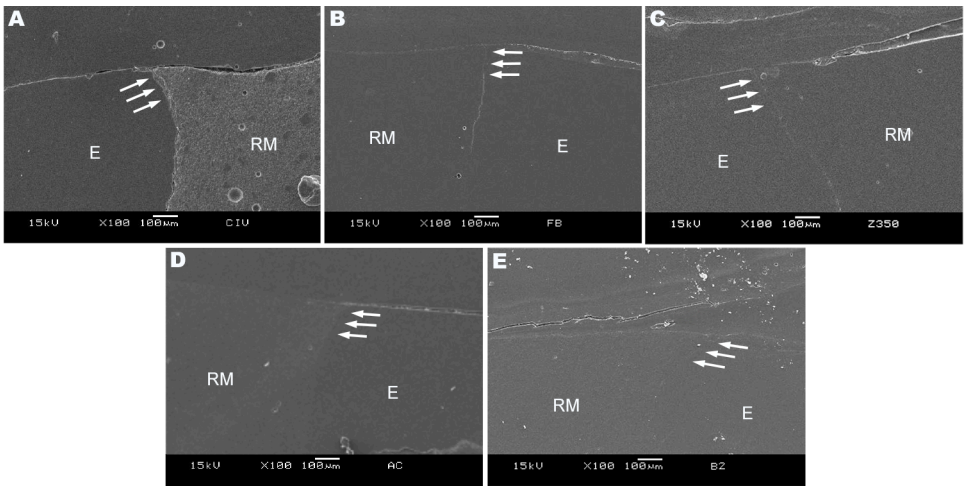

Figure 2. *Representative SEM images of enamel-restorative material interface. (A): Resin-modified glass ionomer - Riva Light Cure. (B): Bulk-fill composite - Filtek Bulk-fill. (C): Conventional composite - Filtek Z350 XT. (D): Bulk-fill bioactive composite - Activa BioACTIVE. (E): Conventional bioactive composite - Beautifil II. Abbreviations: SEM, scanning electron microscope.*

pulp chamber of restored teeth. Therefore, the first hypothesis was rejected. The results suggested that intrapulpal concentration of peroxide was directly related to the concentration of the bleaching agent, as observed previously.[10] In fact, the LSCFM images demonstrated greater degradation of rhodamine B by the high-concentration bleaching agent (35% HP), which possibly displays similar clinical paths of HP penetration.

According to Kwon and others,[19] the immersion of the specimens in rhodamine B for 7 days allowed adequate enamel, dentin, and dentinoenamel junction staining. Therefore, the black areas (interposed by the green channel) may display the paths of HP penetration, by the oxidation of rhodamine B. The spectrophotometry analysis indicated the presence of HP into the pulp chamber, but the images suggested degradation of rhodamine in enamel and low signs of degradation in dentin. This could be a limitation of the method, as the bleaching agent was not able to degrade the whole dye in dentin due to its saturation, but HP was able to reach the pulp chamber. Also, for 9.5% HP, the oxidation pattern of rhodamine B was different when comparing RMGI and the other resin composites tested. This difference can also suggest that RMGI may influence the HP penetration compared to the other materials when the lower concentration of HP is used, although no difference was observed spectrophotometrically.

Penetration of bleaching agents in the pulp chamber can be modulated according to peroxide concentration,[3,4,6,10,18] composition,[20] pH of bleaching gels,[21] presence of restorations,[12,22] and enamel thickness and morphological characteristcs.[23] Previous studies have emphasized that both the concentration and the application time are able to influence the diffusion of HP.[10,21] These results were confirmed by others, who observed that the longer the contact of the bleaching agent with enamel, the greater was the penetration into dental tissues.[4]

It has been observed that not only time, but the pH of bleaching agents influence the rate of HP dissociation. Alkaline bleaching gel increases the degradation rate of HP into reactive oxygen species (ROS), and this could reduce the risk of ROS penetration into the pulp tissue due to the short lifetime of oxygen species.[24,25] Besides, a bleaching agent with an acidic pH may decrease enamel microhardness, increase roughness, and possibly increase clinical sensitivity.[26] The pH of the bleaching agents used hereby exhibited acidic values for both high (pH=3.7) and low (pH=5.7) agents. Therefore, the pH of these agents may increase the intrapulpal concentrations of HP.

The rheological properties of the bleaching agents are among the factors that influence its behavior.[20,27] Kwon and others (2018) suggested that bleaching gels with higher viscosity promoted lower intrapulpal peroxide concentration than low and medium viscosity gels.[27] Therefore, in addition to the other positive aspects of the 9.5% HP agent (lower HP concentration, lower contact time, and higher pH), its greater viscosity could have contributed to the more favorable results.

The type of restorative material directly influenced the intrapulpal HP concentration, as RMGI showed lower penetration than the FB and FZ composites (non-bioactive), when exposed to the high-concentration agent. Based on this fact, the second null hypothesis was rejected. A previous study suggested that the presence of restorations and the use of high-concentration

bleaching agents allow the diffusion and greater amount of peroxide in the pulp chamber.[3] 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, which influence the polymerization stress.[3,13] Even though the literature is still controversial, previous reports observed that the presence of marginal microleakage in enamel restored with composite resin exposed to different concentrations of bleaching agents may favor the diffusion of the agent into the pulp.[28,29]

In this study, RMGI restorations submitted to 35% HP promoted lower HP intrapulpal concentration when compared with non-bioactive materials (FB and FZ). The presence of metal ions in the composition of RMGI (Al^{3+}, SiO_3^{2-}, Ca^{2+}) could have interacted with HP, resulting in the formation of oxides and hydroxyl radicals (OH-), increasing peroxide degradation immediately in the first contact of the restoration with HP.[30] In addition, previous studies have shown that RMGI (Riva Self Cure) presents good physical and mechanical properties due to the arrangement of its particles within the matrix, promoting good marginal integrity, thus lowering the risk of peroxide diffusion at the interface.[31] Although bioactive composites also release metal ions (Ca^{2+}, BO_3^{3-}, Sr^{2+}, Si_3^{2-}, Na^{+}, B^{3-}), these are trapped in charged particles inside a polymeric matrix, possibly impairing their releasing mechanism.

It should be noted that HP is able to alter the physical, mechanical, and esthetic properties of restorative materials. Contact with HP may influence the surface roughness and porosity of the composites,[32] promote crack formation, microhardness reduction[33], and change interface integrity.[34] Bleaching on S-PRG (BII)-based bioactive composites led to an undesirable effect on microhardness and marginal sealing of restorations.[34] However, in another finding, the stable mechanical properties of BII was observed, and these results were attributed to the presence of greater amounts of fillers, including large pre-polymerized (besides S-PRG) fillers.[35] This finding corroborates the fact that bioactive composites exhibit similar behavior to traditional systems against bleaching protocols.

The restorations were submitted to thermal cycles in order to age the interface and simulate a restoration in use. 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.[36] Therefore, the use of materials with a CTE close to the dental structure could potentially reduce the HP penetration. In this context, Sidhu and others (2004) observed that RMGI showed low dimensional changes due to a compensatory effect at high and low temperatures.[37] At high temperatures, a thermal contraction occurs 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,[37] and it was influenced by the amount of inorganic particles, matrix composition, and degree of polymerization.[36] Thus, RMGI possibly 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 for all the resin composites, which did not influence the material behavior. SBU provides adequate bond strength results due to the chemical interaction between the monomer (10-MDP) and the dental substrate.[38] Possibly, the formed adhesive layer prevented higher peroxide concentration from reaching the pulp chamber, confirmed by the integrity of enamel sealing in SEM images (Figure 2). Since enamel bonding is more reliable and durable than dentin bonding, and enamel is the site of interest of the present study, we believe that other adhesive systems could also provide similar results. In addition, the gap observed at the adhesive interface is more related to the resin composite shrinkage after polymerization than to the adhesive itself.[39,40]

Regarding marginal adaptation, most authors evaluate the use of bulk-fill composites in Class II restorations. Clinically, stress distribution at the restorative interface, such as gap formation, depends on the C factor, bonding quality, restorative material volume, and cavity geometry.[39-41] The failures occur mainly in the pulpal floor or in the internal angle of the cavity, as the polymerization contraction vectors point to the occlusal surface.[42-44] In this study, the bioactive bulk-fill composite (AC) indicated a higher gap percentage, which may be related to the fused consistency of this material, since it offers greater polymerization contraction and less inorganic content. In addition, the presence of low molecular weight monomers increases methacrylate group density and polymerization shrinkage.[43,45] However, the integrity of the enamel sealing might have prevented the internal gaps to influence the HP penetration results.

It is important to emphasize that this study has the inherent limitation of an *in vitro* evaluation, and the several physiological conditions affecting the HP diffusion, such as intrapulpal pressure of the dentinal fluid, the presence of peroxidase and catalase enzymes, and cytoplasmic extensions of odontoblasts, were not reproduced.[9-11] Moreover, bovine teeth are thicker and present smaller dentin tubules in comparison to human

teeth.[46] Since the higher permeability of human teeth results in greater susceptibility to hydrogen peroxide penetration, the present results should be cautiously extrapolated to clinical conditions.[47]

Still, within the limitations of this *in vitro* study, we recommend the use of low-concentration agents for bleaching purposes in order to prevent high HP-intrapulpal concentrations. Although restored teeth with resin-modified glass ionomer cement presented satisfactory results, the clinical applicability of this finding is restricted. Therefore, this study does not discourage the use of the other restorative materials tested.

CONCLUSION

In this study, the 35% HP in-office bleaching agent (HP) significantly increased the concentration of hydrogen peroxide into the pulp chamber compared to low-concentration HP gel (9.5%), regardless of the restorative material used. The intrapulpal concentration and diffusion of hydrogen peroxide at the interface of teeth restored with RMGI was significantly lower when exposed to 35% HP compared to the non-bioactive resin composites tested.

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Regulatory Statement

This research was conducted respecting the rules of the local Ethical and Research Committee and the policies of the University.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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