Effects of Bioactive Agents on Dentin Mineralization Kinetics After Dentin Bleaching

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Clinical Relevance

Dental bleaching treatment significantly decreases dentin bond strength. Bleached dentin remineralization treatment seems to effectively promote mineral formation, increase dentin bond strengths, and improve dentin's chemical affinity with adhesive monomers.

SUMMARY

Objectives: This study evaluated effects of Bioglass 45S5 (BG) and Biosilicate (BS) remineralization on the chemical composition and bond strength of control dentin (CD) and bleached dentin (BD) surfaces.

Methods and Materials: Dentin bleaching treatment was performed using the walking bleaching technique with 0.01 g of sodium perborate and 0.5 mL of 3% hydrogen peroxide for 14 days. Remineralization treatment was

carried out by rubbing a remineralization solution (0.015 g of BG or BS diluted in 1.35 mL of distilled water) on the etched dentin surface for 30 seconds. Micro-Raman spectroscopy (MRS) was used to quantitatively analyze the mineral matrix ratios of CD and BD (n=5) after remineralization treatment with BG and BS over 15 days of incubation in artificial saliva. The CD and BD discs (n=10) with and without remineralization treatment with BG and BS were restored using a two-step etch-and-rinse adhesive system (Optibond S, Kerr) and five layers of 1-mm-thick composite resin (Filtek Z250, 3M ESPE). The restored dentin discs were sectioned into nine bonded beams with cross-sectional areas of approximately 0.9 mm^2 and tested for microtensile bond strength (μTBS). The dentin surface of one fractured beam per tooth was submitted to MRS to characterize the physicochemical composition (n=10) at the interface. The data were analyzed using one-way analysis of variance and the Tukey-Kramer post hoc test ($p<0.005$).

Results: MRS bioactive analyses revealed that both BG and BS promoted increased mineral matrix ratios in the CD and BD. Significantly higher μTBS values were found after CD treatment with BG (CD: 57 MPa±11; CD-BG: 78

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MPa±15) and when BG and BS were applied to the BD (BD: 42 MPa±5; BD-BG: 71 MPa±14; BD-BS: 64 MPa±11) (p<0.005). The MRS analysis of the fractured dentin beam showed that the remineralization treatment significantly increased the dentin relative mineral concentration and promoted the appearance of new interface peaks, indicating a chemical interaction (p<0.005).

Conclusion: Remineralization of BD is an effective therapy to restore damage caused by dentin bleaching and acid conditioning. This approach not only increases dentin mineral compounds but also improves dentin's ability to interact chemically with the adhesive system.

INTRODUCTION

Restorative procedures for endodontically treated teeth remain challenging in clinical restorative dentistry due to the high risk of biomechanical failure.[1,2] Internal bleaching is a conservative option to improve the esthetics of discoloured endodontically treated teeth; however, it decreases tooth fracture strength and increases the failure rate of restorative procedures. Additionally, internal bleaching agents chemically interact with dentin compounds,[3] thereby promoting changes in the dentin's organic composition by denaturing the collagen proteins[4] and causing demineralization,[5] thus reducing its adhesion to restorative materials.[6]

The hybrid layer is defined as a three-dimensional polymer/collagen network with a continuous and stable bond between the adhesive monomers and dentin.[7] Previous studies have indicated that this ideal relationship is not achieved due to a discrepancy between the depths of dentin demineralization and adhesive infiltration, resulting in unprotected collagen fiber exposure and susceptibility to deterioration.[8,9] Loss of integrity of the hybrid layer may occur because of water-sorption-induced hydrolysis of the adhesive monomers and as a result of acidic conditioning, which activates collagen fibril degradation through endogenous matrix metalloproteinases (MMPs).[10-12]

The use of agents that promote dentin remineralization, such as calcium sodium phosphosilicate glass (Bioglass 45S5 [BG]: 24.5% Na_2O, 24.5% CaO, 45% SiO_2, 6% P_2O_2 — wt%)[13] and a fully crystalline glass-ceramic (Biosilicate [BS]: 23.75% Na_2O, 23.75% CaO, 48.5% SiO_2, 4.0% P_2O_5 — wt%),[14] has been shown to be an effective alternative for preserving adhesive interface integrity.[15,16] These bioactive compounds induce a progressive dehydration mechanism and replace extrafibrillar and intrafibrillar water, restoring the protective function of the collagen apatites[17-19] and promoting the formation of a hydroxyapatite layer on the dentin surface.[20-23]

Considering that no information is available regarding the use of remineralization agents on bleached dentin (BD) during restorative bonding steps, this study aimed to investigate the remineralization abilities of two bioactive materials and their potential therapeutic effects on the resin-dentin bond when they were used as remineralizing agents on BD. The following null hypotheses were tested: 1) no difference exists in the quantities of mineral components of control dentin (CD) and BD in the presence or absence of remineralization treatment, 2) the dentin bond strength is not altered after internal bleaching and/or remineralization treatment, and 3) dentin remineralization does not change the dentin-adhesive interface composition.

METHODS AND MATERIALS

Specimen Preparation

Eighty extracted unerupted human third molars were used following guidelines approved by the Local Ethics Committee (research protocol: 50615715.1.0000.0104). Using a low-speed diamond saw (Diamond Wheel 012"x fine, South Bay Technology Inc, San Clemente, CA, USA) under water cooling, a flat midcoronal dentin disc of 5 mm was prepared from each tooth. From these 70 dentin discs, 60 were set aside for microtensile bond strength (μTBS) testing and posterior physicochemical analysis using micro-Raman spectroscopy (MRS), while 20 were cut into four small pieces (2×2×5 mm) and used for bioactive tests. The specimens were divided into six groups based on the treatment protocol (Figure 1): 1) the CD group; 2) the CD-BG group, CD remineralized with a calcium sodium phosphosilicate glass (BG, Vitreous Materials Laboratory, São Carlos, Brazil); 3) the CD-BS group, CD remineralized with a fully crystalline glass-ceramic (BS, Vitrovita, São Carlos, Brazil); 4) the BD group; 5) the BD-BG group, BD remineralized with BG; and the BD-BS group, BD remineralized with BS.

Dentin bleaching treatment was performed by simulating the walking bleach technique using a paste made with 0.01 g of sodium perborate (Whiteness Perborato, FGM Produtos Odontológicos,
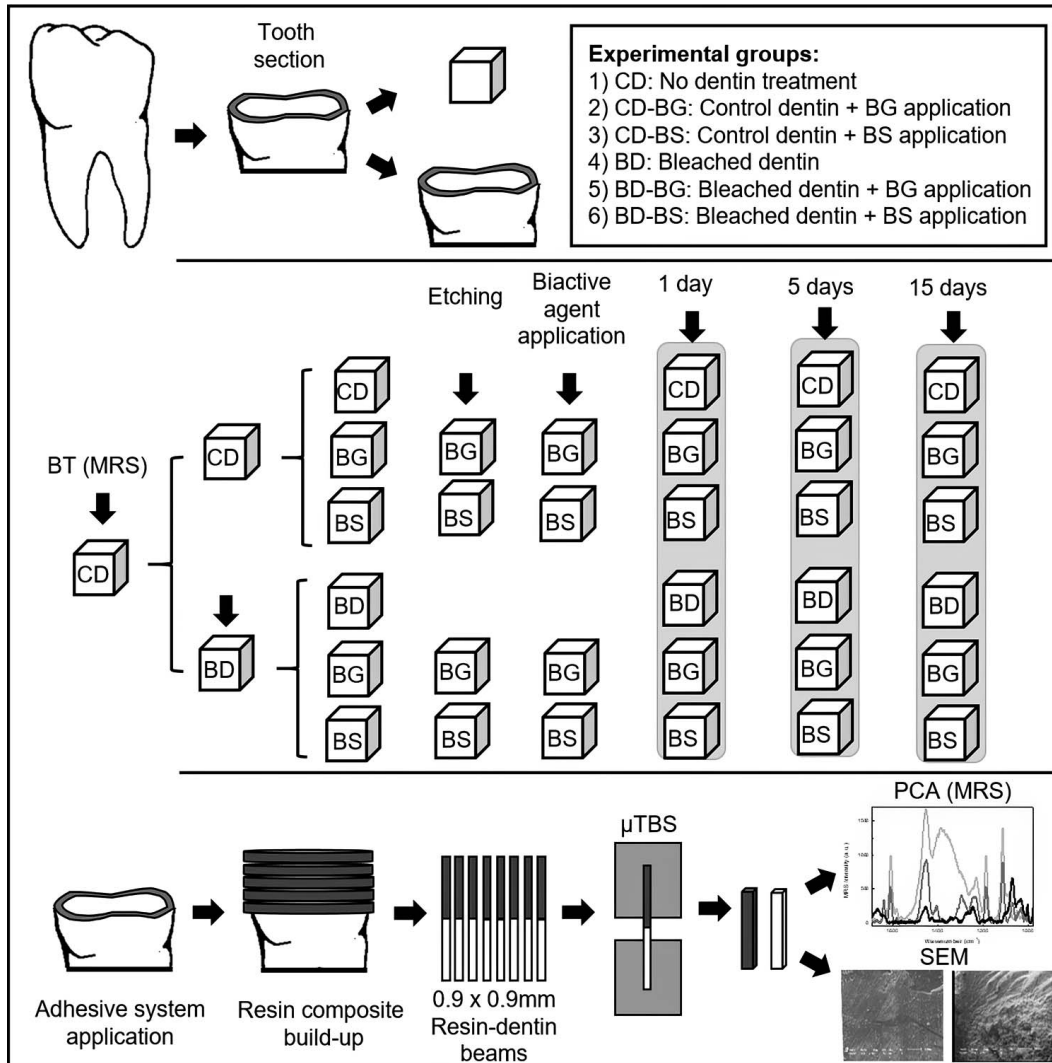
Figure 1.   *Diagram illustrating preparation of the specimens and the analysis sequence.*

Joinville, Brazil) and 0.5 mL of 3% hydrogen peroxide (Rioquímica, São Jose do Rio Preto, Brazil). During the 14 days of bleaching treatment, the specimens were stored in an incubator (ProLab, São Paulo, Brazil) with a 95% ± 5% relative humidity at 37°C, and the bleaching agent was replaced after the seventh day. After the bleaching treatment was finished, the dentin surfaces were washed with distilled water and air-dried for 2 seconds.

Remineralization treatment was performed with two different agents, including the gold standard remineralizing agent, BG,[13,24,25] and the experimental remineralizing crystalline glass-ceramic, BS.[24] The CD and BD surfaces were etched with 37% phosphoric acid (Condac37, FGM Produtos Odontológicos) for 10 seconds, followed by a copious water rinse for 1 minute. After acid conditioning, the

remineralization treatment was carried out to induce mineral bone formation before adhesive application. The remineralization solutions were prepared by diluting 0.1 mg of the remineralizing agent powder in 1 mL of deionized water immediately before application.[20] A micropipette (Monocanal VVCS-10, Digipet, São Paulo, Brazil) was used to apply 10 μl of this solution to the moist dentin surface. Using a microbrush, the solution was gently rubbed on the dentin for 30 seconds. The surface was then washed with deionized water for 15 seconds and gently dried with filter paper to remove excess water.

Bioactive Test

Bioactive analysis (n=10) was performed to investigate the ability of BG and BS to induce dentin

remineralization before or after bleaching treatment. MRS initial spectra were obtained before treatment (as a control group), after the procedures of bleaching and acid conditioning, and after the remineralization agent was applied. To enable the MRS analysis according to storage time after remineralization treatment, the specimens were adapted to fit into a plastic device and immersed in 25 mL of artificial saliva[9] in a 37°C water bath. Then, long-term analyses were carried out after 1 day, 5 days and 15 days of immersion. Before obtaining the spectra, the specimens were rinsed with distilled water for 30 seconds and air-dried.

Raman spectra were obtained with a confocal Raman microscope (Senterra Bruker Optik GmbH, Ettingen, Germany). After the samples were excited by a 785-nm laser source, spectra were recorded in the spectral range of 450 to 1800 cm^{-1}. The laser (power=100 mW) was focused onto the specimen with a 20× magnification lens. The spatial resolution was 3 to 5 cm^{-1}, and the integration time of the detector was 3 seconds. Each curve resulted from an average of 60 spectra. Reference points were determined on the specimen surface to enable the MRS spectra to be gathered at the same position before and after treatment, as well as during the long-term analyses. Four spectra were obtained for each measurement, and the final result for each specimen was calculated by the average of these four spectra.

The spectra were baseline-corrected, and the integrated areas of the dentin organic (amide I: 1650 cm^{-1}) and mineral compounds (phosphate v1: 961 cm^{-1}) were calculated. Then, the dentin structural modifications were characterized by the mineral matrix ratio (961 cm^{-1}/1650 cm^{-1}).[22] To standardize the graphic visual analyses, all ratios were normalized by the values of the control spectra. Additionally, the MRS spectra from BG and BS were obtained to characterize their chemical structures.

Restorative Procedures

An adhesive interface was created to enable μTBS and physicochemical analyses. For the groups in which dentin was tested as a control surface, restorative treatment was conducted immediately after specimen preparation. However, for the groups in which the dentin tissue was bleached, restorative treatment was carried out 14 days after the dentin bleaching treatment was completed to eliminate any residual by-products of the bleaching agent. During those 14 days, the BD specimens were stored in an incubator with a 95% ± 5% relative humidity at 37°C. Restorative procedures were performed ac-

cording to the manufacturer's instructions using a two-step etch-and-rinse adhesive (Optibond S, Kerr, Orange, CA, USA) and five layers of 1-mm-thick composite resin (Filtek Z250, 3M ESPE, AG Dental Products, Seefeld, Germany). Each layer was light-cured for 20 seconds at 1200 mW/cm^2 with a light-emitting diode unit (Radii, Cal-SDI, Bayswater, Australia). Specimens were stored in artificial saliva for seven days at 37°C before testing.

Microtensile Bond Strength Test (μTBS)

The restored dentin blocks (n=10) were longitudinally sectioned across their interfaces in both mesial-distal and buccal-lingual directions to obtain nine bonded beams with cross-sectional areas of approximately 0.9 mm^2. The exact width of each beam was measured with a digital caliper (Zaas Precision; Amatools, São Paulo, Brazil).

The position of the beams was equidistant between the jig claws and perpendicular to the tensile force. Each restoration interface was tested under 0.5 mm/min traction in a universal test machine (EZ Test, Shimadzu, Kyoto, Japan) until the moment of specimen fracture. The tensile force (newtons) was divided by the cross-sectional area in square millimeters to express the bond strength value in megapascals. The μTBS values were computed for each tooth by averaging the values of the nine resin-dentin beams from that tooth. Pretest failures were included in this average by assigning to it the minimum bond strength value found for the corresponding tooth.

The failure modes were evaluated using scanning electron microscopy (Superscan SS-550, Shimadzu) with 100× magnification. The fractured beam surfaces were placed onto aluminium discs and sputter-coated with a gold-palladium alloy (IC-50, Shimadzu). The failure patterns were classified as follows: 1) adhesive fracture when the fracture site was located between the adhesive and dentin; 2) mixed when the fracture involved the dentin-adhesive interface, including cohesive failure of the dentin and composite resin; 3) cohesive dentin fracture; and 4) cohesive fracture in the composite resin.

Physicochemical Analysis of the Fractured Dentin Interface

Physicochemical analysis of the fractured dentin interface was performed on the five tested beams that had the highest μTBS values per experimental group (n=5). The resin-dentin interface modifications due to bleaching and/or remineralization

treatment were analyzed through chemical mapping using the same equipment and configuration described for the bioactive analysis but with a 100×-magnification lens. A scan of the entire area of the fractured dentin (0.9 mm^2) was performed, and MRS mapping was carried out by selecting 10 points on both the X- and Y-axes with a distance of 0.09 mm between them. All spectra were collected systematically under the same conditions.

The MRS spectra were submitted to baseline correction, and an average spectrum was obtained per line on the X-axis of the spectral map. Dentin physicochemical modifications were investigated through analysis of the mineral matrix ratio (961 cm^{-1}/1650 cm^{-1}). Additionally, the Raman spectra of CD, BD and the adhesive system were visually compared with the average spectra obtained from the evaluated fractured interfaces. Comparison of the spectra demonstrated the appearance of three new MRS bands in the dentin interface spectra; these bands were not identified in the CD and BD spectra or in the adhesive spectra. These new MRS bands were centered at 1295 cm^{-1} (C-H bonds),[26] 1405 cm^{-1} (methylene CH$_2$),[27] and 1637 cm^{-1} (methacrylate monomer).[10] The areas of the new bands were measured and normalized to the area of the amide I band of each respective spectrum.

Statistical Methods

Statistical analyses were performed using R i386 3.0.2 software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria). The ratios obtained by the bioactive test and physicochemical analysis were submitted to descriptive statistics and were presented in graphics with their means and standard deviations. The μTBS data were statistically analyzed with the Shapiro-Wilk normality test, two-way analysis of variance and the Tukey-Kramer post hoc analysis (α=0.05).

RESULTS

MRS ratios obtained from the bioactive test are illustrated in Figure 2A,B. The mineral matrix ratio (961 cm^{-1}/1650 cm^{-1}) demonstrated that remineralization treatment with both agents improved the mineral content of BD after the acid conditioning step. The relative mineral composition of the CD remained constant during the 15 days of incubation (Figure 2A), showing that immersion in artificial saliva did not promote mineral formation on the CD surface. Bleaching treatment promoted a reduction in the dentin relative mineral content, which was not restored to its initial value after 15 days of artificial
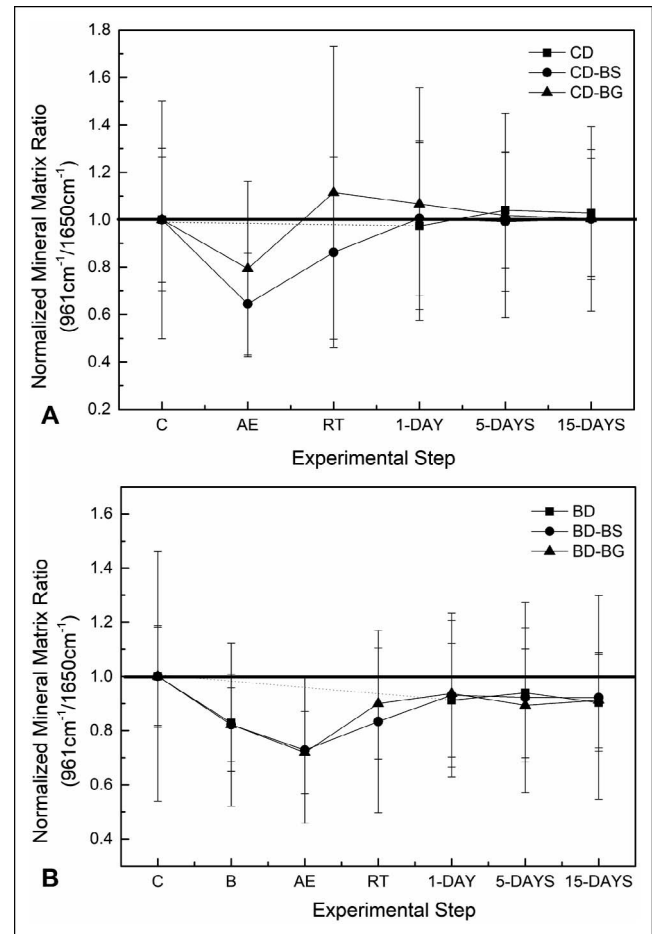

Figure 2.   *Mineral matrix ratio (961 cm^{-1}/1650 cm^{-1}) obtained from the bioactive test under different experimental conditions: B. before any treatment, initial measurement; BL, after bleaching treatment; 1-Day, after one day of artificial saliva incubation; 5-Days, after five days of artificial saliva incubation; 15-Days. after 15 days of artificial saliva incubation AE. after etching; RT, right after remineralization treatment;. (A): Remineralization using a calcium sodium phosphosilicate glass. (B):Rremineralization using a fully crystalline glass-ceramic. Bleaching treatment reduced the dentin relative mineral content. Artificial saliva immersion did not change the mineral composition of CD or BD. The acid conditioning step reduced the mineral compositions of CD and BD. Remineralization treatment using BG and BS improved the mineral contents of CD and BD immediately after agent application in groups CD-BG, BD-BG, and BD-BS and required one day of artificial saliva incubation for CD-BS.*

saliva immersion (Figure 2B). The acid-conditioning step reduced the mineral composition of the CD (Figure 2A) and intensified the loss of the mineral composition of the BD (Figure 2B). Nevertheless, remineralization with BG and BS restored this mineral loss. Mineral deposition on the CD occurred immediately after remineralization treatment with BG (CD-BG), required one day of incubation when BS solution was used (CD-BS), and was stable for both groups after 15 days of artificial saliva incubation (Figure 2A). BD mineral restoration was

Table 1: *Effect of Bleaching and Remineralization Treatment on Dentin Microtensile Bond Strength (MPa) According to Experimental Groups*

Group	Mean±SD	Premature failure	Total Beams*
CD	57.4±11.3	5	85 AB
CD-BG	78.5±15.2	0	90 C
CD-BS	73.7±14.7	0	90 BC
BD	42.2±5.5	10	80 D
BD-BG	70.9±13.7	3	87 BC
BD-BS	63.8±11.0	5	85 BC

Abbreviations: CD, control dentin; CD-BG, control dentin with bioglass; CD-BS, control dentin with crystalline glass-ceramic; BD, bleached dentin; BD-BG, bleached dentin with bioglass and BD-BS, bleached dentin with crystalline glass-ceramic.
* Different capital letters indicate the significant statistical difference ($p<0.05$), after two-way analysis of variance and Tukey tests.

more intense shortly after the BG and BS remineralization treatments (BD-BG and BD-BS) and presented continuous behavior after 15 days of incubation. Although the remineralization approach improved the mineral content of the BD, this treatment was not capable of restoring the dentin mineral components to their initial values (Figure 2B).

Microtensile Bond Strength Test

The μTBS results are shown in Tables 1 and 2 . The statistical analysis indicated that bleaching treatment significantly decreased the dentin μTBS values (CD: 57.4±11.3 MPa; BD: 42.2±5.5 MPa) ($p<0.05$). Remineralization treatment with BG promoted a significant increase in the CD μTBS values (57.4±11.3 MPa; CD-BG: 78.5±15.2 MPa) ($p<0.05$), and both BG and BS treatments resulted in significantly higher BD μTBS values (BD:

Table 2: *Failure Modes Distribution According to Experimental Groups*

Group	Failure Mode, n (%)[a]			
	1	2	3	4
CD	40 (47)	33 (39)	7 (8)	5 (6)
CD-BG	50 (56)	36 (40)	2 (2)	2 (2)
CD-BS	48 (54)	36 (40)	4 (4)	2 (2)
BD	45 (56)	30 (38)	3 (4)	2 (2)
BD-BG	37 (42)	42 (48)	5 (6)	3 (4)
BD-BS	43 (50)	30 (36)	8 (9)	4 (5)

Abbreviations: CD, control dentin; CD-BG, control dentin with bioglass; CD-BS, control dentin with crystalline glass-ceramic; BD, bleached dentin; BD-BG, bleached dentin with bioglass and BD-BS, bleached dentin with crystalline glass-ceramic.
[a] Failure modes: 1, adhesive failure; 2, mixed failure; 3, cohesive failure in the dentin; 4, cohesive failure in composite resin.

Table 3: *Two-way Analysis of Variance*

Source	df	Type III Sum of Squares	Mean Square	F	p*
Bleaching treatment	1	1786.9	1786.9	11.71	0.001
Remineralization treatment	2	6733.21	3366.6	22.06	<0.001
Interaction	3	154.07	77.03	0.5	0.6

Abbreviation: df, degrees of freedom.
* $p<0.05$ indicates significant difference.

42.2±5.5 MPa; BD-BG: 70.9±13.7 MPa; BD-BS: 63.8±11.0 MPa) ($p<0.05$). The 2-way ANOVA (Table 3) indicated a significant effect for the bleaching ($p=0.001$) and the remineralization treatment ($p<0.001$). The interaction term was not significant ($p=0.60$). Scanning electron microscopy analysis of the fractured surfaces revealed that most failures in groups CD, CD-BG, and CD-BS were at the adhesive interface, while the failure patterns were mostly mixed for group BD-BG (Table 2). The types of failures in all experimental groups are illustrated in Figure 3A through D.

Physicochemical Analysis of the Fractured Dentin Interface

A qualitative comparison of the spectra of the dentin surface, adhesive system, and fractured interface is illustrated in Figure 4. Physicochemical analysis of the fractured dentin beam showed an increased mineral matrix ratio (961 cm^{-1}/1650 cm^{-1}) at the interfaces in groups CD-BG and BD-BS (Figure 5A). The new MRS peak ratio analyses revealed that the C-H band (1295 cm^{-1}) was most evident among the new peaks and presented a higher value in the groups treated with the remineralizing agents (CD-BG, BD-BG, and BD-BS) (Figure 5B).

DISCUSSION

This study showed higher dentin mineral matrix ratios when the CD and BD tissues were treated with both of the tested bioactive agents. Dental bleaching treatment significantly decreased the dentin bond strength; however, the mineral formation induced by remineralization treatment not only increased the BD mineral content but also mediated dentin bond strengths. The fractured dentin surface analysis proved that mineral deposition was improved due to remineralization agent application and demonstrated that the dentin tissue presented a greater chemical affinity with adhesive monomers after the remineralization approach. Remineraliza-
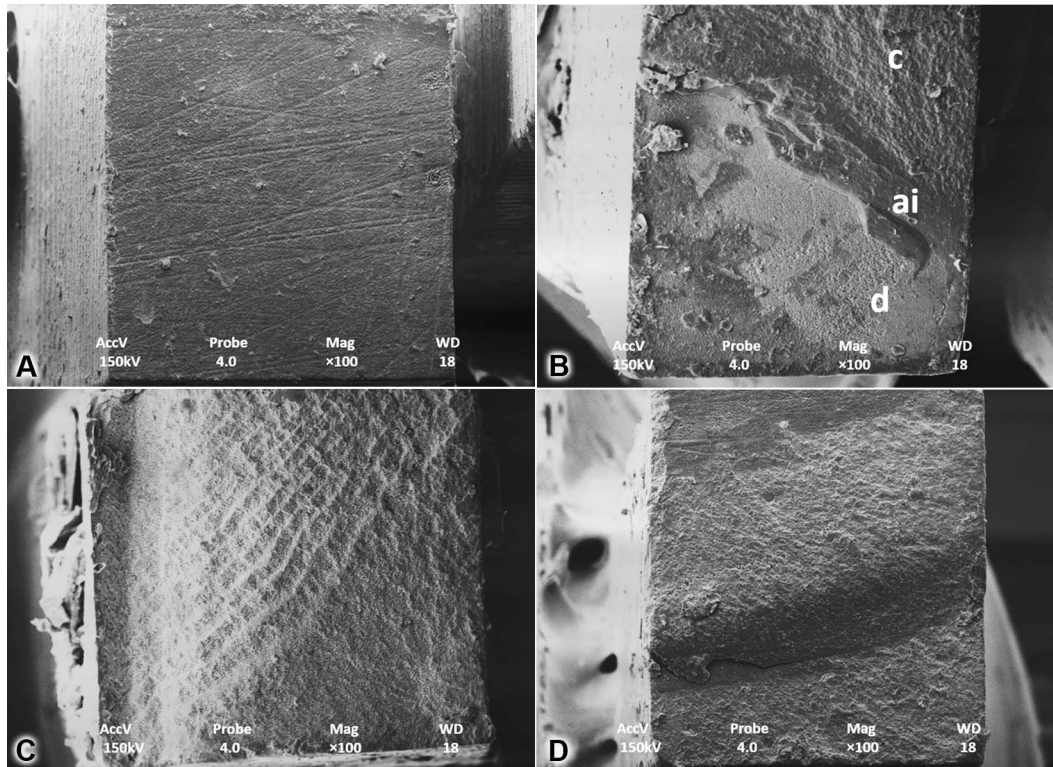
Figure 3. *Scanning electron microscop failure analysis of the fractured specimens (×100). (A) Adhesive failure along the dentin surface for CD-BG. (B) Mixed failure for CD with a fracture at the dentin surface (d) and in the adhesive interface (a), and cohesive failure in the composite resin (c). (C) Cohesive failure within dentin for CD. (D) Cohesive failure within the composite resin for BD-BS.*

tion treatment with BG demonstrated better results as it increased both the CD and BD bond strengths and improved tissue chemical interactions with adhesive monomers. This is the first study to test BD remineralization effects.

Compared with other bioactive materials, BG presents the highest bioactivity index (IB=12.5) and is still considered the gold standard agent.[13,24,25] BS was developed with the goal of combining the high bioactivity of BG with the good mechanical strength and toughness of glass-ceramics.[24] Based on the results of this study, both BG and BS can be used to restore dentin mineral compounds after internal bleaching. The two tested agents promoted mineral deposition on demineralized dentin and BD, increased the BD bond strength, and improved the chemical interactions between this mineral tissue and adhesive monomers. Nevertheless, only BG yielded significant results for CD bond strength and physicochemical interactions with the adhesive system.

Bioactive glass and glass-ceramic remineralization processes involve the exchange of ions (Si^{4+}, OH^-, Na^+, Ca^{2+}, PO_4^{3-}) between the glass silicate network and the surrounding fluid body.[13,14] This process induces calcium phosphate (Ca/P) precipitation and its subsequent crystallization into hydroxycarbonate apatite on the mineral tissue surface.[13,22,28] Glass dissolution during the remineralization reaction depends on the presence of an aqueous medium.[17,24] Considering that fluoride is not a functional compound for biomineralization,[11] artificial saliva was selected as the aqueous medium to simulate the oral environment because its ionic composition is comparable to that of plasma.[29]

Dentin consists of a heterogeneous tissue with high water content, and its remineralization is a challenging process not only because of its mineral and organic composition but also because of its tissue design, that is, the intrafibrillar orientation of the minerals in the collagen network.[14] Improvement of the dentin mechanical properties depends on extra-fibrillar deposition, particularly intrafibrillar minerals, as a consequence of biomimetic remineralization.[30] Mineral deposition around the denuded collagen fibrils protects the resin-dentin interface from water and enzymatic degradation, restores dentin mineral compounds, and consequently improves tissue dynamic behavior.[31]

The bioactive test results led to rejection of the first null hypothesis of the study as the CD and BD remineralization treatments with BG and BS demonstrated increases in the dentin mineral contents. The mineral content of the CD specimens was reduced after the etching step; however, this loss was restored one day after the remineralization treatments were performed. The improved mineral matrix ratio corroborates the findings of an attenuated total reflection Fourier transform infrared spectroscopy study that confirmed the BG remineralization effect on a partially demineralized dentin surface during a seven-day treatment.[22] This long period of time required for evidence of mineral deposition to emerge may be explained by the use of a 0.5-mM ethylenediamine tetra-acetic acid solution rather than a phosphoric acid gel during the demineralization step. Additionally, the ability of BS to induce hydroxycarbonate apatite precipitation on the dentin surface was also evaluated by a Fourier transform infrared spectroscopy study, which was found to occur one day after remineralization treatment.[17]

The application of bleaching agents to dentin tissue may promote changes in the dentin surface morphology and structure.[32] Modifications of the dentin structure not only change the ratio between its organic and inorganic components[33] but also increase dentin collagen degradation by activation of MMPs,[4] which then decrease dentin bonding efficacy.[5,32] Although remineralization treatment of BD with bioactive agents has not been tested previously, different studies[34,35] have evaluated the use of BG and BS as desensitizing agents after enamel bleaching and concluded that remineralization treatment reduced enamel mineral loss and preserved enamel surface integrity.

Dentin bleaching treatment resulted in a decrease in the mineral matrix ratio that was accentuated by the acid conditioning step. Application of both bioactive agents generated the remineralization effect one day after the treatment was completed, restoring the dentin structural components that were lost due to acid etching. Nevertheless, this mineral formation was not sufficient to reestablish the components present in dentin before the bleach-
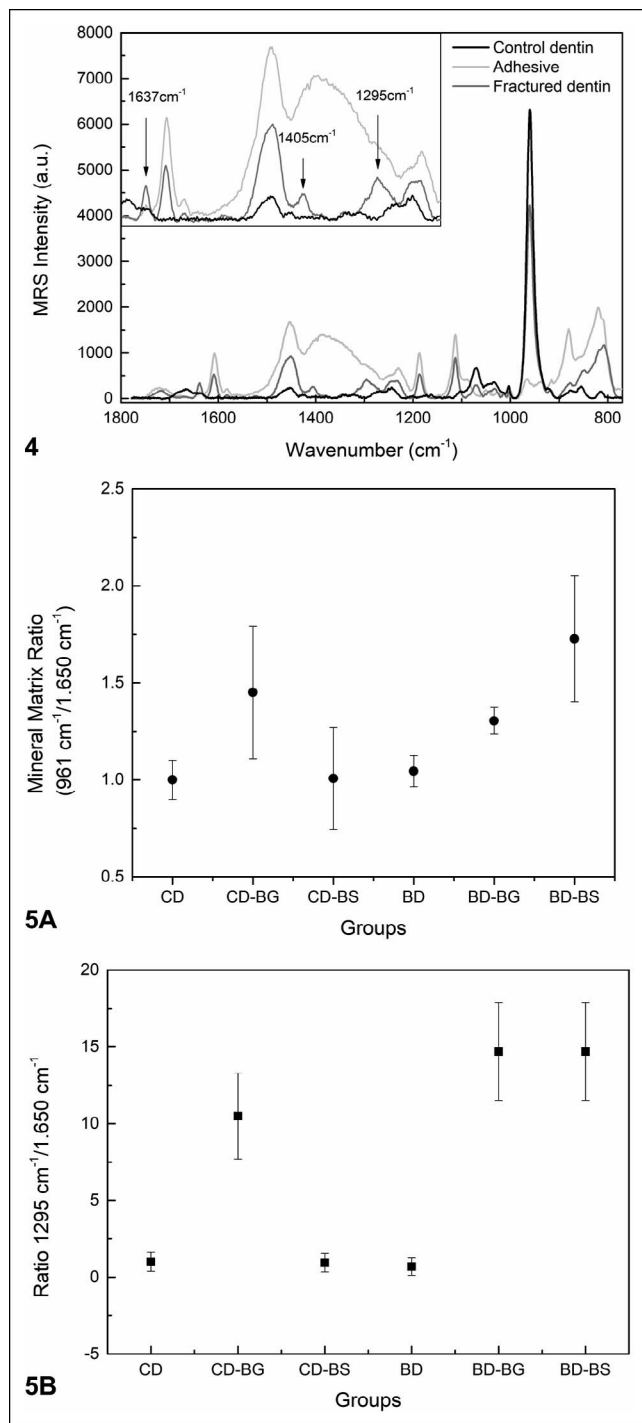

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5A

5B

Figure 4. *Comparison of the MRS spectra of the adhesive system, CD and fractured dentin (interface average spectrum). The new MRS bands are indicated by the black arrows, C-H (1295 cm^{-1}), CH$_2$ of methylene (1405 cm^{-1}) and C=C of methacrylate bonds (1637 cm^{-1}).*

Figure 5. *The mean values (DP) of the fractured dentin MRS physicochemical composition. The vertical axis represents the normalized intensity of the assessed rates, and the horizontal axis presents the experimental groups. (A): Mineral matrix ratio (961 cm^{-1}/ 1650 cm^{-1}). When all groups were compared, the dentin mineral composition was higher for CD treated with BG and BD remineralized*

←

with BS. (B): New MRS peak ratio (1295 cm^{-1}/1650 cm^{-1}). These peak ratio analyses showed that the new band was more evident in CD treated with BG and that BD was remineralized with both BG and BS. The results of both ratio analyses corroborate the microtensile bond strength findings, indicating that when dentin mineral compounds are more intense, the substrate more readily interacts with adhesive monomers, increasing resin-dentin bond strength.

ing treatment. When non-remineralized BD was stored in artificial saliva, the mineral matrix ratio did not change and remained constant during the 15 days of bioactive analysis. The remineralization reaction is known to occur only when bioactive agents are in contact with body fluids.[24] Several liquids have been used to induce this reaction, such as water, simulated body fluid, or artificial saliva.[36] Considering that saliva compounds are readily available to remineralize dental tissue, the use of artificial saliva can be listed as a limitation in a remineralization study. Although fluoride participates in conventional tooth remineralization through a top-down approach, its action on biomineralization has not been proven.[11] This fact corroborates these research results since the bioactive analysis showed that the remineralization effect occurred only in remineralized specimens.

The second null hypothesis of this study was rejected because the dentin bond strength was reduced after dentin bleaching and improved with remineralization treatment. These data are consistent with the findings of other studies reporting that dentin bleaching significantly decreases the dentin bond strength.[37,38] This reduction in the dentin μTBS has been related to adhesive polymerization inhibition caused by oxygen and free radicals resulting from the bleaching reaction. Therefore, some studies have reported that μTBS values were restored when such components were eliminated, which was achieved by postponing the restorative procedures to 7-14 days after bleaching treatment.[39] Nevertheless, the reduced adhesive bonding with BD has also been associated with the oxidizing effect of peroxide,[40] which promotes the dissolution of dentin mineral compounds and dentin collagen denaturation and activates organic enzymatic degradation of dentin by MMPs.[4] The bioactive test results revealed that the decreases in the dentin mineral matrix were not reversible even after 14 days of specimen immersion in artificial saliva, corroborating the μTBS findings that only remineralization treatments were able to improve the BD bond strength values.

Dentin mechanical properties are directly affected by the degree and quality of the dentin mineral content. Mineral deposition resulting from remineralization treatment protects the resin-dentin interfacial integrity from adhesive hydrolysis through its dehydration mechanism and protects denuded collagen from MPP degradation.[14] Although treatment with both bioactive agents increased the μTBS values of CD and BD, the difference was not significant when BS was applied to CD. Microtensile

studies have revealed that dentin treatment with an experimental primer or adhesives containing BG did not change the μTBS values over 24 hours but provided durable resin-dentin bonds when long-term analyses were conducted.[20,28] In the present study, significantly improved μTBS values were obtained seven days after remineralization treatment, which differed from the results when BG was introduced into the adhesive compounds. This fact may indicate that the bioactive components are more reactive when available in a separate solution and are not associated with other chemical molecules. To date, the use of BS to improve resin-dentin bonds has been tested in only one study,[19] which found higher μTBS values after six months when BS was used as a dentin remineralization solution after the acid treatment approach of self-etching and etch-and-rinse adhesives.

Dentin remineralization not only changed the mineral composition ($961 \ cm^{-1}/1650 \ cm^{-1}$) of the fractured dentin interface but also induced new physicochemical interactions of the dentin tissue with adhesive monomers ($1295 \ cm^{-1}/1650 \ cm^{-1}$), thus leading to rejection of the third hypothesis of this study. Physicochemical analysis of the fractured dentin beam confirmed the findings of the bioactive test as both analyses demonstrated the same mineral matrix ratio patterns in the CD and BD before and after remineralization treatment. Micromechanical retention of the etch-and-rinse adhesives is improved when methacrylate monomers and/or carboxylic esters chemically interact with the etched inorganic dentin compounds.[41] Considering that the MRS bands are fingerprints of specific specimen molecules, they provide chemical information expressed by changes in or the appearance of new peaks.[42] Therefore, the new peaks observed in the remineralized groups in the present study (CD-BG, BD-BG, and BD-BS) demonstrated that the adhesive methacrylate monomers are more susceptible to interacting with dentin when its mineral compounds are present in larger quantities. Additionally, the presence of these new peaks was more evident on the dentin beams of the groups with higher μTBS values, proving their association with an improved dentin-adhesive bond.

CONCLUSION

Both bioactive materials tested in this study (the calcium sodium phosphosilicate glass and the fully crystalline glass-ceramic) present a high remineralization ability on bleached dentin surfaces. Remineralization treatment of BD promoted mineral

deposition on the dentin surface, improved dentin's ability to chemically interact with adhesive monomers, and consequently increased the resin-dentin bond strength.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of State University of Maringa Ethics Committee. The approval code for this study is 50615715.1.0000.0104.

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.

REFERENCES

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

2. Samran A, Al-Afandi M, Kadour JA, & Kern M (2015) Effect of ferrule location on the fracture resistance of crowned mandibular premolars: An in vitro study *Journal of Prosthetic Dentistry* **114(1)** 86-91.

3. Azevedo RA, Silva-Sousa YT, Souza-Gabriel AE, Messias DC, Alfredo E, & Silva RG (2011) Fracture resistance of teeth subjected to internal bleaching and restored with different procedures *Brazilian Dental Journal* **22(2)** 117-121.

4. Toledano M, Yamauti M, Osorio E, & Osorio R (2011) Bleaching agents increase metalloproteinases-mediated collagen degradation in dentin. *Journal of Endodontics* **37(12)** 1668-1672.

5. Jiang T, Ma X, Wang Y, Zhu Z, Tong H, & Hu J (2007) Effects of hydrogen peroxide on human dentin structure *Journal of Dental Research* **86(12)** 1040-1045.

6. Swift EJ Jr (2008) Effects of bleaching on tooth structure and restorations, part III: Effects on dentin *Journal of Esthetic and Restorative Dentistry* **20(2)** 1-7.

7. Nakabayashi N, Kojima K, & Masuhara E (1982) The promotion of adhesion by the infiltration of monomers into tooth substrates *Journal of Biomedical Materials Research* **16(3)** 265-273.

8. Spencer P & Swafford JR (1999) Unprotected protein at the dentin-adhesive interface *Quintessence International* **30(7)** 501-507.

9. Hashimoto M (2010) A review-micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions *Journal of Biomedical Materials Research Part B Applied Biomaterials* **92(1)** 268-280.

10. Wang Y & Spencer P (2003) Hybridization efficiency of the adhesive/dentin interface with wet bonding *Journal of Dental Research* **82(2)** 141-145.

11. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, & Tay FR (2011) Limitations in bonding to dentin and experimental strategies to prevent bond degradation *Journal of Dental Research* **90(8)** 953-968.

12. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, & Tezvergil-Mutluay A (2011) State of the art etch-and-rinse adhesives *Dental Materials* **27(1)** 1-16.

13. Hench LL, Splinter RJ, Allen WC. & Greenlee TK (1971) Bonding mechanisms at the interface of ceramic prosthetic materials *Journal of Biomedical Materials Research* **5(6)** 117-141.

14. Zanotto ED, Ravagnani C, Peitl O, Panzeri H, & Lara EH (2004) Process and compositions for preparing particulate, bioactive or resorbable Biosilicate for use in the treatment of oral ailments *Geneve: World Intellectual Property Organization* **September 2** Patent WO2004074199A1.

15. Kim J, Gu L, Breschi L, Tjäderhane L, Choi KK, Pashley DH, & Tay FR (2010) Implication of ethanol wet-bonding in hybrid layer remineralization *Journal of Dental Research* **89(6)** 575-580.

16. Ryou H, Niu LN, Dai L, Pucci CR, Arola DD, Pashley DH, & Tay FR (2011) Effect of biomimetic remineralization on the dynamic nanomechanical properties of dentin hybrid layers *Journal of Dental Research* **90(9)** 1122-1128.

17. Tirapelli C, Panzeri H, Soares RG, Peitl O, & Zanotto ED (2010) A novel bioactive glass-ceramic for treating dentin hypersensitivity *Brazilian Oral Research* **24(4)** 381-387.

18. Tirapelli C, Panzeri H, Lara EHG, Soares RG, Peitl Filho O, & Zanotto ED (2011) The effect of a novel crystallised bioactive glass-ceramic powder on dentine hypersensitivity: a long-term clinical study *Journal of Oral Rehabilitation* **38(4)** 253-262.

19. de Morais RC, Silveira RE, Chinelatti M, Geraldeli S, & de Carvalho Panzeri Pires-de-Souza F (2017) Bond strength of adhesive systems to sound and demineralized dentin treated with bioactive glass ceramic suspension *Clinical Oral Investigations* **22(5)** 1923-1931.

20. Profeta AC, Mannocci F, Foxton R, Watson TF, Feitosa VP, De Carlo B, Mongiorgi R, Valdré G, & Sauro S (2013) Experimental etch-and-rinse adhesives doped with bioactive calcium silicate-based micro-fillers to generate therapeutic resin-dentin interfaces *Dental Materials* **29(7)** 729-741.

21. Sauro S, Thompson I, & Watson TF (2011) Effects of common dental materials used in preventive or operative dentistry on dentin permeability and remineralization *Operative Dentistry* **36(2)** 222-230.

22. Wang Z, Jiang T, Sauro S, Wang Y, Thompson I, Watson TF, Sa Y, Xing W, Shen Y, & Haapasalo M (2011) Dentine remineralization induced by two bioactive glasses developed for air abrasion purposes *Journal of Dentistry* **39(11)** 746-756.

23. Khoroushi M, Mousavinasab SM, Keshani F, & Hashemi S (2012) Effect of resin-modified glass ionomer containing bioactive glass on the flexural strength and morphology of demineralized dentin *Operative Dentistry* **38(2)** E1-E10.

24. Crovace MC, Souza MT, Chinaglia CR, Peitl O, & Zanotto ED (2015) Biosilicate® — A multipurpose, highly bioactive glass-ceramic. In vitro, in vivo and clinical trials *Journal of Non-Crystalline Solids* **432(A)** 90-110.

25. Sauro S, Watson TF, Thompson I, & Banerjee A (2012) One-bottle self-etching adhesives applied to dentine air-abraded using bioactive glasses containing polyacrylic acid: an in vitro microtensile bond strength and confocal microscopy study *Journal of Dentistry* **40(11)** 896-905.

26. Colthup NB, Daly LH, & Wiberley SE (1990) *Introduction to Infrared and Raman Spectroscopy* 3rd edition Cambridge, Massachusetts/EUA.

27. Xu J, Stangel I, Butler IS, & Gilson DFR (1997) An FT-Raman spectroscopic investigation of dentin and collagen surfaces modified by 2-hydroxyethylmethacrylate *Journal of Dental Research* **76(1)** 596-601.

28. Sauro S, Osorio R, Watson TF, & Toledano M (2012) Therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface *Journal of Materials Science. Materials in Medicine* **23(6)** 1521-1532.

29. Nanci A & Ten Cate AR (2013) *Ten Cate's Oral Histology: Development, Structure, and Function* **8th edition** Elsevier, Amsterdam, Netherlands.

30. Bertassoni LE, Habelitz S, Kinney JH, Marshall SJ, & Marshall GW (2009) Biomechanical perspective on the remineralization of dentin *Caries Research* **43(1)** 70-77.

31. Tay FR & Pashley DH (2009) Biomimetic remineralization of resin-bonded acid-etched dentin *Journal of Dental Research* **88(8)** 719-724.

32. Uysal T, Er O, Sagsen B, Ustdal A, & Akdogan G (2009) Can intracoronally bleached teeth be bonded safely? *American Journal of Orthodontics and Dentofacial Orthopedics* **136(5)** 689-694.

33. Plotino G, Buono L, Grande NM, Pameijer CH, & Somma F (2008) Nonvital tooth bleaching: A review of the literature and clinical procedures *Journal of Endodontics* **34(4)** 394-407.

34. Deng M, Wen HL, Dong XL, Li F, Xu X, Li H, Li JY, & Zhou XD (2013) Effects of 45S5 bioglass on surface properties of dental enamel subjected to 35% hydrogen peroxide International *Journal of Oral Science* **5(2)** 103-110.

35. Rastelli ANS, Nicolodelli G, Romano RA, Milori DMBP, Perazzoli ILO, Ferreira EJ, Pedroso ACB, Souza MT, Peitl O, & Zanotto ED (2016) After bleaching enamel remineralization using a bioactive glass-ceramic (BioSilicate®) *Biomedical Glasses* **2(1)** 2299-3932.

36. Kokubo T & Takadama H (2006) How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials* **27(15)** 2907-2915.

37. Demarco FF, Turbino M L, Jorge AG, & Matson E (1998) Influence of bleaching on dentin bond strength *American Journal of Dentistry* **11(2)** 78-82.

38. Elkhatib H, Nakajima M, Hiraishi N, Kitasako Y, Tagami J, & Nomura S (2003) Surface pH and bond strength of a self-etching primer/adhesive system to intracoronal dentin after application of hydrogen peroxide bleach with sodium perborate *Operative Dentistry* **28(5)** 591-597.

39. Cavalli V, Sebold M, Shinohara MS, Pereira PNR, & Giannini M (2018) Dentin bond strength and nanoleakage of the adhesive interface after intracoronal bleaching *Microscopy Research and Technique* **81(4)** 428-436.

40. Tam LE & Noroozi A (2007) Effects of direct and indirect bleach on dentin fracture toughness *Journal of Dental Research* **86(12)** 1193-1197.

41. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, & De Munck J (2010) Relationship between bond-strength tests and clinical outcomes *Dental Materials* **26(2)** e100-e121.

42. Zhang Y & Wang Y (2013) Effect of application mode on interfacial morphology and chemistry between dentine and self-etch adhesives *Journal of Dentistry* **41(3)** 231-240.