

## RESEARCH ARTICLE

# Effect of strong tooth-bleaching with 38% hydrogen peroxide on marginal seal of dental restorations using self-etch and total-etch adhesives

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**ABSTRACT**

The purpose of this study was to evaluate the distance and/or thickness of dye penetration at enamel and cementum/dentin margins of restorations before and after vital bleaching. Class V cavities were prepared on extracted human premolars and restored with light-cured resin composite using either AQ-Bond or Single-Bond2 adhesives. Four conditions: no bleaching (control), restored before bleaching (RB), bleached and immediately restored (BIR), bleached and stored in artificial saliva for 7 days then restored (B7R), were used for each adhesive group. After soaking in basic fuchsin dye for 24 hours, all specimens were vertically sectioned through the middle of restorations to measure the distance and/or the thickness of dye penetration. No microleakage was found either on enamel and cementum/dentin margins of control group using AQ-Bond. Whereas leakage was found in the control group using Single-Bond2 and bleached groups. Statistically significant differences in leakage distance and/or thickness were found among groups at the cementum/dentin margin ( $P < .05$ ). Dye could penetrate into the pulp chamber of BIR (60%) and B7R (15%) specimens.

**Clinical Significance**

The negative control or leakage-free group is very important when discussing the effects of bleaching on marginal seal or microleakage. Bleaching using 38% H<sub>2</sub>O<sub>2</sub> increases the microleakage distance and dye penetration area of cementum/dentin margin more than that of enamel margin. The bleached tooth is not a good substrate for reliable bonding and complete seal and it is also a channel for external stimuli reaching the pulp.

**KEYWORDS**

marginal seal, microleakage, tooth-bleaching

## 1 | INTRODUCTION

Vital tooth bleaching has been shown to be an effective and conservative technique to improve the esthetics of discolored teeth.<sup>1-4</sup> Hydrogen peroxide, in various concentrations and forms, for example, carbamide peroxide agent, sodium perborate, is the primary material used by clinicians in the bleaching process. It is an unstable liquid which easily transforms to water and nascent oxygen that effectively penetrates into enamel and dentin, and chemically reacts with chromogens and tooth components.<sup>5</sup> Hydrogen peroxide can oxidize both inorganic and organic components of tooth structures resulting in lower surface

hardness,<sup>6,7</sup> tensile strength,<sup>8,9</sup> fracture toughness,<sup>10</sup> and an increase in surface roughness, porosity, and sensitivity of tooth tissue.<sup>1,8,9,11-20</sup>

Most studies have reported adverse effects of bleaching agent on the physical properties of bleached tooth including bond strength at the tooth-restoration interface.<sup>7,9,13,21,22</sup> Few of them have studied microleakage between tooth and restoration that could be related to vital tooth bleaching.<sup>23-25</sup> Microleakage is the major contributor to tooth-hypersensitivity, tooth staining, dental caries, and pulp infection.<sup>26,27</sup> TEM examination confirmed that exposed collagen fibrils in the remaining demineralized dentin under dental restorations, where microleakage had taken place was stained by dye.<sup>28</sup> It was

hypothesized that strong tooth-bleaching that increased the porosity of tooth substrate could affect the leakage level of restorations which could then be prevented or limited.

The objective of this present study was to evaluate the effect of 38% hydrogen peroxide on the distance and/or thickness of dye penetration at enamel and cementum/dentin margins of Class V resin-composite restorations using two adhesive systems, self-etch and total-etch with a moist technique, before and after filling manipulation.

## 2 | MATERIALS AND METHODS

Forty previously frozen sound human premolars without any visible defects extracted for orthodontics purpose were collected and randomly divided into eight groups of five teeth. Each tooth was used to prepare two Class V box cavities at the CEJ, one each on the buccal and lingual aspects, sized 3.0 × 2.0 mm and 1.5 mm deep with a high speed cylindrical diamond burs (836KR Series ISO size 014, Intensiv, Switzerland). A cavosurface angle of approximately 90° was prepared at both the enamel and cementum/dentin margins. Two groups (control) of 10 cavities each were filled with light-cured resin-composite (Filtek, Supreme Restoratives, 3M ESPE, Minnesota) either using self-etch adhesive (AQ-Bond, Sun Medical, Shiga, Japan) (C-AQ group) or total-etch adhesive with a moist technique (Single-Bond2, 3M ESPE, Minnesota) (C-S group) following the manufacturer's instructions (Table 1). An AQ sponge soaked in 1 drop of AQ-Bond base liquid was scrubbed on the entire cavity surface for 20 seconds, air-blown for 3 to 5 seconds then a second coat was applied, gently air-blown for 5 to 10 seconds and light-cured for 10 seconds. For the Single-Bond2 adhesive, the cavity surface was conditioned with 35% phosphoric acid for 10 seconds, rinsed with water for 10 seconds, kept moist, conditioned with Adper Single-Bond2 for 15 seconds, air-blown for 5 seconds and light-cured for 10 seconds. Each bonded cavity was filled with resin-composite using a bulk technique and light-cured for 40 seconds. All the margins of restorations were finished using fine diamond burs (K2ff, GC International, Tokyo, Japan) in a high-speed handpiece. The other six groups of samples were bleached with 38% hydrogen peroxide (Opalescence Boost, Ultradent, South Jordan) before and after being bonded and filled with light-cured resin-composite

**TABLE 1** Filling techniques using self-etch and total-etch (moist technique) adhesives in control groups ( $n = 10$ )

Control group (C)	Filling manipulation
C-AQ	The entire cavity was scrubbed with an AQ sponge (soaked in 1 drop of AQ-Bond base liquid) for 20 s, and air-blown for 3 to 5 s. A second coat was applied, gently air-blown for 5 to 10 s and light-cured for 10 s. The cavity was filled with resin-composite using a bulk technique and light-cured for 40 s.
C-S	The entire cavity was etched using 35% phosphoric acid for 10 s and rinsed with water for 10 s. A cotton pellet was used to absorb the excess water and keep the cavity surface moist. Adper Single-Bond2 was applied on the entire surface for 15 s, air-blown for 5 s and light-cured for 10 s. The cavity was filled with resin-composite using a bulk technique and light-cured for 40 s.

**TABLE 2** Treatment sequence in bleached groups ( $n = 10$ )

Bleached groups	Sequence
BIR-AQ	Bleached, <sup>a</sup> then prepared cavity and immediately restored with resin-composite using AQ-Bond.
BIR-S	Bleached, <sup>a</sup> then prepared cavity and immediately restored with resin-composite using Single-Bond2.
B7R-AQ	Bleached <sup>a</sup> and stored in artificial saliva for 7 days at 37°C, then prepared cavity and filled with resin-composite using AQ-Bond.
B7R-S	Bleached <sup>a</sup> and stored in artificial saliva for 7 days at 37°C, then prepared cavity and filled with resin-composite using Single-Bond2.
RB-AQ	Prepared cavity and filled with resin-composite using AQ-Bond, then bleached. <sup>a</sup>
RB-S	Prepared cavity and filled with resin-composite using Single-Bond2, then bleached. <sup>a</sup>

<sup>a</sup>Using Opalescence Boost for 20 min and agitate every 5 min, rinse with water, then repeat same bleaching process 3 times.

(Table 2). The composition details of relevant products used as disclosed by the manufacturer in instructions for use are presented in Table 3.

All specimens were coated with two layers of nail varnish except for the restorations and the area 1 mm away from the occlusal and gingival margins and stored in artificial saliva (CU products, Bangkok, Thailand) for 24 hours at 37°C before being immersed in 0.5% basic fuchsin dye aqueous solution (VWR international, Ltd., Poole, England) for 24 hours at 37°C. All specimens then were cleaned with running water for 1 minute. Diamond discs were used to section each tooth longitudinally through the center of the restoration from the buccal to the lingual aspects to expose a cross-sectional surface which was manually polished with abrasive paper up to #2000 grit size. The distance or thickness of dye penetration was evaluated at the gingival (cementum/dentin) and occlusal (enamel) margins of each Class V restorations using a polarizing microscope (Nikon Eclipse Model E400Pol, Nikon Corporation, Tokyo, Japan) at x50, x100, x200 magnifications. A digital image of each specimen was taken with the attached camera (Nikon990, Nikon Corporation, Tokyo, Japan). The dye penetration distances and thicknesses along the tooth-resin interface in all groups were measured using Image-Pro plus 4.5 (Leeds Precision Instruments, Inc., Texas). Penetration data were statistically analyzed using the SPSS 13 (SPSS, Inc., Illinois) at  $P < .05$ .

## 3 | RESULTS

Dye penetration distances of all groups are shown on Figure 1 and Table 4. No microleakage at the enamel and cementum/dentin margins of C-AQ specimens was found (Figure 2A) whereas dye penetration and/or leakage in the control group using Single-Bond2 (C-S) (Figure 2B) and bleached groups (Figures 3 and 4) was demonstrated. Three-way ANOVA showed that bleaching, tissue and intercept between bleaching and tissue had effects on the leakage distances. Turkey's test showed no significant difference in leakage distance at the enamel margin between groups using each adhesive. At the cementum/dentin margin, significant

TABLE 3 Composition of the relevant used products

Product	Manufacturer	Composition	Lot No.
Opalescence Boost 38%	Ultradent Products, Inc., South Jordan, UT	Hydrogen Peroxide, 1.1% fluoride and 3% potassium nitrate	B069
AQ-Bond	Sun Medical, Shiga, Japan	Methacrylate monomers: methyl methacrylate (MMA), 4-methacryloxyethyltrimellitic acid anhydride (4META), dimethacrylate, 2-hydroxyethyl methacrylate (HEMA), Acetone, Water; Sponge: polyurethane foam, sodium p-toluenesulfonate (p-TSNa), aromatic amine	L L 2
Adper Single-Bond2	3M ESPE, St. Paul, MN, USA	Etchant: 35% H <sub>3</sub> PO <sub>4</sub> , BisGMA, HEMA, dimethacrylates, ethanol, water, nanofiller, a methacrylate functional copolymer of polyacrylic and polyitaconic acids	N267397
Filtek Supreme Restoratives	3M ESPE, St. Paul, MN, USA	Zirconia silica filler, Triethylene glycol dimethacrylate (TEGDMA), Bis-GMA (bisphenol A diglycidyl ether dimethacrylate), UDMA (Urethane dimethacrylate), Bis-EMA (Bisphenol A polyethylene glycol diether dimethacrylate)	5 A A
Basic Fuchsin (certifiable grade)	VWR International Ltd., Poole, England	C.I. No. 42510 > 85%, C <sub>20</sub> H <sub>20</sub> C <sub>1</sub> N <sub>3</sub> , Molecular weight 337.86 g/mol	K32573428 417
Artificial saliva (6.68–7 pH)	CU products, Bangkok, Thailand	Potassium chloride BP = 0.75 g., Magnesium chloride BP = 0.07 g., Calcium chloride BP = 0.199 g., Di potassium hydrogen phosphate USP = 0.965 g., Potassium dihydrogen phosphate = 0.439 g., Sodium fluoride BP = 0.005 g., Sodium carboxymethylcellulose 1500 = 6 g., Soribital 70% BP = 36 g., Sodium benzoate = 2.4 g, Deionized water to make 1200 ml.	–

differences in leakage between groups: BIR-AQ and RB-AQ; C-S and BIR-S; BIR-S and RB-S were revealed. Significantly, more leakage at the cementum/dentin margin than that of enamel margin was shown for all groups.

The thickness of the leakage pathway could be measured at the cementum/dentin margin in groups: BIR and B7R for both adhesives. Two-way ANOVA showed that the type of adhesive, waiting time after bleaching, and their interaction had effects on the thickness of the leakage pathway. Statistically significant differences in thickness was

revealed between BIR-AQ and the other three groups: B7R-AQ; BIR-S; and B7R-S (Table 5). Sixty percent of BIR and 15% of B7R groups demonstrated dye penetration into the pulp chamber (Figure 4).

## 4 | DISCUSSION

The effect of tooth-bleaching on microleakage is still controversial.<sup>23–25</sup> This might be because there was no negative control, the microleakage-free restorations, for comparison in the previous studies.

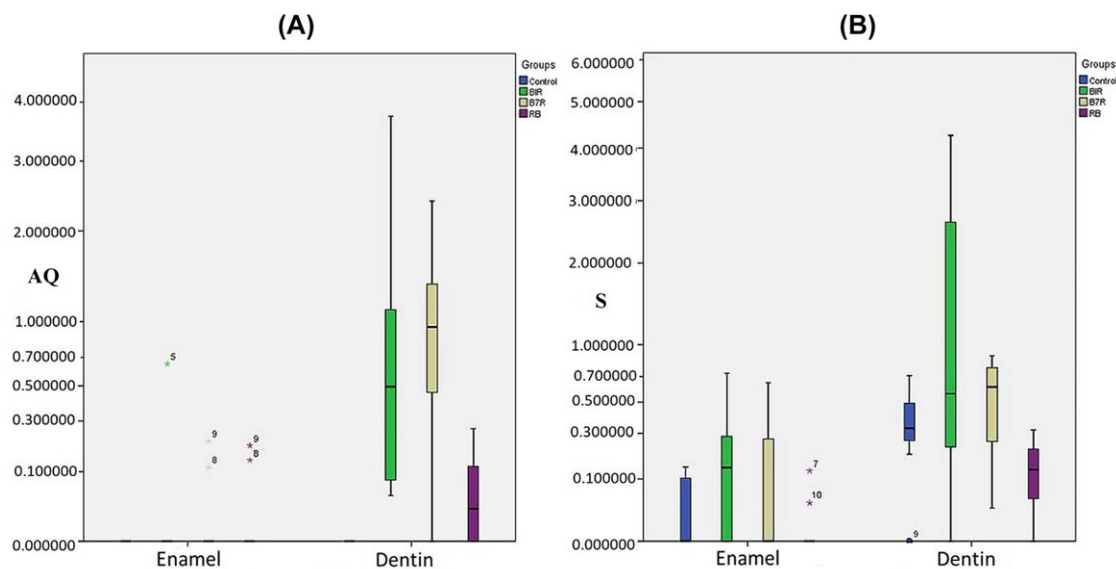


FIGURE 1 A graphic showing the individual dye penetration distances (mm) of all groups using AQ-Bond (A) and Single-Bond2 (B)

TABLE 4 Dye penetration distances (mean  $\pm$  SD) in mm at enamel and cementum/dentin margins for all groups

Groups	AQ-Bond (AQ)		Single-Bond2 (S)		
	Enamel	Cementum/dentin	Enamel	Cementum/dentin	
Control	0.000	0.000	0.037 $\pm$ 0.061	–	0.357 $\pm$ 0.197 <sup>a</sup>
BIR	0.065 $\pm$ 0.207	–	0.189 $\pm$ 0.230	–	1.538 $\pm$ 1.704 <sup>b</sup>
B7R	0.032 $\pm$ 0.071	–	0.145 $\pm$ 0.226	–	0.553 $\pm$ 0.303 <sup>a,b</sup>
RB	0.033 $\pm$ 0.069	–	0.016 $\pm$ 0.041	–	0.131 $\pm$ 0.107 <sup>a</sup>

0 = no leakage.

<sup>a,b</sup>Different superscript letters indicate significant differences in mean distances between groups ( $P < .05$ ).

– Indicates significant differences in mean distances between groups. ( $P < .05$ ).

The AQ-Bond self-etch and Single-Bond2 total-etch adhesives provided microleakage-free and microleakage margins, respectively<sup>29</sup> thus they were selected for the negative and positive controls respectively in this study. The results showed microleakage-free restoration using AQ-Bond in non-bleached tooth (control, C-AQ), whereas microleakage was found in the bleached-tooth for all groups (Table 4). This suggests that tooth-bleaching has an effect on microleakage. When using Single-Bond2 which could not provide microleakage-free restorations, there was no significant difference in microleakage data between non-bleached (control, C-S) and bleached groups at the enamel margin and among groups: control (C-S), B7R-S and RB-S at the cementum/dentin margin. The results of the Single-Bond2 groups suggest that tooth-bleaching has no effect on microleakage in some circumstances such as bleached after restoration (RB)<sup>24,25</sup> or restored after bleaching at least 7 days (B7R). However, a significant difference between the dye penetration of bleached groups compared with the leakage-free control groups using AQ-Bond in this study suggest that the peroxide ions

could affect the permeability of the tooth substrate and penetrate through tooth-resin interfaces and lead to microleakage.

The leakage-free control specimens using AQ-Bond confirmed the results reported recently.<sup>29</sup> Complete hybridization occurs when monomers can penetrate contiguously to envelope the exposed collagen fibrils and/or the hydroxyapatite of the underlying dentin. After complete polymerization, hybridized dentin was formed and could provide microleakage-free restoration without any remaining defects or demineralized dentin, which can create a leakage pathway.<sup>28,29</sup> When demineralized dentin was left under restorations it can be visualized or detected by dye solution.<sup>28,29</sup> Single-Bond2, the total-etch adhesive using phosphoric acid for etching left demineralized dentin visibly moist so preventing the collapse of collagen fibrils. The primer was applied to replace the remaining water in the demineralized dentin. However, when it is not completely displaced, the content of resin inside the hybrid layer can be affected resulting in a remaining defect or demineralized dentin as the leakage pathway.<sup>29–31</sup> This study showed the dye

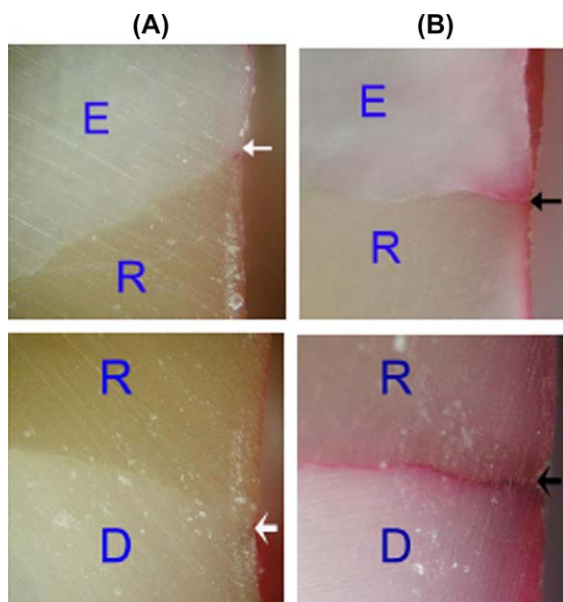


FIGURE 2 Control specimens demonstrating: A, No leakage (white arrow) at enamel and cementum/dentin margins using AQ-Bond; B, Leakage (black arrow) using Single-Bond2. (original  $\times$  200, R = restoration, E = enamel, D = dentin)

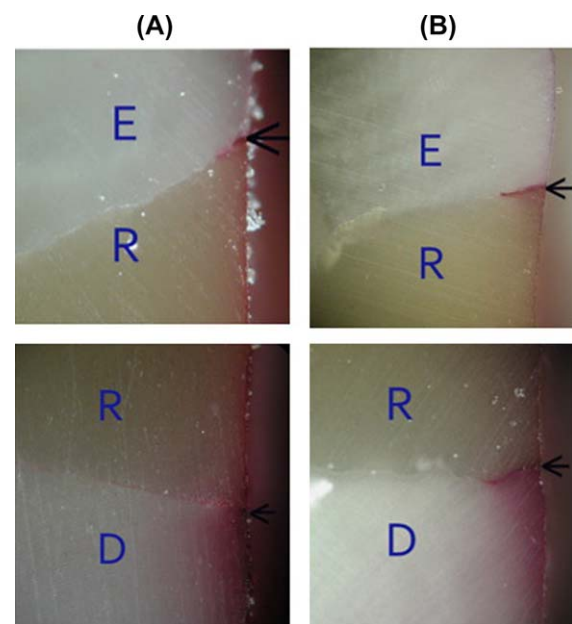
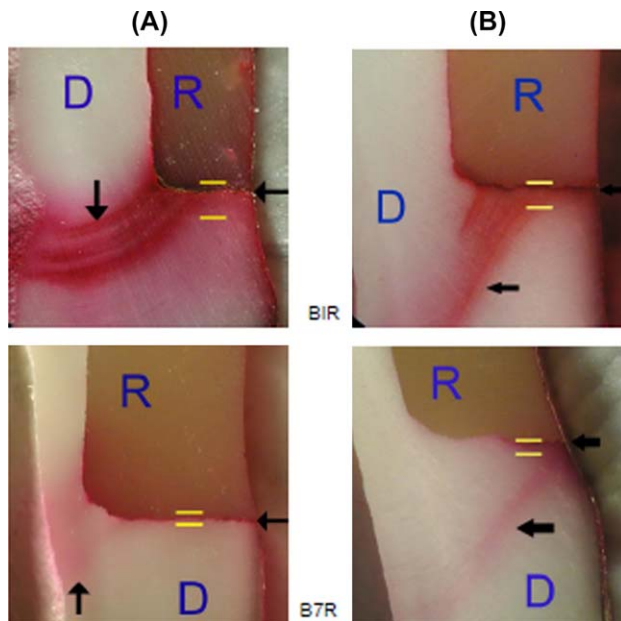


FIGURE 3 Leakage at enamel and cementum/dentin-resin interface (arrowed) of RB groups using AQ-Bond (A) and Single-Bond2 (B). (original  $\times$  200, R = restoration, E = enamel, D = dentin)



**FIGURE 4** Dye penetration distance (arrowed) and thickness (=) at gingival margins of BIR and B7R groups using AQ-Bond (A) and Single-Bond2 (B). (original  $\times 25$ , R = restoration, D = dentin)

penetration distance of control specimens using Single-Bond2 (C-S) both at the enamel and cementum/dentin margins (Table 4, Figure 1B), which suggests that Single-Bond2 could not provide complete hybridization or a defect-free barrier to resist microleakage or dye penetration as same as the other moist bonding system using phosphoric etched dentin.<sup>29,32</sup>

Microleakage was found in a restored tooth that was susceptible to bleaching either before (BIR, B7R groups) or after filling (RB group) both at the enamel and cementum/dentin margins using AQ-Bond. There was no significant difference in microleakage distance between BIR, B7R, and RB groups at the enamel margin. These results suggest that hydrogen peroxide, a strong oxidizing agent, could penetrate into the enamel-resin interface and create a microleakage pathway in specimens that were restored before bleaching (RB group). As hydrogen peroxide can decompose hydroxyapatite crystals making more porosity,<sup>8</sup> roughness<sup>19</sup> and thinner enamel rods which can be stained by Caries Detector.<sup>9</sup> Specimens that were bleached before restoration (BIR, B7R groups) could also not provide good hybridization by the resin to prevent microleakage. Significant differences in greater leakage distance at the cementum/dentin margin than that of the enamel margin were found in bleached specimens either before or after filling. This assumes that hydrogen peroxide penetrates and disrupts hydroxyapatite in cementum/dentin deeper than in enamel. Significantly, higher leakage distance at the cementum/dentin margin on BIR than RB whereas no significant difference between BIR and B7R groups suggests that hydrogen peroxide not only destroyed the inorganic and organic components<sup>33,34</sup> but also had an effect on the bonding mechanism of the resin adhesive especially on cementum and dentin substrates more than on enamel. The strong concentration of hydrogen peroxide in both vital and non-vital bleaching could expose the collagen fibrils in intertubular and peritubular dentin.<sup>9</sup> The exposed collagen fiber

network is easily collapsed when air-dried which then obstructs monomer diffusion for a complete seal.<sup>28</sup> The residual nascent oxygen from the oxidation reaction of hydrogen peroxide also interferes with the resin polymerization via a free-radical mechanism.<sup>35,36</sup> The lower inorganic and greater organic contents of dentin contributed to the greater effect of hydrogen peroxide on microleakage than for enamel.

BIR-AQ specimens demonstrated greater thickness of dye penetration along the dentin-resin interface than BIR-S specimens (Table 5). This might be because the acidic monomer of AQ-Bond was applied for a longer period (50 seconds) than phosphoric acid (10 seconds) in the Single-Bond2 group, therefore the acidic monomers could diffuse deeper into dentin. When the bonding mechanism was not completed because of the effect of hydrogen peroxide, thicker demineralized dentin resulted when visualized or stained by dye solution. Piemjai and Surakamporn<sup>9</sup> also reported that bleached dentin following either intra-coronal or extra-coronal tooth bleaching could be stained by Caries Detector, an organic dye. This study results imply that the combination of tooth bleaching and immediate application of self-etch or total-etch adhesive monomers could result in severe leakage into the pulp chamber (Figure 4BIR). The thickness of dye penetration might demonstrate an area of destruction by hydrogen peroxide that could reach the pulp chamber as previously reported<sup>37,38</sup> and acids from bonding procedures.

No significant difference in leakage distance between BIR and B7R groups indicated that seven days storage in artificial saliva after external bleaching with 38% hydrogen peroxide could not significantly decrease the leakage distance (Table 4), whereas there was significant reduction in leakage thickness at the dentin-resin interface of group B7R-AQ than BIR-AQ groups (Table 5) and smaller number of specimens with dye penetration into the pulp chamber of B7R (15%) compared with BIR (60%) groups were revealed. These results suggest that seven days soaking in artificial saliva after bleaching could reduce the dye penetration into the destruction area resulting from the oxidation reaction of hydrogen peroxide rather than the microleakage pathway resulting from incomplete hybridization of resin. This also suggests that the mineral deposition of potassium chloride, magnesium chloride, calcium chloride, potassium hydrogen phosphate, and sodium fluoride, the main components in artificial saliva, might occlude the bleached dentin especially the tubules and help resist the penetration of acid and dye. Also the residual contents of oxygen and hydrogen peroxide after tooth storage in artificial saliva for 7 days might completely decay to interrupt the resin polymerization. These results indicated that seven

**TABLE 5** Mean  $\pm$  SD of maximum dye penetration thickness (mm) at dentin/cementum margin of groups: BIR-AQ, B7R-AQ, BIR-S, and B7R-S

Groups	Adhesives	
	AQ-Bond	Single-Bond2
BIR	0.141 $\pm$ 0.07 <sup>a</sup>	0.060 $\pm$ 0.06 <sup>b</sup>
B7R	0.049 $\pm$ 0.02 <sup>b</sup>	0.032 $\pm$ 0.01 <sup>b</sup>

<sup>a,b</sup>Different superscript letters indicate significant differences in mean thickness between groups ( $P < .05$ ).

days storage in artificial saliva was not sufficient to reduce the effect from extra-coronal bleaching with 38% hydrogen peroxide on microleakage. Further studies to provide microleakage-free restoration of bleached teeth is necessary when tooth-bleaching is still needed.

## 5 | CONCLUSIONS

A negative control or leakage-free restoration is very important for revealing the effects of bleaching on marginal seal or microleakage. Restored teeth using self-etch AQ-Bond provided leakage-free control specimens, whereas extra-coronal tooth bleaching with 38% hydrogen peroxide either before or after restoration placement initiated marginal leakage of the restorations. Storing in artificial saliva for seven days was not sufficient to reduce the effect of bleaching on microleakage at the resin-tooth interface using either self-etch or total-etch with moist bonding adhesives.

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