Effect of different surface pretreatment methods on repair bond strength of resin composite subjected to pH-cycling

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of two different repair methods (Er:YAG laser and bur) with or without silane application on the microtensile bond strength of a nanohybrid resin composite aged with two different aging methods (pH cycling and thermocycling).

Material and Method: Resin composite blocks (Clearfil Majesty Esthetic, Kuraray, Japan) were randomly assigned into two groups for aging process: (a) pH cycling (b) thermocycling (5,000 cycles). After aging, the blocks were assigned to one of the following repair procedures: (1) Er:YAG laser (LightWalker STE-E, Fotona Medical Lasers, Ljubljana, Slovenia) (2) Er:YAG laser+silane (3) bur (4) bur+silane and (5) no-pretreatment group and (6) Cohesive control (cohesive strength of the resin). Resin composite (Clearfil Majesty Esthetic) was bonded to the conditioned substrates incrementally and light polymerized. Repaired samples were thermocycled (5,000 cycles). The microtensile bonding test was performed. The data were analyzed using Scheirer-Ray-Hare, Kruskal-Wallis Mann-Whitney U tests, Chi-square and Z tests with Bonferroni correction (p=0.05).

Results: No statistically significant difference was found between the aging methods applied to filling material (p=0.821) and the interaction of applied surface treatments and aging (p=0.289). All repair procedures achieved bond strength values higher than the no-pretreatment group but they did not reach the resin composite's cohesive bond strength. Failure modes distribution was found statistically different according to repair procedure and also aging methods (p<0.05).

Conclusion: The bond strengths of the resin composites were similar to those of applied thermal cycling and the pH cycling model with no difference between the different repair methods.

Keywords: Aging, dental restoration repair, lasers, pH, silanes

INTRODUCTION

In recent years, resin composites have shown significant developments regarding their technical and aesthetic properties. The mechanical/physical properties and diversity of these restorative materials vary depending on the size, morphology, amount, distribution and chemical composition of the filler (1). After the introduction of nanotechnology into the field of dentistry, nano-filled resin composites that can be better polished, have higher fracture and wear resistance, lower polymerization shrinkage, and can be used in both anterior and posterior restorations with more aesthetic properties have been introduced to the market (2).

Restorative dentistry does not only show its conservative approach in the treatment of caries with conservative restorations, but also maintains this attitude with the repair of the existing restoration instead of replacing the defective restoration completely. Restoration repair is more practical and economical approach, with less compromise on tooth hard tissues (3). In addition, it is timesaving, requires less extensive cavity preparation thus reduce the risk of pulp exposure, less traumatic to the tooth. It has been reported that the repair of resin composite restorations yields promising results in terms of longevity and quality of the restoration (4).

Bonding between the two composite layers is achieved by the presence of an oxygen inhibition layer over the unpolymerized resin. The oxygen inhibition layer is a layer of unreacted monomers that increase inter-material adhesion. The amount of unreacted monomers present in the restoration decrease with aging (5). Therefore, the bond formed between the old and new resin composite during the repair procedure could be unreliable. When
restorations are exposed to the oral environment, absorb water and the activity of free radicals inside the material terminates (6). In order to increase the repair bond strength between the old and new composite materials, many techniques such as bur (7-14), silane (6-8,11,15-21), bonding agent (6-9,11,13-15,17,19,22,23) and Erbium Yttrium Aluminum Garnet (Er:YAG) laser (7,10,12,24,25) pretreatment modalities are used.

Artificial aging is preferred to mimic the intraoral environmental changes and helps to evaluate the effect of various aging factors on the resin composite. For simulating intraoral temperature changes, thermal cycling (12,15,18,19,24,26,27) is frequently used in vivo. It is one of the most commonly employed methods, which is found to be much more effective than other aging methods for mimicking aging by creating stress on the bonding interface (18). Generally, samples are subjected to extreme temperatures of 5 - 55 °C. High temperatures are known to weaken the physicochemical properties of resin composites. Additionally, temperature changes can also reduce the number of unreacted double bonds within the composite or on the composite surface, thereby can negatively affect the composite-to-composite repair bond strength (18).

In the oral cavity, resin composite restorations are under the impact of many dynamics such as water, saliva, thermal stress, chemical attacks, and chewing forces. Intraoral pH varies depending on the organic acid in the plaque, bacterial metabolism, saliva and eating habits (28). These factors, individually or collectively, may degrade the resin composite or lead to inter-material debonding. Although pH cycling models have been used widely to create artificial demineralization and remineralization (29,30), little is known about their effects on dental materials (31,32). Different in vitro artificial aging methods such as thermal cycling and pH cycling may have different effects on the degradation of resin composites.

The aim of this study is to evaluate the effect of two different repair methods (Er:YAG laser and bur) applied with and without silane on the microtensile bond strength of nanohybrid resin composite (Clearfil Majesty Esthetic, Kuraray, Japan) aged using two different methods (pH cycle and thermal cycle). The null hypotheses tested were that (1) the success of the repair bond strength of resin composites is not dependent on the surface treatments evaluated, and (2) the aging conditions will not have the same effect on the repair bond strength of resin composites.

**MATERIAL AND METHOD**

Ethics committee approval is not required for in vitro material studies that do not use human and animal subjects. All procedures were performed adhered to the ethical rules and principles of the Helsinki Declaration. The materials used in this study are shown in Table 1. The experimental design is outlined in Figure 1.

**Preparation of Aged Resin Composites**

Thirty resin composite blocks with dimensions of 6x6 mm and 5 mm in height were built up incrementally with three increments of nanohybrid resin composite (Clearfil Majesty Esthetic, Shade A1, Kuraray, Tokyo, Japan) placed inside a silicone matrix (Elite HD Putty soft setting, Zhermack, Italy) according to the manufacturer’s instructions. Each 2-mm increment was light-cured for 20 seconds with a LED lightcuring unit (Elipar Freelight 2, 3M ESPE, Germany). After the samples were removed from the mold, curing light was applied to the bottom and sides of the composite that were previously in contact with the silicone mold for a total of 100 seconds to ensure their full polymerization. All surfaces of the specimens to be treated were polished with 320-grit silicone carbide papers under cooling, and then the specimens were ultrasonically cleaned for 3 min using distilled water.

**Table 1. Materials and contents used in the study.**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Manufacturer</th>
<th>Batch no.</th>
<th>Composition Filler loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Majesty Esthetic (A1, A3,5 shade)</td>
<td>Kuraray, Tokyo, Japan</td>
<td>00033A B50001</td>
<td>Silanated barium glass (mean particle size 0.7 μm), pre-polymerized organic hiler including nanofiller, Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic methacrylate 78% by weight 66% by volume</td>
</tr>
<tr>
<td>Clearfil Ceramic Primer</td>
<td>Kuraray, Tokyo, Japan</td>
<td>2S0001</td>
<td>MPS, MDP, ethanol</td>
</tr>
<tr>
<td>Clearfil SE Bond</td>
<td>Kuraray, Tokyo, Japan</td>
<td>000093</td>
<td>Primer: MDP, HEMA, hydrophilic dimethacrylate, water, photoinitiator Bond: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, silanized colloidal silica, photoinitiators</td>
</tr>
</tbody>
</table>

****Bis-GMA: Bisphenol A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloyloxydecyl dihydrogenophosphate, MPS: 3-Methacryloxypropyltrimethoxysilane.

**Fig. 1. Study design.**
As the cohesive control group and to determine the inherent cohesive strength of the resin composite, six resin composite blocks (6×6×10 mm) were prepared and cured in a similar manner as previously described using the same composite (Clearfil Majesty Esthetic (Shade A1)).

Aging of the Resin Composites
The resin composite blocks were then randomly and equally divided into two groups, according to the aging method applied.

In pH cycling model for the cariogenic challenge specimens were immersed in demineralizing solution [2.0 mmol/L Ca(NO\textsubscript{3})\textsubscript{2}, 4H\textsubscript{2}O, 2.0 mmol/L Na\textsubscript{2}HPO\textsubscript{4}, 75 mmol/L acetate buffer, 0.04 ppm F, pH 4.7] for 6 h (30 mL per specimen) and in remineralizing solution [1.5 mmol/L Ca(NO\textsubscript{3})\textsubscript{2}, 4H\textsubscript{2}O, 0.9 mmol/L Na\textsubscript{2}HPO\textsubscript{4}, 2H\textsubscript{2}O, 150 mmol/L KCl, 0.02 mol/L Tris buffer, 0.05 ppm F, pH 7.0] for 18 h (30 mL per specimen) at 37°C. This sequence was repeated for 5 days. In the last two days, the specimens were kept in the remineralizing solution only. Both solutions were replaced daily (30).

Other samples were thermally aged in distilled water between 5°C and 55°C for 5000 cycles with a dwell time of 30 seconds and a transfer time of 5 seconds.

Conditioning of the Aged Resin Composites
After the aging protocols resin composite blocks were randomly and equally divided into five subgroups (n=3) and different surface pretreatment methods were employed to one surface of the samples as follows:

Er:YAG laser: An Er:YAG laser (LightWalker STE-E, Fotona Medical Lasers, Ljubljana, Slovenia) with a wavelength of 2940 nm coupled with a handpiece (R02-C) having a spot size of 0.9 mm in diameter in a noncontact mode under continuous water spray (40–60 mL/min) at a focal distance of 7 mm from the target point was used. Laser was applied with the following parameters; 5 W power, 250 mJ energy, 20 Hz pulse repetition rate and 100 μs pulse duration (10).

Er:YAG laser+Silane: Er:YAG laser application was performed as described in the Er:YAG laser only group followed by, Clearfil Ceramic Primer application as described in Er:YAG laser + Silane group.

No-pretreatment group: No surface treatment was performed on the surface of resin composite blocks (5 mm × 6 mm × 6 mm) to be repaired.

Cohesive Control: Longer resin composite blocks (10 mm × 6 mm × 6 mm) were used to measure the cohesive strength of the resin composite. No surface pretreatment was performed, and no repair composite was bonded. Specimens were directly subjected to tensile forces.

Bonding Procedure and Specimen Storage
Following pretreatment procedures, specimens were rinsed with water and dried with air. A two-step self-etch adhesive system (Clearfil SE Bond, Kuraray, Okayama, Japan) was then applied to all the pretreated surfaces except cohesive control group. Self-etching primer was applied to the surface and left undisturbed for 20 seconds, followed by drying with mild air flow for 5 seconds. Bond was applied and gently air-thinned with to provide a uniform resin film and cured for 10 seconds with the LED lightcuring unit.

The samples were then placed inside the bottom portion of the 10 mm-deep silicone mold, with the adhesive applied surface on top. To distinguish between the new repair and the aged composite, a different shade (A3.5) nanohybrid resin composite of the same brand (Clearfil Majesty Esthetic) was placed with 2 mm-thick increments. Each increment was light-cured for 20 seconds with the LED lightcuring unit. Then, the mold was removed, and samples were post-cured for a total of 80 seconds from all four lateral surfaces.

All specimens, including the cohesive control groups, were subjected to 5000 thermal cycles in distilled water bath between 5°C-55°C before the microtensile bond strength test.

Microtensile Bond Strength Test
The samples were glued to the L-shaped acrylic blocks with cyanoacrylate adhesive for micro-sectioning, then were placed in a precision cutting device (Micrcut Precision Cutter 201, Metkon, Turkiye) for sectioning. Serial sections were taken at a cross-sectional area of 1mm x 1mm (approximately 1 mm\textsuperscript{2}) under water cooling with a low-speed diamond saw. At least 13 test sticks were obtained from each sample, resulting in at least 40 test sticks for each group.

For the microtensile bond strength test, test sticks were glued to the microtensile test device (Microtensile tester, Bisco, Schamburg, USA) from both ends with cyanoacrylate adhesive (Polibond, Polidol, Istanbul, Turkiye) parallel to the long axis of the device. The loading speed of the test
device was determined as 0.5 mm/min until failure. The results were recorded as megapascals (MPa).

After the microtensile bond strength test, the failure modes of the samples were determined by examining the failed bonding interface under a stereo microscope (Leica MZ12; Wetzlar, Germany) at x40 magnification. Failure modes were classified as cohesive failure (within the filling material or repair material) and adhesive failure (between the filling and repair material).

Statistical Analysis
Normality of data distribution and homogeneity of group variances were evaluated with Levene's test. After observing non-homogeneous variance the non-parametric counterpart of the two-way ANOVA, Scheirer-Ray-Hare analysis was used. The interaction between the surface treatment and aging methods was not statistically significant, therefore Kruskal-Wallis test was used to compare the microtensile bond strength values according to the surface pretreatment methods used. Mann-Whitney U test with Bonferroni correction was used for the multiple comparisons. Chi-square tests were used to compare failure patterns in the aging and surface pretreatment groups. Bonferroni corrected z-test was used for pairwise comparisons (α=0.05).


RESULTS
No statistically significant interaction was found between the aging methods and surface treatments (p=0.289). While aging methods had no significant effect on repair bond strength (p=0.821), surface pretreatment had (p<0.001) (Table 2). Comparison of µTBS values according to the surface pretreatment methods are given in Table 3. Cohesive strength of the resin composite was significantly higher than all repaired composite groups (p<0.05). No-pretreatment group had significantly lower µTBS values than that of the cohesive control group, Er:YAG laser+silane and bur+silane groups. (p<0.05). Er:YAG laser-only, Er:YAG laser+silane, bur-only and bur+silane groups had similar µTBS values (p>0.05).

Comparison of µTBS values according to the aging methods are given in Table 4. Cohesive strength of the resin composite was significantly higher than all repaired composite groups and no-pretreatment group had significantly lower µTBS values than that of the cohesive control group, Er:YAG laser+silane and bur+silane groups in both pH cycle and thermal cycle aging methods (p<0.05).

The distribution of failure modes is shown in Figure 2. There is a statistically significant difference in terms of failure modes according to aging methods (p=0.007). Adhesive failures were significantly fewer and cohesive failures (in repair material) were significantly more frequent in pH cycle compared to thermal cycle (p<0.05). Adhesive failure was statistically significantly more frequent in Er:YAG laser and no-pretreatment group compared to other groups (p<0.05). When the surface

<table>
<thead>
<tr>
<th>Effects</th>
<th>H statistic</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Aging methods</td>
<td>11.25</td>
<td>0.821</td>
</tr>
<tr>
<td>Surface treatments</td>
<td>71.660</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aging methods*Surface treatments</td>
<td>-2.197</td>
<td>0.289</td>
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<table>
<thead>
<tr>
<th>Table 2. Comparison of aging methods and surface treatments applied to the filling materials.</th>
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<tr>
<td>Effects</td>
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<tr>
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<tr>
<td>Aging methods</td>
</tr>
<tr>
<td>Surface treatments</td>
</tr>
<tr>
<td>Aging methods*Surface treatments</td>
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<tr>
<th>Table 3. Comparison of the overall microtensile bond strength values according to the surface pretreatments applied to the filling materials.</th>
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<tbody>
<tr>
<td>Surface treatments</td>
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<td>---------------------</td>
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<tr>
<td></td>
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<tr>
<td>Er:YAG Laser Group</td>
</tr>
<tr>
<td>Er:YAG Laser + Silane Group</td>
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<tr>
<td>Bur Group</td>
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<tr>
<td>Bur+Silane Group</td>
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<tr>
<td>No-pretreatment Group</td>
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<tr>
<td>Cohesive Control Group</td>
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<td>Cohesive Control Group</td>
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</table>

* Data are given as mean ± standard error (Mean ± S) and median (Minimum-Maximum) (Min-Max). There is a statistically significant difference between groups with the same number (**4,4,4,4,4,4,7** p<0.05).

<table>
<thead>
<tr>
<th>Table 4. Comparison of microtensile bond strength values according to aging methods.</th>
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<tbody>
<tr>
<td>Aging methods</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Surface pretreatments</td>
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<tr>
<td>Er:YAG Laser Group</td>
</tr>
<tr>
<td>Er:YAG Laser + Silane Group</td>
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<tr>
<td>Bur Group</td>
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<tr>
<td>Bur+Silane Group</td>
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<tr>
<td>No-pretreatment Group</td>
</tr>
<tr>
<td>Cohesive Control Group</td>
</tr>
</tbody>
</table>

** Data are given as mean ± standard error (Mean ± S) and median (Minimum-Maximum) (Min-Max). There is a statistically significant difference between groups with the same number (**4,4,4,4,4,4,7** p<0.05).
treatments were compared in terms of failure modes among the aging methods, adhesive failure in both pH cycle and thermal cycle applied samples was statistically significantly more frequent in the Er:YAG laser group compared to Er:YAG laser+Silane, Bur and Bur+Silane groups (p<0.05).

DISCUSSION

Dental resin composites can degrade over time due to mechanical factors such as wear, abrasion and fatigue; or chemical factors such as enzymatic hydrolytic and acidic action or due to temperature changes. This degradation may result in discoloration, microleakage, marginal misadaptation or minor fractures of the restoration and may require repair or replacement of the restoration (17). Restoration repair is less time consuming and less costly than replacement (3,4).

Özcan et al. (18) found that the 5000 thermal cycles they applied were a more effective aging method than other aging methods in reducing the repair bond strength of the resin composite. When the repair bond strength studies are examined, it is seen that 5000 thermal cycles are generally performed (19,26,27,33), therefore resin composites before and after the repair was thermally aged for 5000 cycles in our study. On the other hand, there are limited studies examining the effects of pH cycling on dental materials (31,32), and these pH cycling models used to evaluate artificial caries formation or remineralization (29,30) differ in terms of solution pH and application periods. Since the enamel surface is the first surface exposed to the oral environment and the histological and chemical changes of caries are observed on this tissue first, with the pH cycle model applied to the enamel tissue for cariogenic challenge, the pH of the remineralization solution in which we kept our samples for 18 hours was determined as 7 and the pH of the demineralization solution, which we kept for 6 hours, was determined as 4.7 (30).

In composite repair, bonding between the old and new resin composite is achieved through three mechanisms such as chemical bonding to organic matrix, chemical bonding to exposed filler particles, and micromechanical adhesion (8). Micoretentive interlocking is the most important factor that provides bonding between the new and old resin composite and increases the bonding between the resin matrix and the exposed filler particles (8,17). Roughening the surface increases the availability of free carbon atoms on the surface (34). Due to the fact that the most frequently used material in daily dental applications is diamond burs and researchers have focused on the effectiveness of Er:YAG lasers for composite repair in recent years, but there are limited studies on this subject (7,10,12,24,25,35), the burs and Er:YAG laser were used as different roughening methods in our study. The repair bond strengths of nanohybrid resin composites have been evaluated in a limited number of studies using the Er:YAG laser applied at 1.5W, 25 Hz, 75 μs (7), 50 mJ and 200 mJ, 10 Hz and 10 seconds (35). Since there are not enough studies examining the effect of Er:YAG lasers on the repair bond strength of nanohybrid resin composites, the Er:YAG laser we used in our study was applied with parameters 250 mJ, 20 Hz, 5 W, wavelength 2.94 μm and pulse duration 100 μs (very short pulse) to determine whether the parameters we chose for these resin composites are suitable or not and to shed light on other studies.

In addition to micromechanical adhesion, it has been reported that chemical bonding to the exposed filler particles and organic matrix is effective in the repair process between the old and new resin composite (8). For this purpose, silanes were frequently evaluated in many studies (6-8,11,13,15-20). Resin composite restorations may lose the silane layers around the fillers due to both aging in the oral environment after polishing and finishing, and mechanical surface applications such as pretreatment with bur, airabrasion, lasers etc. It is reported that with the use of silane, covalent bonds are re-established between the inorganic fillers of the resin composite and the monomers in the adhesive system, and at the same time, the wettability of the adhesive increases and it infiltrates the surface irregularities more easily (8,16,18,20). In our study, silane was used to evaluate its effect of roughening methods on bond strengths.

Temperature changes and water absorbed by the resin composites as a result of thermal cycling can cause the resin composite organic matrix to swell, lead to microcracks, resin destruction, disintegration of the silane layer on the surface of the filler particles, separation of the filler particles, and a decrease in the number of unreacted double bonds on the resin composite surface or inside the composite, which may affect the composite-composite
In a study comparing the roughness of the diamond bur and the Er:YAG laser applied at different power on microhybrid resin composite using scanning electron microscopy, it has been shown that the diamond bur forms smear layer and grooves on the surface of the resin composites and produces much lower roughness surfaces compared to the surfaces treated with the Er:YAG laser and the Er:YAG laser applied up to 5W exhibits more irregular and microporous surfaces, while the Er:YAG laser applied at 6W causes degradation in the resin composite (10). In studies (25,35), where higher roughness values were obtained with Er:YAG laser application compared to burs, it was stated that surface pretreatment methods performed with burs on resin composites did not provide a significant increase in bond strength. This outcome was confirmed in our study.

Consistent with our study, in a study in which different energy parameters of the Er:YAG laser were evaluated in order to determine the best surface treatment for the repair bond strength of the microhybrid resin composite, it was shown that there was no statistically significant difference between the experimental groups including the control group with no treatment (25). Er:YAG laser applied with 150 mJ 10 Hz, 1.5 W 0.119 W/mm² and a pulse duration of 700-ms was reported to provide repair bond strength similar to that of the bur treatment in microfilled hybrid resin composite thermally aged (39). This result is in accordance with our study. Contrary to our study, another study evaluating the repair bond strength of microhybrid resin composite aged with 6000 thermal cycles using 75, 100, 200 and 300 mJ Er:YAG laser energies, found that the highest shear bond strength values (25.98 MPa) in the laser groups were in the group using 75 mJ power and the lowest bond strength values were in the groups using 200 mJ and 300 mJ, and these bond strength values were statistically different compare to the control group with no treatment (24). It was found that Er:YAG laser with 1.5W, 25 Hz, 75 µs increased the repair shear bond strength of nanofilled resin composite aged with 500 thermal cycles compared to the control group with no treatment, unlike our study (7). When the repair bond strengths of nanofilled resin composites applied with 50 mJ and 200 mJ 10Hz and 10 seconds Er:YAG laser were compared, it was seen that the Er:YAG laser groups were not statistically different from the diamond bur group in accordance with our study, but unlike the no-pretreatment group, contrary to our study (35). Differences in the bond strength test method used in our study, the materials, the aging methods used before and after the repair process, and the application parameters of the Er:YAG laser may have led us to obtain different results compared to other studies (7,24,35).

In this study, statistically higher repair bond strength of silane applied bur and Er:YAG laser compared to the no-pretreatment group and the repair bond strength of the bur and Er:YAG laser applied with and without silane is higher than the ones without silane, with no statistical difference between them and the repair bond strengths of the silane-free bur and the Er:YAG laser exhibit statistically similar bond strengths with the control group with no treatment shows the specific effect of silane on repair bond strength. For this reason, null hypothesis tested that the success of the repair bond strength of resin composites is not dependent on the surface treatments evaluated was rejected. Our finding that the use of silane increases the repair bond strength is also supported by other studies (15,23,33).
Failure modes provide important information that allows estimation of possible clinical performance limits of the tested material. In this study, failure patterns were mostly cohesive (repair material/filling material), while adhesive failures were more frequent in Er:YAG laser and no-pretreatment group of samples aged with both pH and thermal cycling. It shows that the cohesive failures represent the weak point in the resin composites because of its own composition or the presence of voids or contamination between composite layers, whereas the bond between the filling material and the repair resin composite is reliable (26). In our study, we think that aging methods affect the mechanical and physical properties of resin composites. In the Er:YAG laser group, we believe that the adhesive failures, which were seen intensely similar to the no-pretreatment group, were that the laser parameters we used in the study could not provide sufficient morphological changes on the resin composites, and therefore, the surface change created by the laser was not much different from that of the no-pretreatment group.

CONCLUSION

Within the limitations of this in vitro study, it was observed that the bond strengths of resin composites were similar to those applied with the pH cycle model and thermal cycle. Although there was no difference between the other repair methods such as Er:YAG laser-only or bur-only, surface pretreatment methods with silane such as Er:YAG laser+silane and bur+silane groups had significantly higher µTBS values than that of no-pretreatment group.

ETHICAL DECLARATIONS

Ethics Committee Approval: No interventional procedure was performed with the method and study protocol infrastructure of the study. Due to the absence of clinical studies, ethics committee approval is not required for in vitro material studies that do not use human and animal subjects.

Informed Consent: Because the study was designed on materials, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES