Effect of grape seed extract against biodegradation of composite resin-dentin shear bond strength

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Abstract. This study aimed to analyze the effect of grape seed extract (GSE) on resin-dentin shear bond strength. A group of 48 dentin samples were divided into 6 groups. The six groups, each with eight specimens, included group 1 (control), group 2 (control + NaOCl 10%), group 3 (2.9% GSE application before etching), group 4 (2.9% GSE application before etching + NaOCl 10%), group 5 (2.9% GSE application after etching), and group 6 (2.9% GSE application after etching + NaOCl 10%). Shear bond strengths were measured using a universal testing machine. Statistical analysis was done with the Kruskal-Wallis test and the Mann-Whitney U test. The highest median value was in group 3, and the lowest value was in group 5. GSE can improve the shear bond strength ($p = 0.002$ and 0.001), but it has no effect on reducing biodegradation ($p = 0.141$).

1. Introduction
Based on a study conducted over many years, bond strength degradation has been shown to occur in the resin-dentin bond. Biodegradation between the dentin and resin cause restoration failures. Mai et al stated that composite resin restoration failures occurred in more than 50% of cases after 10 years, the cause of composite resin restoration failures is due to biodegradation between the dentin and resin that results from mechanical loads and the tooth’s chemical environment [1,2].

Effective bonding between the bonding agent and dentin determines the success of a composite resin restoration. The bond that results from resin and dentin is stronger compared to enamel because the dentin’s histological structure is more complex and contains more water and organic tissue than enamel. The formation of a smear layer from the tooth preparation for restoration requires hydroxyapatite crystal and collagen fiber, which decrease the permeability of dentin tubules and inhibit the infiltration of monomer resin [3,4]. Monomer resin infiltration into the collagen network in dentin tubules and intertubules will form a micromechanical retention region called the hybrid layer. Micromechanical retention between the resin and dentin is the main mechanism of the resin-dentin bond. The bond strength between the resin and dentin is determined by the integrity of dentin collagen fibers with the resin monomer [4,5].

The use of a composite resin with the total-etch and self-etch techniques produces hybrid layers between the resin and dentin. In the total-etch technique, a collagen layer with the proper humidity is needed for the monomer resin to penetrate and produce a good collagen layer. However, the excess or lack of water on collagen surfaces can also reduce the resin-dentin bond strength. The self-etch technique has more tolerance to collagen humidity, but produces a shallower hybrid layer than the
total-etch technique, resulting in a lower bond strength. The use of water and etch in the total-etch and self-etch techniques in vital tooth restoration procedures can activate the matrix metalloproteinase (MMP) enzyme that degrades the resin-dentin bond strength [6]. According to Srinivasulu et al., the mechanical properties of collagen and its resistance to enzymatic degradation can be improved by applying crosslink collagen agents [4,7]. Chlorhexidine and glutaraldehyde are crosslink collagen agents that have been widely studied and are used today. Despite their ability to induce resin-dentin bonding, these agents also have weaknesses that include cytotoxicity, tooth discoloration, and a short effect potency. Therefore, crosslink collagen agents, which have no weaknesses similar to the previous agents, are needed [8,9].

Grape is an excellent source of phenol, particularly in its skin and seeds. The most abundant phenol compound found in grapes is proanthocyanidin [10]. Based on a study by Macedo et al, the application of grape seed extract (GSE) with 6.5% proanthocyanidin in dentin can increase dentin collagen stability and resistance [11]. Proanthocyanidin is a natural crosslink collagen agent that can retain the stability of the dentin collagen matrix and increase dentin biodegradation resistance. Proanthocyanidin can be linked with an adhesive system through its ability to increase collagen crosslinks. Moreover, proanthocyanidin can also increase collagen resistance to collagenolytic enzymes in vital teeth. The increase of collagen resistance is expected to inhibit biodegradation in the hybrid layer [12]. Based on a study by Green et al, in non-vital post extraction teeth, a 5% proanthocyanidin application can increase collagen biodegradation resistance and maintain hybrid layer stability [13]. In addition, according to Angelina, GSE can clean the smear layer in the dentin wall and increase dentin permeability to a monomer resin solution [14].

2. Materials and Methods

A total of twenty four extracted teeth were cleaned and root portions were removed. The enamel in the buccal and palatal/lingual regions was ground flat under flowing water with a low speed carborundum disc to expose the dentin. Each crown was sectioned vertically in the buco-palatal direction into two parts to obtain 48 samples. The tooth crown was then dried and mounted in a self-cured acrylic resin inside a pipe medium with a 2-cm diameter and height. The dentin surface had to be exposed without contamination from the acrylic. All samples were divided into 3 groups, each with 16 specimens. Each group received a different treatment after being planted in acrylic. The first group, after etching, received a primer-bonding and cylinder composite resin with a 3-mm diameter and a 2-mm length. The second group was soaked in a 2.9% GSE solution for 10 minutes and etched, and then the primer-bonding agent and cylindrical composite resin were applied with a 3-mm diameter and a 2-mm length [7]. The third group, after being etched, was rinsed and soaked in 2.9% GSE for 10 minutes [8], and then primer and bonding were applied followed by a cylindrical composite resin with a 3-mm diameter and a 2-mm length.

Specimens from each group were then stored in a saline water medium and incubated at 37 °C for 24 hours. Storing the specimens in saline water was a simulation of the oral conditions for 1 hour. Half of the specimens from each group were soaked in a 10% NaOCl solution for 1 hour, which aimed to simulate time in the oral cavity and results in a 50% decrease in bond quality [4,7].

All specimens were consecutively divided into six groups, each with eight specimens. These groups included group 1 (control), group 2 (control + NaOCl 10%), group 3 (2.9% GSE application before etching), group 4 (2.9% GSE application before etching + NaOCl 10%), group 5 (2.9% GSE application after etching), and group 6 (2.9% GSE application after etching + NaOCl 10%).

For all specimens, the exposed dentin surface was etched with a 36% phosphoric acid gel for 15 seconds, then rinsed with water for 10 seconds, and dried with slightly pressurized air. Bonding agents were applied with a micro-brush for 10 seconds, then sprayed with slightly pressurized air for 5 seconds, followed by light-curing polymerization for 15 seconds. Ceramix nano composite resin was formed with a cylinder-shaped plastic mold with a 3-mm diameter and a 2-mm length placed in manipulated dentin and polymerized for 20 seconds. Specimens were incubated for 24 hours and tested for shear bond strength. In group 1 there was no additional intervention. In group 2, 4, and 6,
after incubation, specimens were soaked in a 10% NaOCl solution for 1 hour, then tested for shear bond strength. In group 3 and 4, the exposed dentin surface was soaked in GSE for 10 minutes and rinsed with water before being etched. In group 5 and 6, after the dentin surface was etched, specimens were then soaked in GSE for 10 minutes and rinsed with flowing water.

Shear bond strength was tested with a universal testing machine, a blade with a chisel shape was placed in a composite with a 0.5 mm per minute pressure ratio until the composite resin detached. Shear bond strength was recorded in kilogram-force (kgf) units and converted to megapascal (MPa) units. Statistical analysis was performed with Kruskal Wallis test followed by the Mann-Whitney U test.

3. Results and Discussion

3.1 Results
The highest median for shear bond strength was in the group that received a 2.9% GSE application before etching with a shear bond strength of 13.69 MPa. The lowest was in the group that received a 2.9% GSE application after etching with a shear bond strength of 1.84 MPa (Table 1).

| Group(s) | Median | Min-Max Value | Mean ± SD |
|----------|--------|---------------|----------|
| 1        | 6.56   | 5.07-10.48    | 6.95 ± 1.83 |
| 2        | 4.06   | 3.08-6.31     | 4.44 ± 1.83 |
| 3        | 13.69  | 9.95-15.85    | 13.34 ± 2.15 |
| 4        | 5.01   | 3.54-7.87     | 5.33 ± 1.82 |
| 5        | 1.84   | 1.39-2.85     | 2.01 ± 0.49 |
| 6        | 2.19   | 1.80-5.90     | 2.63 ± 1.38 |

Table 2 shows significant relationships for shear bond strength between groups that had not biodegraded due to the application of 10% NaOCl. There were significant differences between the control group and the group that received a 2.9% GSE application before etching (p = 0.002) and the group that received a 2.9% GSE application after etching (p = 0.001). The difference in shear bond strength values in the group that received a 2.9% GSE application before etching and the control group, as well as the significant relationship of the shear bond strength, shows that the 2.9% GSE solution can increase the resin–dentin shear bond strength.

In addition, the before and after treatment surface roughness values were compared using the Mann-Whitney test. Table 3 presents the results. Table 3 shows significant relationships for shear bond strength values between groups that had not biodegraded due to the application of 10% NaOCl. The shear bond strength value in the group that received a 2.9% GSE application before etching in addition to 10% NaOCl was higher than the 10% NaOCl control group. However, statistically, both
groups had no significant relationship \((p = 0.141)\), suggesting that the application of a 2.9% GSE solution before etching does not inhibit biodegradation of the resin-dentin shear bond strength.

| Table 3. | Significance values between groups after a 10% NaOCl application before or after etching |
|----------|----------------------------------|
| Group 2  | Group 4  | Group 6  |
| Group 2  | -       | 0.141    |
| Group 4  | 0.141   | -        |
| Group 6  | 0.006*  | 0.004*   |

*Mann-Whitney \(p < 0.05\)

3.2 Discussion

Proanthocyanidin can increase the resin-dentin shear bond strength and collagen resistance to biodegradation by increasing the physical properties and dentin collagen resistance [9]. Proanthocyanidin can form hydrogen bonds with a bridge-shape in the hydroxyl ion, carboxyl, amines, and dentin collagen amide. Hydrogen bonds can increase the stability of collagen fibers. In the process of hydrogen bond formation, the proanthocyanidin molecule can replace water molecules in the extrafibrillar collagen space. Proanthocyanidin can inhibit the production and activation of the MMP enzyme. The proanthocyanidin mechanism for inhibiting MMP production occurs through phosphorylase reduction, which is a key intracellular kinase [12,15].

In general, there are two methods for applying cross linking collagen agents to the dentin surface. Agents can be applied before or after etching. Applying GSE for 10 minutes before etching is based on a study by Srinivasulu et al that showed increased resin-dentin shear bond strength. Alternatively, applying GSE after etching was based on work by Al Ammar et al. [7,8].

This study was a preliminary study to examine the effectiveness of GSE, which contained 2.9% proanthocyanidin, to increase the bonding between the composite resin and dentin, as well as its ability to inhibit biodegradation of the bond. GSE is a solution that has been produced and sold on the market. To measure the value of the resin-dentin bond and its ability to inhibit biodegradation in restorations, the shear bond strength test can be done to determine the bond’s strength before and after a restoration has defects from mechanical and chemical processes. Shear bond strength measurements are one method to measure bonding efficiency between resin and dentin [16]. When in vitro conditions are applied to simulate oral cavity conditions, Yamauti suggests that storing the teeth in a 10% NaOCl solution for 1 hour can mimic the conditions of bonding biodegradation for 50% of the conditions observed during bonding biodegradation [17].

In this study, the groups prior to a 10% NaOCl application (shown in Tables 1 and 2) show that groups with a 2.9% GSE application beforeetching had a greater shear bond strength value (13.69 MPa) than the control group (6.56 MPa). Furthermore, there was a significant difference \((p = 0.002)\) between these groups, suggesting that a 2.9% GSE application before etching improves shear bond strength. These results are similar to a study by Srinivasulu et al. where a GSE application with 6.5% proanthocyanidin for 10 minutes increased the resin-dentin shear bond strength [7]. A significant difference between the group with a 2.9% GSE application before etching and the control group supported the first hypothesis that stated that 2.9% GSE applications could improve the resin shear bond strength on the dentin surface.

The group with an etching application method based on Al Ammar et al had a shear bond strength median value of 1.84 MPa. However, the shear bond strength value between the current study and Al Ammar et al was different. In the Al Ammar et al study, the shear bond strength values were lower. The lower values of the shear bond strength in their study were due to the molecular deposit of proanthocyanidin on the dentin surface. Proanthocyanidin molecules can be deposited on the dentin surface because of their large molecular weight and strong attachment to collagen, resulting in difficulties when rinsing with water. Proanthocyanidin molecules can inhibit resin polymerization and
reduce shear bond strength between resin and dentin. This finding concurs with a study by Liu who reported that the remaining proanthocyanidin on the dentin surface can inhibit resin polymerization [18].

Data groups after the application of 10% NaOCl described in Tables 1 and 3 show that the median value of the shear bond strength between the 2.9% GSE application before etching + 10% NaOCl (5.01 MPa) was greater than the control group + 10% NaOCl (4.06 MPa), however, this difference was not significant (p = 0.141). These results are in contrast with findings reported by Green et al who stated that the GSE may inhibit the collagen biodegradation processes. The differences in the results in this study were because the GSE solution used in this study was not a pure GSE. Statistical values were not significant (p = 0.141) between the groups receiving 2.9% GSE applications before etching + 10% NaOCl and the control group + 10% NaOCl.

4. Conclusion

GSE application with 2.9% proanthocyanidin on the dentin surface before etching can increase the resin-dentin shear bond strength, but it cannot reduce the resin-dentin shear bond strength biodegradation. Further studies are needed to assess the effectiveness of pure GSE on shear bond strength and resin-dentin bonding resistance with more proanthocyanidin and a shorter application time.

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