Original Article

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Micro-tensile Bond Strength of Self-etch and Etch and Rinse Adhesives to Dentin Following Surface Treatment With and Without Cross-linking Agents after 3 Months Aging: (In-Vitro Study)

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Abstract:

This study aimed to assess the impact of grape seed extract as a crosslinking agent on the micro-tensile bond strength of self-etch and etch-and-rinse adhesives to dentin after 24 hours and a 3-month aging period. 64 beams for micro-tensile bond strength testing derived from extracted sound human molars which were chosen and subsequently randomized into four primary groups. The teeth were categorized into two equal groups, each including four teeth, based on the surface treatment applied. Group (A1) denotes the control group that does not utilize cross-linking agents. Group (A2) comprises specimens subjected to treatment with grape seed extract. Each group will be further separated into two equal subgroups based on the adhesive method employed, with subgroup (B1) indicating the use of a total-etch adhesive system. Subgroup (B2) pertains to the application of a self-etch adhesive method. Subsequently, each group will be separated into two equal classes based on the aging period. T1 denotes aging after 24 hours, whereas T2 signifies aging after 3 months. The null hypotheses tested is that neither the surface treatment with grape seed extract as a cross-linking agent nor time interval affect the micro-tensile bond strength of composite resin restorations. The results indicated that etch and rinse adhesives exhibit significantly higher bond strength compared to self-etch adhesives. Additionally, grape seed extract markedly enhances the micro-tensile bond strength in self-etch groups relative to their control, whereas in the etch and rinse group, grape seed extract does not significantly improve bond strength compared to the control group. The bonding efficacy of etch-and-rinse adhesives surpasses that of self-adhesives, and pre-treatment of dentin with 6.5% GSE w/v can sustain superior bond strength during storage in artificial saliva.

Key words: Proanthocyanidin, Grapseed extract , Micro tensile bond strength.

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INTRODUCTION:

Proanthocyanidin is a plant-derived flavonoid often found in pine bark, elm trees, and grape seeds [(1)]. They are also prevalent in vegetables and fruits, but in diminished amounts. As a powerful antioxidant, PA lacks the harmful effects associated with glutaraldehyde. Numerous studies indicate that grape seed extracts, mostly consisting of PA, enhance the ultimate tensile strength and stiffness of demineralized dentine collagen [(2)] and increase the long-term stability of the dentine matrix [(3)]. Moreover, PA treatment enhanced the resin-dentine binding strength in both sound and caries-affected dentine.

Dentine is a hierarchically structured biological substance possessing elastic qualities attributed to its greater organic composition relative to enamel. It protects enamel against occlusal pressures. The organic matrix of dentine predominantly comprises type 1 collagen, along with several non-collagenous proteins, including proteoglycans, matrix metalloproteinases (MMPs), cysteine cathepsins, phosphoproteins, and additional dentine matrix proteins. The organic matrix is augmented by nanometer-scale hydroxyapatite mineral crystals deposited both extra-fibrillarly and intra-fibrillarly[(4)]. As caries progress, bacterial acid production demineralizes the extra-fibrillar and intra-fibrillar mineral compartments, therefore exposing the collagen matrix. The decrease in pH levels in demineralized dentine activates collagen-bound enzymes, including MMPs and cysteine cathepsins, which commence the proteolytic breakdown of the collagen-matrix.

The use of slightly acidic etch-and-rinse and self-etch adhesives on demineralized dentine may activate endogenous dentine matrix metalloproteinases (MMPs) and cysteine cathepsins as caries advance, resulting in further breakdown of collagen within the hybrid layer. Consequently, for reparative operations, it is essential to suppress or inactivate these protease enzymes and restore the mechanical capabilities of the collagen matrix to reverse the damage inflicted by the caries process and to improve the durability of resin-dentine linkages [(6)].

MATERIALS AND METHODS:

Selection and grouping of teeth:

A total of eight sound human extracted molars were utilized in the investigation. The teeth were meticulously cleansed under water and subsequently scaled with a sharp hand scaler to eliminate any plaque, calculus, or soft debris.

The teeth were preserved in physiological saline until used. The teeth will be categorized into two equal groups (four teeth per group) based on the surface treatment. Group (A1) denotes the control group that does not utilize cross-linking agents. Group (A2) denotes specimens subjected to treatment with Grape Seed extract. Subsequently, each group will be

separated into two equal subgroups based on the adhesive system employed, with subgroup (B1) denoting the utilization of a total-etch adhesive system. Subgroup (B2) refers to the application of a self-etch adhesive method. Subsequently, each group will be separated into two equal classes based on the aging period. T1 denotes aging after 24 hours, whereas T2 denotes aging after 3months. The teeth were abraded from their lingual surface to eliminate surface enamel, followed by the removal of 1 mm of the occlusal surface, as measured by pencil, below the dentin-enamel junction of the molars using a low-speed diamond saw under copious-coolant. A 6.5% grape seed extract solution was formulated by dissolving 6.5 grams of grape seed extract powder, sourced from capsules (Puritan's Pride Inc, Oakdale, NY, USA), in 100 ml of distilled water. [3]

Material's application:

Adhesive and GCE were administered in accordance with their respective groups.

Group 1: (control group utilizing etch and rinse adhesive) Etching and adhesive application were conducted in accordance with the manufacturer's guidelines.

Group 2: (GSE utilizing etch-and-rinse adhesive) Scotchbond™ Universal etchant was utilized for 15 seconds on the whole dentin surface. Pre-treatment with 6.5% GSE for 10 minutes following etching and before to adhesive application.

Group 3: (Control group utilizing self-etch adhesive) A self-adhesive bonding solution, Single Bond Universal, was employed in accordance with the manufacturer's instructions.

Group 4: (GSE utilizing ***self-etch adhesives)***

6.5 % GSE for 10 min painted using micro-brush, excess solution was absorbed using a cotton pellet.

Self-adhesive bonding system single bond universal (3M ESPE , St Paul ,MN,USA) was used according to manufacture instructions. The adhesive was applied to the entire tooth structure into 2 consecutive coats using micro-brush (Micro-brush-International, Grafton,USA) and rubbing each coat for 20 s. a Direct gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely.

Light curing for 10 s was performed using LED light-curing unit (Elipar S10,3M ESPE ,Paul,MN,USA)with light intensity 1200 mW/cm2 in standard mode using manufacture instructions.

Pre-treatment with 6.5% GSE for 10 minutes following etching and before to adhesive application. A circular Teflon mold with a height of 4 mm was constructed for the incremental assembly of composite blocks.

Regarding Micro-tensile bond strength testing of samples, the teeth were sectioned perpendicularly to the bonding surface with an automatic water-cooled precision diamond saw, both vertically and longitudinally to obtain rectangular sticks (1.0 mm _

1.0 mm wide; 8–9 mm long).8 beams from the same tooth were evaluated at each storage interval: 24 hours and 3 months. Specimens were preserved in synthetic saliva. The storage media were replaced daily.

Upon the completion of the storage period, the specimens were affixed with cyanoacrylate adhesive to a metallic fixture, which was then positioned on a micro-tensile testing apparatus subjected to tensile forces at a crosshead velocity of 1 mm/min until detachment occurred.

RESULTS:

Relation between different time periods in each group (Table 1):

Control with etch and rinse adhesive (Group1):

The findings indicated that the maximum mean value was recorded after 24 hours (26.79 ± 5.44), whilst the lowest mean value was seen after 3 months (17.62 ± 1.43).

A statistically significant change was seen at 24 hours and at 3 months (p=0.005).

GSE with etch and rinse adhesive (Group2):

Results revealed that the highest mean value was found (After 24 hours) (29.08 ± 4.02) while the lowest mean value was found (After 3 months) (28.15 ± 2.94).

There was no statistically significant difference between (After 24 hours) and (3 months) (p=0.865).

Control with Self etch adhesive (Group3):

The results indicated that the maximum mean value was seen after 24 hours (14.48 ± 1.44), while the lowest mean value was recorded after 3 months (11.94 ± 3.59), with no statistically significant difference identified between the two time periods.

GSE with Self Etch adhesives (Group4):

Results revealed that the highest mean value was found in (After 24 hours) (22.20 ± 2.97) while the lowest mean value was found in (After 3 months) (19.18 ± 2.52).

There was no statistically significant difference between (After 24 hours) and (After 3 months) where (p=0.119).

Variables	Micro-tensile strength				
	After 24 hours		After 3 months		*p-value*
	Mean	SD	Mean	SD	
Control etch and rinse (Group1)	26.79^{abA}	5.44	17.62^{bB}	1.43	**0.005 ***
GSE etch and rinse (Group2)	29.08^{aA}	4.02	28.15^{aA}	2.94	**0.865 ns**
Control Self etch (Group3)	14.48^{cA}	1.44	11.94^{cA}	3.59	**0.151 ns**
GSE Self Etch (Group4)	22.20^{bA}	2.97	19.18^{bA}	2.52	**0.119 ns**
p-value	**<0.001***		**<0.001***		

Table (1) Means with different small letters in the same column indicate statistically significance difference, means with different capital letters in the same row indicate statistically significance difference. *; significant (p<0.05) ns; non-significant (p>0.05)

DISCUSSION:

While bonding to enamel has demonstrated reliability, the longevity of resin-dentin bonds has been comparatively inadequate [(7)]. The bonding method to dentin employed by current adhesive technologies is fundamentally micromechanical, relying on the creation of a hybrid layer [(3)]. Furthermore, endogenous enzymes, including matrix metalloproteinases (MMPs), embedded within the hybrid layer may be activated by acid etching or acidic bonding agents, potentially compromising the stability of the bond between the adhesive and dentin by degrading the hybrid layer.

Diverse methods for stabilizing the resin-dentin contact, including the use of collagen cross-linking agents, have significant promise. The enhancement of collagen fibrils by the creation of supplementary inter- and intra-crosslinks, prompted by external factors, might augment their resistance to biodegradation. Prior research on collagen cross-linking agents includes glutaraldehyde, riboflavin, proanthocyanidins, and carbodiimides. Nonetheless, several factors such as biocompatibility, usability, clinical feasibility, and application duration must be considered.

This study employed a two-step etch and rinse adhesive (Adper Single Bond 3M ESPE), as this category of adhesives is often utilized. They include a distinct etching phase, succeeded by priming and bonding phases. These adhesives are technique-sensitive due to the existence of two independent stages and are prone to failure if the bonding surface is contaminated before the application of the restorative substance. Nonetheless, if the bonding is executed without technical faults, the resin-dentine bonds formed are dependable and long-lasting [(9)].

The study utilized Self-etch adhesive (Single Bond Universal 3M ESPE), the latest iteration of adhesive systems available. The reduction to a single step is considered the most advantageous application in therapeutic contexts. Due to its capacity for simultaneous self-etching and dentine infiltration, separate conditioning and priming processes are unnecessary[(9)].

The current investigation utilized grape seed extract at a concentration of 6.5%. Proanthocyanidins (PA) are naturally occurring polyphenolic compounds consisting of flavan-3-ol subunits mostly linked by C4–C8 (or C6) linkages. PA has been utilized in dentistry for the cross-linking of dentin collagen, the inactivation of endogenous collagen proteases, and the enhancement of resin-dentin bond strength. **Castellan et al. (2013)** demonstrated that a 6.5% GSE solution, high in PA, enhances the bond strength and stability of dentin bonding without inducing discoloration of the restoration. In the present investigation, a 6.5% w/v grape seed extract pre-conditioner has been employed, which is a recognized and established source of PA [(11)]. **Srinivasulu et al. (2012)** proposed that a ten-minute

therapy using cross-linkers is less time-intensive, more clinically viable, and can attain its objectives at elevated doses [12]. Nonetheless, **Carina S. (2013)** indicated that a ten-minute therapy remains clinically impracticable [13].

This study employed μTBS testing to mitigate the primary limitations of tensile bond strength assessment by removing tooth reliance and minimizing test variation. The μTBS values are determined by the tensile load at failure per unit area of the whole cross-sectional surface area of the bonded contact. To ensure the validity of bond strength values, a consistent and uniaxial stress must be applied. The benefits and drawbacks of using μTBS have been documented by [14]. The benefits encompassed the measurement of elevated interfacial bond strengths, an increase in adhesive failures relative to cohesive failures, the capability to analyze means and variances for an individual tooth, the ability to test irregular surfaces or smaller areas, and the examination of failed surfaces using scanning electron microscopy and transmission electron microscopy.

The study hypothesis, which posited that grape seed extract enhances the micro-tensile bond strength of both etch-and-rinse and self-etch adhesives, was accepted. The findings indicated a statistically significant enhancement in micro-tensile bond strength in the GCE group relative to the control group after 24 hours and three months. The efficacy of PA pre-treatment for 30-60 seconds in protecting the resin-dentine bond for an extended duration is uncertain, as it lacks a constant supply of PA to the dentine collagen. It has been suggested that gradual delivery techniques of PA into the resin-dentine interface might enhance the durability of the resin-dentine bond. Consequently, PA was included into an adhesive to guarantee the prolonged preservation of dentine collagen. The inclusion of 6.5% GCE in a resin adhesive has demonstrated the ability to safeguard collagen fibrils in the hybrid layer against collagenase degradation [15].

Additionally, it was agreed with **Srinivasulu et al. (2012)**, who asserted that collagen cross-linking agents enhance the bond strength of composites by increasing the number of cross-links, thereby augmenting dentin collagen stability. This procedure can be performed chairside to enhance the bond strength of composites to deep dentin. **Castellan et al. (2013)** said that cross-linking agents can enhance the strength of the dentin matrix without diminishing resin-penetration.

Furthermore, the examination of collagen cross-linking agents on the dentin-bonded interface in situ revealed a synergistic effect between the antibacterial activity that indirectly diminished demineralization and the exposure of the collagen matrix, along with the exogenous cross-linking of collagen, which elucidated the stable resin-dentin bond strength. **Fang et al. (2012)** sought to determine if transient

pre-treatment with PA-based preconditioners could enhance the resin-dentin bonds of various etch-and-rinse adhesives, revealing that collagen cross-linking treatment with PA-based preconditioners resulted in a concentration- and time-dependent enhancement in dentin bond strength, even with reduced application duration[16].

The interaction of GCE with proteins occurs via four mechanisms: covalent connections, ionic interactions, hydrogen bonding interactions, or hydrophobic interactions. PA, upon interacting with collagen, can induce inter- and intra-fibrillar, along with inter-microfibrillar cross-links within the collagen matrix [17].

PA has many free phenyl hydroxyl groups capable of forming bridge-type hydrogen interactions with the side chains of hydroxyl, carboxyl, amino, or amide groups in collagen molecules [18]. Hydrogen bonds confer stability to PA-collagen interactions. Furthermore, during the formation of hydrogen bonds, PA molecules may replace water molecules associated with collagen in the extra-fibrillar compartment. PA establishes ionic and covalent interactions with collagen fibrils by positioning itself between collagen molecules [19].

Covalent connections enhance the durability of collagen fibrils against endogenous protease activity, with the primary mechanism of most matrix metalloproteinases (MMPs) and cysteine cathepsins being the silencing of catalytic and allosteric sites (20). Consequently, PA, being a potent cross-linker, may directly cross-link the catalytic and allosteric regions of these enzymes, thereby limiting their functionality[21].

The pre-treatment of dentine with 6.5% GSE before bonding with self-etch adhesive cement did not appreciably enhance bond strength. **Broyles et al. 2012**.

The PA pre-treatment was confined to the surface regions of dentine, resulting in the cross-linking of the smear layer by PA, which enhanced resistance to breakdown by acidic monomers. This leads to inadequate penetration of adhesive resin through the remaining smear layer and demineralized dentin, leading in diminished bond strengths. The results of GSE dentine pre-treatment with a self-etch adhesive were contingent upon both the material and the primer used [22].

CONCLUSIONS:

Within the limitations of this study, the following conclusions can be drawn.

1) The bonding performance of etch and rinse adhesives is better than self- adhesives

2) Pre-treatment of dentin with 6.5 % GSE w/v for 10minute, before the application of the adhesive significantly improves the bonding performance for self-etch adhesives.

3) Pre-treatment of dentin with 6.5 % GSE w/v could maintain higher bond strength after storage in artificial saliva.

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