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Effect of Naturally Derived Dentin Bio-modifiers on Immediate and Long-Term Bond Strength of Etch and Rinse Adhesive to Dentin

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ABSTRACT

Purpose: To investigate the effect of 10% Proanthocyanidin (PA) rich grape seed extract (GSE) and cardanol oil applied for 60s on immediate and long-term shear bond strength (SBS) of etch and rinse to dentin. **Materials and methods**: The occlusal surfaces of forty two samples were ground to expose flat superficial dentin surfaces. The samples were divided into 3 groups; Group A1 (control group) without any pre-treatment, Group A2 (10 % GSE), Group A3 (cardanol). Pre-treatments were applied for 60s and then washed away for 30s. Each group was further randomly subdivided into two groups (n=7) according to the storage time (immediate 24 hours) and (long-term six months period). Shear bond strength (SBS) was tested using a universal testing machine at a cross head speed of 0.5mm/min. Data was tabulated and presented as mean and standard deviation, one-way ANOVA, post hoc Tukey's test for pairwise and Paired student t-test was performed between paired groups. **Results**: All pre-treatment groups showed statistically no significant SBS values after 24 hours of storage. While at 6 months storage, group A3 showed the highest SBS mean value followed by group A2 while Control Group recorded the lowest shear bond strength mean value. **Conclusion:** Natural dentin Bio-modifiers are considered effective in improving the shear bond strength of the bonded interface and the Bond strength of dentin is affected by the storage time.

KEYWORDS

Grape seed extract, Cardanol, Natural bio-modifiers

INTRODUCTION

Dentin is a mineralized tissue made up of tubules that extend from the pulp to the DEJ, as well as intra-tubular and peri-tubular dentin. Fibrillar type I collagen makes up 90% of the organic matrix in dentin, with non-collagenous proteins including phosphor-proteins and proteoglycans accounting for the remaining 10%. Acid etching

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serves as a framework for resin infiltration in the dentin resin bond system. The resin then infiltrates the exposed collagen fibres, which should be completely covered. It is widely acknowledged that the resin-dentin bond established by dentin adhesive systems deteriorates with time and is susceptible to hydrolytic breakdown by activated host-derived proteases matrix metallo-proteinases (MMPs) and cysteine cathepsins and the longevity of the bonded restoration will be decreased as an outcome[1].

Dentin bio-modification has recently been used to generate a more stable and long-lasting adhesive contact. To bio-modify and increase the mechanical properties of the dentin substrate, these bio-modifiers operate as MMP inhibitors and collagen cross-linkers[2]. Natural bio-modifiers are naturally occurring substances that have gotten a lot of attention in the last decade because of their potential dental applications. When compared to synthetic agents, the most appealing properties are their very low toxicity bio-modifiers and renewable/ sustainable resources[3]. Natural condensed tannins, also known as proanthocyanidins (PACs) (extract from grape seed), are a category of plant-derived polyphenols with a high crosslinking capability to collagen, which provides bio-stability and improves the mechanical properties of the organic matrix of the dentin[4].

Cardanol and cardol, two phenols found in cashew nut shell liquid oil, make up the majority of the oil. Cardanol is the primary component, accounting for 60–65 percent (w/w) of the total, with a long carbon-chain phenol derived from industrial cashew nut shell oil. Cardanol is a non-cytotoxic chemical with antioxidant properties at low concentrations. Cardanol inhibits matrix metalloproteinase-2 and matrix metalloproteinase-9, just like PACs[5,6]. Despite the fact that several plant-derived polyphenols as proanthocyanidins have been tested for the bond strength of a simple etch and rinse adhesive system to dentin, there has been little research on the effect of cardanol from cashew-nut shell liquid on the bond strength of an etch and rinse adhesive system to dentin[2,7].

The study initial null hypothesis indicated that cardanol from cashew-nut shell liquid had no effect on the etch and rinse adhesive binding strength to dentin. After age, dentin surfaces pre-treated with natural cross-linkers demonstrate bio-degradation, according to the second null hypothesis.

MATERIALS AND METHODS

Preparation of proanthocyanidins (grape seed extract) (Liquid/Liquid Extraction with Ethyl Acetate): Grape seeds were obtained from fresh red grapes then washed under tap water, dried and freezed for 24 hours in the freezer -18^{0}c. The grape seeds were grounded to 20 grams of brownish powder to obtain maximum amount of proanthocyanidins (up to 90%) released from the extract. The powder was added in a separation funnel with (10% ethyl acetate as a solvent prepared at (Al-Azhar University, regional center for mycology and biotechnology) (9ml of ethyl acetate: 1ml of water) (>90% ethyl acetate). Then, shaking of the mixture in the separation funnel was done followed by soaking overnight for 24 hours to ensure complete release of the polyphenolic substance. After that, the mixture was placed in an electric oven at 50^{o}c for 20 minutes to concentrate GSE and produce homogenous mixture. 10% of grape seed extract was placed at room temperature until time of use as (Fig. 1)[8].

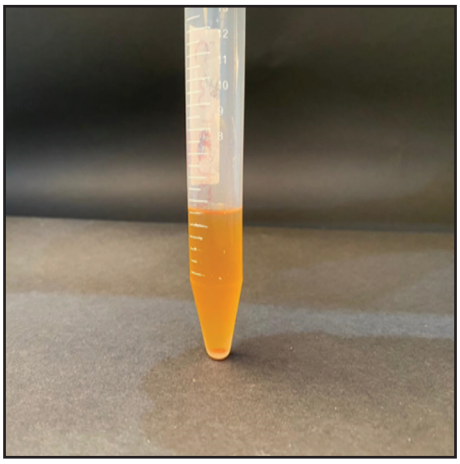

Figure (1) Grape seed extract as a natural bio-modifier

Cardanol oil (Cashew nut shell oil extract): A natural cross-linker (cashew nut extract) was purchased from (deve herbes, New Delhi, India) and contained in a glass bottle as (Fig. 2). Artificial saliva (pH 7.11) for the immediate and long-term storage of the samples was prepared by adding 0.5% w/v sodium carboxymethyl cellulose to 30% w/v glycerine and a flavoured base. 0.2% sodium azide (pH 7.31) was further added to prevent bacterial growth and to be changed every two weeks. This was confirmed through the maintenance of the clear color of artificial saliva during the storage period[9].

Research ethics committee approval (REC) was obtained from faculty of dental medicine for girls Al-Azhar University at code REC-OP-21-08. All teeth obtained from patients who signed consents of approval of using their teeth. A total number of forty-two human sound premolars were collected from patients aged between 20-45 years. The selected teeth were cleaned from calculus and soft tissue remnants using an ultrasonic scaler (Cavitron, Dentsply, USA). All teeth were stored in normal saline[10] at room temperature to be used within one month.

Figure (2) Pure cardanol oil as a natural bio-modifier

The teeth were divided into three main groups (14 teeth each) (A1, A2 and A3) according to surface treatments, where group A1 is the control group as dentin surface did not receive any surface treatment. Group A2 where dentin surface was pre-treated with 10% grape seed extract (GSE). Group A3 where dentin surface was pre-treated with Cardanol. Each group was further subdivided into two subgroup (7 teeth each) (B1 and B2) according to storage time into immediate (24 hours in storage media) and long-term (6 months period in storage media).

The occlusal surface of each sample was ground using grinding machine (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water coolant until the level of dentin-enamel junction (DEJ). The level of superficial dentin was marked using unerasable Marker at a level of 1mm below DEJ and by using the grinding machine the superficial dentin was exposed[11]. All flat dentin surfaces were acid etched for 15 seconds with 30-40% phosphoric acid (N-Etch, Ivoclarvivadent, USA) followed by surface pre-treatements according to each group. Group A1: did not receive any surface pre-treatment. Group A2 and A3: the dentin surface was pre-treated with 1mm coat of grape seed extract and cardanol for 1 minute using disposable applicator brush at and then vigorously rinsed by water for 30 seconds to remove the unbound cross-linkers.

After that, two consecutive coats of the adhesive bond (a total etch i-BONDING LCN bond, i-dental Medicinos linija, UAB) were Light cured (LEDition curing unit 3M, ESPE elipar, USA) for 10 seconds. A metal ring surrounding Teflon mold with a central hole (2.5 mm in diameter x 2 mm in height) was placed around the bonded dentin surface to produce a standardized composite resin (nano-hybrid FILTEK Z250 XT composite, 3M ESPE, ST.PAUL, MN, USA)[9,12]. Half of the samples (B1) (immediate testing group) were then stored in artificial saliva at 37°C for 24 hours for completion of

polymerization before immediate testing. The remaining half samples (B2) from each group (delayed testing group) were stored in artificial saliva at room temperature in an incubator for 6 months before shear bond strength test. Moreover, storage solution was changed every 2 weeks[9].

Shear bond strength was tested using a universal testing (Instron Industrial Products, Norwood, USA) with a load cell of newton (5 KN). The shear bond strength results were tabulated and presented as mean and standard deviation. One-way ANOVA was done for comparison between groups followed by post hoc Tukey's test for pairwise comparison between mean values when ANOVA was significant. Paired student t-test was performed between paired groups.

RESULTS

Mean values with the same superscript uppercase letters within each column indicated statistically non-significant difference (p>0.05), mean values with different superscript uppercase letters within each column indicated statistically significant difference (p<0.05) within same storage time

Table 1: *The mean ± SD and P-value for the effect of different treatment agents on shear bond strength at different storage time intervals according to the paired tukey's test and two way ANOVA test.*

Variables	24 hours storage time	6 months storage time
Control group	8.32 ± 0.91^{A}	7.45 ± 0.83^{C}
GSE group	9.48 ± 1.26^{A}	14.91 ± 1.92^{B}
Cardanol group	9.76 ± 1.74^{A}	20.34 ± 2.13^{A}
P-value	0.4014 NS	$<0.001^{*}$

**: significant P≤0.05; NS; non-significant P>0.05*

Mean values with the same superscript uppercase letters within each column indicated statistically non-significant difference (p>0.05), mean values with different superscript uppercase letters within each column indicated statistically significant difference (p<0.05) within same group.

Table 2*: The mean ± SD and P-value for effect of storage time intervals on shear bond strength of different dentin treatment groups according to the paired student t-test*

Variables	Control group	GSE group	Cardanol group
24 hours storage time	8.32 ± 0.91^{A}	9.48 ± 1.26^{B}	9.76 ± 1.74^{B}
6 months storage time	7.45 ± 0.83^{A}	14.91 ± 1.92^{A}	20.34 ± 2.13^{A}
P value	0.2648 NS	0.001*	$<0.0001^{*}$

**: significant P≤0.05; NS; non-significant P>0.05*

DISCUSSION

Cardanol oil is a potent antioxidant and natural bio-modifier with a long 15-carbon alkyl side chain, which provides more hydrophobic interaction with the dentin substrate and a lower molecular weight than grape seed extract, which may achieve greater and faster penetration into the dentin collagen matrix, resulting in an increase in the mechanical properties of the dentin collagen matrix. When grape seed extract is compared to cardanol, the additional hydroxyl group in proanthocyanidin increases hydrophilicity, resulting in fewer hydrophobic bonds. Collagen fibrils linked to cardanol molecules may become more hydrophobic, preventing water seepage over the 4-week storage period and the collagen degradation[12].

Grape seed extract is a natural bio-modifier as it contains bio-compatible polyphenols (proanthocyanidin) that belong to condensed tannins which have outstanding potentials as bio-modification agents and matrix metallo-proteinase inhibitor[12]. The building blocks, such as catechin and epigallo-catechin gallate in GSE, have been identified as potent collagenase inhibitors in addition to the four proposed mechanisms (covalent, ionic, hydro-

gen bonding and hydrophobic) for interaction with dentin collagen matrix. Grape seed extract induces more cross-links via bonding to ε-amino groups of peptidyl and hydroxyl residues of collagen fibrils forming more strong bonds. PACs in the GSE is belonging to the condensed tannins group so they have high molecular weight allowing the formation of more complex structures with collagen matrix[11,13].

Regarding the effect of treatment agents on shear bond strength at 24 hours storage time, one way ANOVA followed by pair-wise Tukey's post-hoc tests showed that there was no statistically significant difference in mean shear bond strength between different dentin treatment agents. The result proved that there was no additional bond formation between resin and dentin interface in the bio-modifier treated groups after 24 hours storage and proved the effectiveness of the polymerization process and stable hybrid layer formation without bond degradation. In this study, PA was used as a separate step similar to previous research showed that direct application of PA to dentin as a separate step preserved the hybrid layer stability[14].

While at 6 months storage time, the treatment agents had statistically significant difference effect on shear bond strength where cardanol Group recorded the highest shear bond strength mean value followed by GSE Group while control group recorded the lowest shear bond strength mean value. It has been proposed that the application of cross-linkers as pre-treatments promoted significant bonding stability to dentin compared with the no pre-treatment group. The characteristics of grape seed extract and cardanol resulted in more bond formation (hydrophobic, ionic, covalent and hydrogen) with the collagen matrix resulting in increasing the shear bond strength of bio-modifier treated groups[12,15,16]. This study was in agreement with other studies that showed a decrease in the shear bond strength mean value and bio-degradation of the bonded interface in the control group because it lacked the benefits of bio-modifiers[14,17].

Regarding cardanol as a bio-modifier, few research used cardanol oil in the dental field as a natural bio-modifier. In 2017, a study showed a significant increase in mechanical properties immediately after treatment with cardanol which doesn't coincide with the result of this study. Their study used molars with a bigger surface area that may synergistically increase the mechanical properties[12].

Also, this study conflicted with another study that showed no significant difference after one-year of storage. This could be related to using wet cotton pellet to remove acid etch and excess bio-modifier solution which might have compromised the bonding procedure with collagen matrix[14].

Regarding the effect of storage time on shear bond strength within each dentin treatment group, Paired student t-test showed that there was statistically significant difference in mean shear bond strength between different storage times within Cardanol oil Group and grape seed extract group. While in control group there was no statistically significant difference in mean shear bond strength and. In cardanol group, shear bond strength mean values at 6 months storage time recorded statistically significant higher shear bond strength than that at 24 hours storage time. In grape seed group, shear bond strength mean values at 6 months storage time recorded statistically significant higher shear bond strength than that at 24 hours storage time. While in control group, shear bond strength mean values at 6 months storage time recorded no statistically significant lower shear bond strength from that at 24 hours storage time.

The result could be attributed to the characteristics of grape seed extract and cardanol groups which might have maintained the hybrid layer without degradation and resulted in more bond formation with the collagen dentin matrix. More bonds formation (hydrophobic, ionic, covalent and hydrogen) with the collagen matrix resulted in increasing the shear bond strength of bio-modifier treated

groups after aging[18]. This result agreed with a study showed no degradation in cardanol treated group after aging when compared with the immediate tested group and showed statistically significant decrease in bond strength of control treated group after aging than the immediate tested group[19].

This study contradicted with other study that showed a statistically significant decrease in the mechanical properties after aging when compared to immediate dentin pre-treated groups. In their study they used only 6.5% of GSE while in this study 10% of grape seed extract was used[12].

CONCLUSION

Natural dentin Bio-modifiers are considered effective in improving the shear bond strength of the bonded interface and the Bond strength of dentin is affected by the storage time.

RECOMMENDATIONS

Natural dentin bio-modifiers can be regarded in terms of dentin cross-linking and long-term dentin-resin bond stability.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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