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EVALUATION OF SHEAR BOND STRENGTH OF RESIN ADHESIVE/ TOOTH INTERFACE AFTER MODIFICATION WITH GRAPE SEED EXTRACT: AN *IN-VITRO* STUDY

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ABSTRACT

Objective: To evaluate the influence of a grape seed extract (Proanthocyanidin)-based preconditioners on shear bond strength (SBS) of restorative resin-composites bonded to the dentin of human premolar teeth.

Materials and Methods: unrestored extracted human premolars (n = 130) with sound buccal surfaces were divided into five groups: one control (n = 10) and four experimental groups (n = 30 each). Crowns of all teeth were cut and embedded horizontally in the middle of a plastic cylinder of a self-curing polymethyl methacrylate measuring 4 cm in diameter and 3 cm in depth. Exposure of the buccal dentin surface was done using a high-speed diamond bur with abundant cooling to avoid dentin burning and smoothed with wet silicon carbide (SiC) sandpapers, grits 180-320, and running tap water. Dentin surfaces of all specimens were etched with 37% phosphoric acid for 15 s, rinsed and gently dried with oil-free air. For the control group (I), a fully saturated brush tip of All-Bond Universal (BISCO, USA) was utilized to apply two coats to the dentin surface then light-cured. For the intervention groups, four concentrations of Proanthocyanidin (PA)-based preconditioners were applied to the etched dentinal surfaces: specimens of group II were treated with 5% PA-based gel, group III with 10% gel, group IV with 15% gel and group V with 20% gel. Specimens of each of the intervention groups were further subdivided into three subgroups (n=10) according to the application time of PA-based gel; 20 s, 40 s and 60 s. All treated surfaces were then washed and gently dried to receive the All-Bond Universal as the control group. A resin-composite (Grandio, VOCO GmbH-Cuxhaven, Germany) disc specimen (4 mm in diameter × 2 mm in thickness) was built to each dentin surface using Teflon molds and light-activated for 40 s and stored in distilled water at 37°C for 24 h before debonding that was carried out using a universal testing machine (model 3365, Instron, High Wycombe, UK) at a crosshead speed of 1 mm/min. A two-way ANOVA followed by one-way ANOVA and Bonferroni *post-hoc* test were applied for data analysis.

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Results: Statistical analysis revealed significant differences between the investigated PA-concentrations at the application times selected: at 20 s ($p = 0.013$), at 40 s ($p = 0.009$) and at 60 s ($p = 0.04$). Dependent on PA concentration and application time, the mean value of SBS ranged from 23.87 MPa to 38.65 MPa. At a given concentration (15%) of PA-based gel, linear regression analysis revealed a strong positive correlation ($R^2 = 0.956$) between SBS and application time. However, a moderate positive correlation ($R^2 = 0.484$) between SBS and PA concentration was revealed when linear regression analysis was applied at a given application time (60 s).

Conclusion: Treatment of dentin surfaces with PA-based preconditioners significantly enhanced SBS of resin composite/tooth structure. SBS results showed a direct increase with the application time of PA-based preconditioners while 15% concentration of PA recorded the highest values.

KEYWORDS: Shear Bond Strength; Grape Seed Extract; Proanthocyanidin; Resin Adhesive; Resin-composite.

INTRODUCTION

Establishing a truly adhesive bond between a restorative material and the natural tooth structure is one of the most crucial goals of modern dental researches. Conservation of tooth structure during restoration of teeth as well as increasing the clinical durability are two important sequelae of developing such a powerful bond between restoration and tooth material [1]. Bonding to enamel and dentin has become so critical to the extent that it is no longer acceptable that a restorative material is just required to replace the defective dental structure, but it is also mandatory to adhere, seal, provide a durable, long-lasting surface in a very harsh environment, and yet biocompatible [2].

The development of adhesive systems has caused profound changes in the dental practice [3]. The primary aim of dental adhesives is to provide retention to resin-composite fillings or cements. Prevention of leakage along the restoration margins as well as withstanding mechanical forces, particularly shrinkage stress from the lining composite, are two main tasks of a successful adhesive system. Clinically, failure of restorations occurs more often due to inadequate sealing, with subsequent discoloration of the cavity margins, than due to loss of retention [4,5].

It was a great work by Buonocore, 1955, when he established the basis for modern restorative dentistry by developing a technique for bonding acrylic materials to enamel using phosphoric acid [6]. The strong adhesive joints formed by bonding resins to etched enamel resulted in the widespread use of “acid-etching techniques” rather than pins for the retention of resin-composite restorations [7,8]. At first, it was thought that “acid-etching” simply increases the microscopic surface area available for resin retention. Upon looking closer at the tooth-restoration interface, John Gwinnett reported that adhesive resins could penetrate into acid-etched enamel prisms where they could actually envelop apatite crystallites rendering them acid-resistant [9]. With regard to dentin bonding, Nakabayashi et al. [10] were the first to demonstrate true hybrid layer formation in acid-etched dentin. This was best observed by transmission electron microscopy and later demonstrated by scanning electron microscopy following argon ion beam etching [11].

Many approaches have been tried to enhance the resin-dentin bond and to minimize its deterioration. Münchow and Bottino [12] stated that adhesively-bonded restorations may be benefited from the use of some biomolecules, nanocompounds or alternative bonding approaches in order to minimize bond strength degradation. They presented an overview about the main agents and strategies to improve or stabilize resin-dentin bonding. These include: i) use of collagen crosslinking agents that may form stable covalent bonds with collagen fibrils, thus strengthening the hybrid layer, ii) use of antioxidants that may allow further polymerization reaction overtime, iii) use of protease inhibitors which may inhibit or inactivate matrix metalloproteinases (MMPs), iv) reinforcement of the resin matrix with inorganic fillers and v) utilization of remineralizing agents that may positively enhance physico-mechanical properties of the hybrid layer.

Lately, proanthocyanidins (PA) were used to enhance resin-dentin bonding. Proanthocyanidins are any oligomeric polyphenolic compound composed of flavonoids that have the ability to interact with proteins such as collagen. Naturally occurring proanthocyanidin is an extract from grape seeds. Four mechanisms for interaction between proanthocyanidin and proteins have been proposed. These include covalent interactions, ionic interactions, hydrogen bonding interactions and hydrophobic interactions. The application of grape seed extract PA was said to be beneficial to enhance the mechanical properties of adhesive restoration interfaces to withstand degradation overtime [13]. Also, it was reported that when the grape seed extract PA was applied to demineralized dentin collagen matrix, the size and number of interfibrillar spaces were reduced and the dentin matrix exhibited remarkably low collagen solubilization [14,15].

Prevention of collagen matrix degradation, with the aid of available materials and clinically applicable techniques, can be achieved in several ways to retain hybrid layer integrity and strong dentin bonding. Cross-linking of dentin matrix collagen could render hybrid layer more resistant to degradation. In addition, inhibition of the matrix metalloproteinases (MMPs) associated with matrix degradation is a promising approach to improve hybrid layer preservation and bond strength durability [16]. The use of PA-based extracts proved capacity of improving and stabilizing collagen matrices through induction of exogenous cross-links, thus enhancing the long-term stability of dentin matrix [12]. Proanthocyanidins were found to increase collagen synthesis and accelerate the conversion of soluble collagen to insoluble collagen during development and have the potential to give rise to a stable hydrogen-bonded structure [17, 18].

Upon evaluating the mechanical characterization of proanthocyanidin-dentin matrix interaction, Castellan et al. [19] reported an enhancement of the elastic modulus of demineralized dentin after treatment with grape seed extract cross-linking agent. In addition, resin-dentin bond was significantly increased regardless the application time and adhesive system used. According to the authors, an increase in the mechanical properties and dentin matrix stability can be accomplished by the use of PA-rich collagen cross-linkers most likely due to formation of a PA-collagen complex.

In the current study, PA was added to a commercially adhesive system and utilized in resin-dentin bonding to investigate the capability of PA to enhance the bond strength of resin-composite restoration. Our two null hypotheses were; (i) addition of PA to dentin adhesive system will not affect the bond strength, and (ii) varied PA concentration and different application time of PA-based preconditioners will not affect the bond strength.

MATERIALS AND METHODS

Tooth preparation

Testing of shear bond strength (SBS) was carried out on sound dentin surfaces of 130 extracted human premolars for orthodontic reasons (first and second, upper and lower premolars) with unaffected buccal surfaces. The teeth were stored in normal saline at 4°C, until further use. To make the handling of specimens easier, the crowns of all teeth were cut and embedded horizontally in a self-curing polymethyl methacrylate (PMMA; Esschem Co., PA, USA), that was mixed according to the instructions of the manufacturer. Each tooth crown was centered in the middle of a plastic cylinder measuring 4 cm in diameter and 3 cm in depth and then the PMMA mixture was poured around the cut crown. Care was taken during the embedding process so that the buccal surface of each tooth crown was projected 3mm above the surface of the set PMMA.

Dentin surface preparation

Exposure of the buccal dentin surface was done using a high-speed diamond bur (8-3 Kiyohara Industrial Park, Utsunomiya, Tochigi, Japan) with abundant cooling to avoid dentin burning. Wet silicon carbide (SiC) sandpapers, grits 180-320, and running tap water were used to flatten dentin surfaces. All specimens were cut and flattened by only one operator to guarantee standardization. The prepared surfaces were dried for 3-5 s before etching.

Preparation of Proanthocyanidin-based preconditioners

This step was carried out at the laboratory of Faculty of Science by an experienced chemist where PA-based preconditioners were prepared by adding powdered proanthocyanidin-rich grape seed extract at different ratios to demineralized water and then incorporated in carbomer gel base. Four concentrations were prepared: 5, 10, 15 and 20% (w/w) of grape seed extract (GSE). The pH of all these concentrations was adjusted to be slightly alkaline (7.4) by addition of tri-ethanol amine and stirred slowly until a gel was obtained. All these preparations were produced in the form of gels which were dark brown in color. Table 1 shows the composition of the four concentrations of GSE preconditioners used in his study.

TABLE (1) Materials and concentrations used for preparation of PA-based preconditioning gels. The pH of the prepared gels was adjusted using a pH meter.

Grape seed extract (g)	Carbomer (g)	Demineralized water (100g)
5	0.2	94.8
10	0.2	89.8
15	0.2	84.8
20	0.2	79.8

Preparation of dentinal surfaces for adhesion

For the control group (group I, *n*=10) - that was treated with conventional bonding without PA-based preconditioners - the dentin surfaces were etched with 37% phosphoric acid for 15 s. Etched surfaces were then rinsed and gently dried with oil-free air. A fully saturated brush tip of All-Bond Universal (BISCO, USA) was utilized to apply two coats to the dentin surface then light-cured. For the intervention groups (II-V, 30 specimens each), four concentrations of PA-based preconditioners were applied to the etched dentinal surfaces: group II specimens were treated with 5% PA-based gel, group III was treated with 10% gel, group IV was treated with 15% gel and group V was treated with 20% gel. Specimens of each of the intervention groups were subdivided into three subgroups (*n*=10) according to the application time of PA-based gel; 20 s, 40 s and 60 s. All treated surfaces were then washed and gently dried to receive the All-Bond Universal as the control group.

Building up of resin-composite specimens

A resin-composite (Grandio, VOCO GmbH-Cuxhaven, Germany) disc specimen (4 mm in diameter× 2 mm in thickness) was built to each dentin surface using Teflon molds and light-activated for 40 s using the tungsten-halogen curing unit Translux Energy (850 mW/cm^2, Heraeus

Kulzer, Hanau, Germany) and stored in distilled water at 37°C for 24 h before testing.

Mechanical testing (debonding)

A universal testing machine (model 3365, Instron, High Wycombe, UK) was used to measure shear bond strength (SBS) at room temperature. The test assembly was prepared so that the long axis of the tooth crown was set vertically and the shearing blade (stainless steel chisel with a pointed end measuring 2 mm in thickness) contacted the upper part of the resin-composite disc at the dentin-resin interface. Loading of specimens was carried out at a crosshead speed of 1 mm/min until debonding of the resin-composite disc. Specimens with adhesive failure were considered and those with cohesive failure were excluded. Values of shear bond strength for all test groups were recorded and compared.

Statistical analysis

A two-way ANOVA was applied to determine the interaction between factors: PA concentration (at 4 levels) and application time (at 3 levels). As the interaction was significant ($p < 0.05$), the comparison between groups was conducted at each application time using a one-way ANOVA. The significance level was set at ($p \leq 0.05$). Levene's test for homogeneity of variance was carried out for the data of each application time ($p \leq 0.05$). As equal variances were confirmed ($p = 0.802$) for data at all etching time groups, the Bonferroni *post-hoc* test was used multiple comparison between groups. Linear regression analysis was performed to investigate relationship between bond strength and PA concentration and between bond strength and application time of PA-based gel of all groups.

RESULTS

The two-way ANOVA revealed a statistically significant interaction ($P < 0.05$) between the two factors: PA concentration and application time. This meant that the data for each concentration or time had to be considered separately. Data of bond strength (mean and standard deviation) at different PA concentrations and different application times are presented in Table 2 and shown in Figure 1. Superscript letters and numbers are shown in Table 2 to demonstrate statistical differences between investigated subgroups.

The strongest shear bond strength (SBS) was showed by specimens treated with 15% PA-based gel and application time of 60 s (38.65 MPa). The weakest SBS was recorded for specimens treated with 5% PA-based gel for 20 s (23.87 MPa) followed by those of the control group (24.46 MPa).

With the exception of specimens treated with 5% PA-based gel for 20 s, all specimens showed SBS greater than that recorded by the control group. Longer application time of PA-based gel demonstrated a systematic increase in SBS in all groups. A systematic increase in SBS results was also shown with greater concentrations of PA-based gel between 5-15%. Increasing PA concentration to 20% produced lower SBS values than specimens treated with 15% but still greater than those of the control group and those treated with 5% PA-based gel.

At a given concentration (15%) of PA-based gel, linear regression analysis revealed a strong positive correlation ($R^2 = 0.956$) between shear bond strength (MPa) and application time (s). However, a moderate positive correlation ($R^2 = 0.484$) between SBS and PA concentration was revealed when linear regression analysis was applied at a given application time (60 s).

Statistical analysis revealed that significant differences were found between the investigated PA-concentrations at the three application times selected: at 20 s ($p = 0.013$), at 40 s ($p = 0.009$) and at 60 s ($p = 0.04$).

TABLE (2) Mean data and standard deviations (in parentheses) of shear bond strength (MPa) for all control and intervention groups (at different PA concentration and application time). Each bond strength value represents the mean of ten measurements. Different superscript letters indicate statistically significant differences ($p \leq 0.05$) between subgroups, compared with the control group.

Groups	**Preconditioning with PA-based gel**		**Shear Bond Strength (MPa) (Mean & St. deviation)**
	Concentration (%)	**Application Time (s)**	
Group I (*control*)	-	-	24.46 (2.14) [a, i, A]
Group II	5	20	23.87 (1.07) [a]
		40	24.65 (1.81) [i]
		60	25.19 (2.12) [A]
Group III	10	20	27.17 (2.21) [a]
		40	30.22 (1.63) [ii]
		60	31.69 (1.55) [B]
Group IV	15	20	35.26 (1.21) [b]
		40	36.34 (1.42) [iii]
		60	38.65 (1.13) [C]
Group V	20	20	30.82 (2.03) [b]
		40	32.11 (1.93) [ii]
		60	32.79 (2.18) [B]

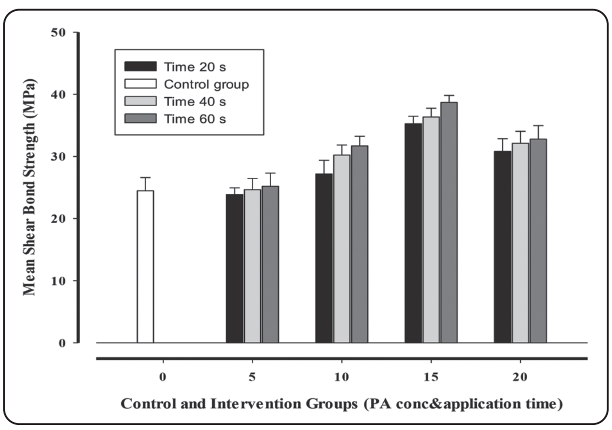

Fig. (1) Error bar of shear bond strength (MPa) of control and intervention groups (at different PA concentration and application time).

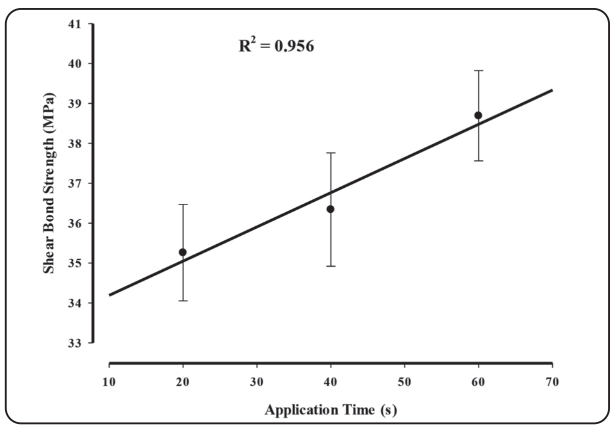

Fig. (2) At a given concentration (15%) of PA-based gel, linear regression analysis revealed a strong positive correlation (R^2= 0.956) between shear bond strength (MPa) and application time (s).

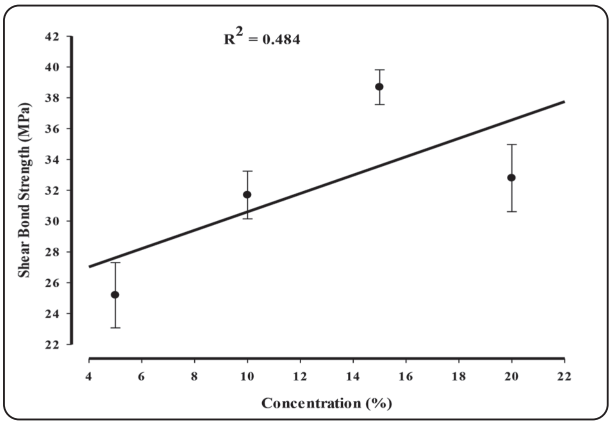

Fig. (3) At a given application time (60 s) of PA-based gel, linear regression analysis revealed a moderate positive correlation (R^2= 0.484) between shear bond strength (MPa) and PA concentration (%).

DISCUSSION

Many dental investigations can be carried out both in the patient's mouth as well as in the laboratory. However, in case of testing bond strength between tooth structure and resin-composite materials, the *in-vitro* screening tests may be superior to the clinical trials provided that these laboratory tests are conducted under conditions simulating those of the clinical situation. The ability to evaluate a single variable when other variables are kept constant, patient's independence, ease of achievement and saving money and time are some of the advantages that *in-vitro* tests possess when compared with *in-vivo* ones [20].

An enhanced clinical behavior of a resin-based restorative material is greatly dependent on an effective adhesion that blocks, or at least reduces, the interfacial microleakage together with the minimal and conservative tooth preparation that preserve the bulk of the tooth structure [21]. This adhesion that provides retention and sealing of a restoration involves joining of two dissimilar surfaces: a restorative material and a tooth structure [22]. The adhesives applied join materials together to resist debonding and transmit stresses across the bonds. It is well-established between authors that the etching strategy, type of adhesive and kind of restorative material applied significantly affect the "tooth-restoration" bond strength [23]. In addition, it is widely recognized that the characteristics of the bonding substrate greatly affect the bond strength. Such clinically relevant substrates include caries-affected, caries-infected, sclerotic, deep, and bur cut dentin [24].

In the current study, shear bond strength of resin-composite discs bonded to dentin surfaces treated with a grape seed extract, proanthocyanidin (PA), was evaluated. The main purpose of this investigation was to study the promoting effect of PA-based preconditioners on shear bond strength of resin adhesives/tooth interface. When dentin is etched before the application of primer/adhesive system, collagen becomes exposed. Upon the application of adhesive monomers that penetrate into the expanded collagen network, a hybrid layer is formed which is essential for successful dentin bonding. That is why, for durable and clinically predictable bonding, the stability and maintenance of collagen matrix are mandatory [16]. Improper impregnation of dentin collagen with the adhesive system or existence of porosity within the hybrid layer can accelerate the process of dentin/restoration interface degradation leading to reduced bond strength over time and increased leakage at the interface [25]. Thus, several cross-linking agents were applied. Some of them, however, have some drawbacks such as toxicity, instability and difficulty to control. Proanthocyanidin (PA) compound was claimed to be an agent with cross-linking abilities and biocompatibility. Proanthocyanidins were found to enhance collagen synthesis and accelerate transformation of soluble collagen into insoluble one during development by inducing additional intra- and inter-molecular crosslinks [17, 26].

In the present study, PA-based preconditioning gels were applied on the prepared and acid-etched dentin surfaces before adhesive application to investigate their effect on the shear bond strength of resin-composite/tooth interface. Different concentrations were prepared and applied ranging between

5% and 20% PA. These PA-based gels were applied for varied time periods starting from 20 s to 60 s. As the statistical analysis revealed significant differences ($p < 0.05$) between the control (untreated) group and those treated with PA-based preconditioning gels, the first null hypothesis was rejected. It was reported that PA compound can interact with the dentin matrix and change its characteristics, particularly the mechanical ones, through four mechanisms. These include covalent interactions, [18] ionic interactions,[19] hydrogen bonding interactions and hydrophobic interactions[27].

When the ability of PA, as a crosslinking agent, was investigated to enhance the stiffness of demineralized dentin, there was a significant improvement of the stiffness of dentin matrix compared to the untreated specimens. The authors, consequently, revealed that it is possible to use PA-based preconditioners to increase the resin-dentin bond strength by bringing the stiffness of the collagen matrix closer to that of adhesive resin in the hybrid layer [28]. Moreover, following a study conducted by Carrilho et al. [29], they reported that resin-dentin bond strength exhibited a significant decrease over time *in-vivo* because of collagen degradation in the hybrid layer by endogenous collagenolytic enzymes. The use of PA-based preconditioner as a cross-linking agent, according to the authors, can produce collagen crosslinks that may be resistant to endogenous collagenases and provide more durable hybrid layers than untreated controls.

As there was a significant effect of varying the concentration of PA-based gels and their different application time on SBS, the second null hypothesis was rejected as well. The results showed that the best mean values of bond strength were recorded by the concentration 15% PA. This might be explained on the basis that lesser concentrations (5% and 10%) have not had much PA molecules to produce enough and effective cross-linking with dentin matrix collagen and hence lower bond strength compared to that recorded by 15% PA group. On the other hand, greater concentration (20%) may be too viscous to the extent that penetration into the micropores of dentin surface becomes lower, compared to 15% PA group, that affects both cross-linking with dentin collagen and bond strength value. With regards to the application time of PA-based gels, results showed that longer application time produced better bond strength. Linear regression analysis, at a given concentration (15%) of PA-based gel, revealed a strong positive correlation ($R^2= 0.956$) between shear bond strength (MPa) and the application time. In the clinical situation, application of such a conditioner for more than 60 s may result in longer chairside time and inconvenience to the patient. That is why the time of application in this study was not increased beyond 60 s.

Our findings are in agreement with a study conducted by Liu et al. [30] where the authors prepared PA-based preconditioners by adding powdered proanthocyanidins-rich grape seed extract to various solvents at different concentrations. Specimens were preconditioned for varied time periods (20-120s). Upon comparing the study groups with different concentration and preconditioning time, with regard to micromorphology, cross-linking degrees and mechanical properties, results exhibited time and concentration-dependent increase after preconditioning with PA-based gels. When treated for the same exposure time, specimens after 15% proanthocyanidins preconditioning showed the highest ultimate tensile strength. In the same stream, Bedran-Russo et al. [28] carried out a study to evaluate the influence of PA-based preconditioners on the elastic modulus of dental tissues investigated. They used different concentrations and varied application time. It was reported that the use of PA-based preconditioners resulted in a rapid and continuous increase in the elastic modulus with increasing the application time. In addition, increasing concentration of PA significantly improved the modulus of elasticity, where the higher concentration of PA recorded the highest mean stiffness values.

CONCLUSIONS

- Treatment of dentin surfaces with PA-based preconditioners significantly enhanced the shear bond strength of resin composite/tooth structure.
- Within the parameters selected in this study, SBS results showed a direct increase with the application time of PA-based preconditioners while 15% concentration of PA recorded the highest SBS.

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