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The Effect of Synthetic Grape Seed Extract (GSE) on the Shear Bond Strength of composite resin to Dentin

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ABSTRACT

Proper bonding to dentin depends on efficient infiltration of resin into the demineralized microporous collagen and formation of hybrid layer. Grape Seed Extract (GSE) has up to 97% PA and can potentially stimulate collagen cross linking and increase the stiffness of dentin. This study was designed to evaluate the efficacy of GSE on composite-dentin bond strength in different times and concentrations. In this experimental study, 60 freshly sound extracted teeth selected. The dentin surface of each specimen was acid-etched with 37% Phosphoric acid gel for 15 seconds and then they were rinsed for 10 s with distilled water. All teeth were divided to five groups (each group consists of 12 teeth). In control group GSE was not applied and in other groups different concentrations of GSE were applied for different times. The micro-shear bond strength of composite-dentin interface was evaluated using universal testing machine at a crosshead speed of 1 mm/min. Data were statistically analyzed using ANOVA test by SPSS software ver.15 (α=0.05). The highest bond strength of one-day group was 17.28 MPa and the lowest bond strength in one-hour and one-minute group was 3.70 MPa. The difference between control group and one-day group, one-hour group and one-minute group was significant (p values 0.002, 0.024 and 0.012 respectively). Mann-Whitney revealed also that there was no significant difference between the experimental groups. We concluded that the produced GSE material could not improve the bond strength between adhesive and dentin.

Keywords: Deep Dentin, Grape Seed Extract, Shear Bond Strength
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INTRODUCTION

Adhesive resin composite systems have found their niche in the field of esthetic dentistry and most of the lost dental tissues are replaced by composite in the esthetic zone. Although composite restorations rely on adhesive systems to replace teeth lost structures, achievement of durable bond to underlying tooth structure has been a dilemma for clinicians as the average replacement time of tooth colored restorations was estimated 5.7 years [1].

Dentin is a complex hydrated structure that its properties vary with the preparation depth of tooth [2]. Dentin consists of an organic phase of type one collagen and inorganic phase saturated with hydroxyapatite crystals [3]. Proper bonding to dentin depends on efficient infiltration of resin into the demineralized microporous collagen and formation of hybrid layer [4-5] and stable hybrid layer between resin and collagen microfibers assure a durable bond [6].

Although new bonding systems warrant a durable bond and proper marginal seal, this bond starts to deteriorate 3 to 6 months after use of bonding systems [7-8]. It has been proven that inappropriate infiltration of resin into collagen micro fibrils, makes the dentin-resin

bond vulnerable to degradation [9]. The deterioration of resin-dentin bond can be attributed to enzymatic degradation of collagen micro fibrils or adhesive resins [8, 10]. It was shown that deterioration of any component of hybrid layer, facilitate the infiltration of dentinal fluid into hybrid layer and accentuate the degradation phase [11]. Also the activation of matrix metaloproteinases (MMPs) after acid etching procedure can be a potential cause of bonding degradation [12-13].

To control the deterioration of dentin-resin bond, the quality of two different component of hybrid layer should be enhanced. Most of the researches have focused on resin properties and also improved bonding agents and techniques but there is no promising investigation regarding the improvement and structural durability of collagen component of dentin-resin bond [3]. Bond's stability depends on mechanical features of collagen fibers and any attempt to enhance collagen properties and stabilize collagen component, can directly affect the durability of bond [14].

Type I collagen is prominent in dentin and this collagen can be modified by cross-linking agents. The efficacy of cross-linking agents has been investigated in different tissues and it was declared that these agents can enhance nano-mechanical features like hardness and modulus of elasticity and increase the denaturation temperature of collagen fibrils [15-17].

Researchers claimed that these agents can increase the collagen biodegradation resistance in the dentin-adhesive interface and can improve the resin-dentin bond properties consequently [18]. Glutaraldehyde and proantocyanidin (PA) are two cross-linking agents that have shown promising results in the field of dentistry. Glutaraldehyde is which can stabilize collagen efficiently but its drawbacks like toxicity restricted its use [19]. In contrary to glutaraldehyde, PA is a natural plant flavonoid which is available in fruits, flowers, nuts and especially in grape seed extracts from VINIS VITIFERA [19]. Grape Seed Extract (GSE) have up to 97% PA and can potentially stimulate collagen cross linking [16]. This agent also increase the stiffness of dentin [20] and can inhibit the activity of MMPs [21-22].

There are numerous studies which investigated the effect of GSE on the collagen stability [3], nano-mechanical feature [15] and biodegration resistance [18] and most of them represented promising results and showed that GSE can enhance the mechanical feature of collagen but there are no many studies which evaluate the efficacy of GSE on resin-dentin bond strength in practical condition. Srinivasula et al. [23] concluded that the use of 6.5% PA can significantly increase the bond strength while in another study PA could not reveal promising results [24]. As there are conflicting results regarding the efficacy of PA on dentin-resin bond strength and there is no definite practical protocol for the use of PA, this study was designed to evaluate the efficacy of GSE on composite-dentin bond strength in different times and concentrations.

MATERIALS AND METHODS

Study design

In this experimental study, 60 freshly extracted teeth had been selected after the patients' informed consent had been obtained after approval by the ethical committee of Isfahan University of medical science.
Included teeth were intact, carries free human molar teeth and teeth with any sign of crack or developmental defect were excluded. Teeth had been stored in 0.2% Thymol solution for one month after extraction.

Specimen preparation

The occlusal enamel was removed using a water cooled, low speed trimmer at 90°to longitudinal axis of teeth to expose the underlying dentin. The exposed dentine was polished wet with 180-grit silicon carbide paper for 15 s to create a standardized smear layer on the surface of dentine.

Preparation of experimental grape seed extract

Grape's seeds from Vitis Vinifera Species were extracted by percolation method using ethanol solvent. The extract then was concentrated by rotary device and the extract reached to its desired concentration of 100% (this 100% concentration differs from 100% concentration in another study as the effective materials are 1/3 of what other studies had used).

200cc grape seed extract was gained from 700gr grape seed and this 200cc was considered 100% concentration in the present study. As it was mentioned, the amount of effective agents of the prepared extract is 1/3 of what similar studies had used, so 6.5% concentration which was gained in other studies was considered 3x6.5%≈20% in the present study. In order to

prepare 20% extract, 20 Deg Bitrex free ethanol was used. It means 20 units GSE was added to 80 units 20 Deg Bitrex free ethanol. According to adequate wetting of 100% extract, this concentration was also used in the present study.

Dentin surface preparation

The dentin surface of each specimen was acid-etched with 37% Phosphoric acid gel for 15 seconds and then they were rinsed for 10 s with distilled water. The etched dentin was blot-dried according to wet bonding technique.

All teeth were divided to five groups (each group consists of 12 teeth) according to the protocol which was used to apply GSE on demineralized dentin:

First group: GSE was not applied on the dentin (control group).

Second group: GSE with concentration of 100% was applied on the demineralized dentin for 1 minute.

Third group: GSE with concentration of 20% was applied on the demineralized dentin for 1 minute.

Fourth group: GSE with concentration of 100% was applied on the demineralized dentin for 30 seconds.

Fifth group: GSE with concentration of 20% was applied on the demineralized dentin for 30 seconds.

GSE was applied on dentin surface using micro brush and then air thinned for 2s

Bonding procedure

According to the manufacture instruction two layers of Single Bond adhesive was applied on the dentin surface and was cured for 20s by LED light curing device (500 mW/cm^2). Tygon tube with 0.9 mm diameter and 1mm height was used for placing the Z 250 resin composite. The composite filled tygon tube was placed on the dentin surface and was cured for 20 seconds. Then tygon tube was cut away from composite cylinders and all specimens were kept in distilled water at 37 8C for 24 h. Micro-shear bond strength testing The micro-shear bond strength of composite-dentin interface was evaluated using universal testing machine at a crosshead speed of 1 mm/min. Data were statistically analyzed using ANOVA test by SPSS software ver.15. (α=0.05).

RESULTS

In the present study the highest and the lowest mean shear bond strength of adhesive to dentin was for control group and one-day experimental group (9.19 ± 2.35 and 7.17 ± 3.48 respectively). The highest bond strength of one-day group was 17.28 MPa and the lowest bond strength in one-hour and one-minute group was 3.70 MPa (Table 1).

Because the data were not hemogen Kruskal-Wallis test was used it showed that there is a significant difference among groups (p= 0.015). Mann-Whitney revealed that the difference was between the control group and the three other groups. The difference between control group and one-day group, one-hour group and one-minute group was significant (p values 0.002, 0.024 and 0.012 respectively). Mann-Whitney revealed also that there was no significant difference between the experimental groups.

DISCUSSION

The inherent stiffness of type-I collagen is derived from extra-cellular formation of covalent inter-molecular cross-links [25], which are claimed to enhance the bond strength of adhesives to dentin [26]. PAs interact with proteins through hydrogen bonding, covalent interaction, ionic interaction and hydrophobic interaction [27]. The primary mechanism of collagen stabilization with PA is the formation of hydrogen bonding [15].

The high affinity of PAs to proline-rich proteins like collagen is proved (Long-term bonding effectiveness of simplified etch-and-rinse adhesives to dentin after different surface pre-treatments). Proline, an amino acid with a carbonyl oxygen adjacent to a secondary amin nitrogen, is a very good hydrogen bond acceptor [16].

Cross-links are therefore anticipated to occur when applying PA-rich GSE to collagen-rich dentin matrix [28] which leads to stabilization of demineralized dentin collagen. Since an increase in the mechanical properties of collagen in the hybrid layer likely results in a prolonged life of restoration, the induction of cross-linking with PA compounds may be beneficial for restorative dentistry [29]; however the results of the present study demonstrated that pretreatment with the

Table 1:Mean, standard deviation, minimum and maximum bond strength in different groups

Groups	N	Minimum (MPa)	Maximum (MPa)	Mean ± Std. Deviation (MPa)
Oneday group	11	4.93	17.28	7.17 ± 3.48
Onehour group	11	3.70	14.81	7.28 ± 3.23
Oneminute group	11	3.70	14.81	7.28 ± 3.75
Control group	11	6.17	14.81	9.19 ± 2.35

produced GSE on demineralized dentin not only did not increase the shear bond strength of adhesive to dentin, but also reduced the shear bond strength compared to the control group.

In the present study the highest concentration of GSE with a viscosity similar to Single Bond was produced to benefit the highest amounts of PA. This concentration is almost 5 times higher than Mega Natural Grape Seed Extract. Although it is suggested that higher concentrations of GSE result in higher mean stiffness values of dentin [20], the insufficiency of the resultant extract can be explained by the fact that higher concentrations of PAs might not penetrate into the tissues efficiently, reducing the chance for providing cross-links in collagen matrix [16].

The primary distinction between the various PA-rich plant extracts that are available on the market today, such as GSE, is their underlying extraction process, representing one of the key pharmacognistic parameters. The sourcing of start material and the extraction process of the products have profound effects on the composition, potency and PA content of resulting extract [25, 30]. It is reasonable that the variations of chemical structures and overall compositions affect their characteristics and cross-linking potential in the collagen matrix [28, 30]. In the present study vitis vinifera species of Gewurztramine type were selected for extracting their seeds. We used percolation method because it is able to solubilize prianthocyanidin more sufficiently (reference: Proanthocyanidins: Extraction, Purification, and Determination of Subunit Composition by HPLC). As ethanol is added to water (to provide solvent), the entropy of mixing increases and the structure of the solvent mixture becomes less ordered, favoring the interactions of the solute with the solvent mixture [31], thus ethanol was selected as solvent in the present study; however it may have had an adverse effect on the bond strength of adhesive to dentin collagen. More details are explained below.

Collagen is a fibrous biopolymer that normally exists in an aqueous environment [32]. Inter-peptide H-bonding is important for maintaining the helical structure of collagen (33). Water is the strongest known hydrogen bonding (H-bonding) solvent [34]. It forms clusters around the collagen residues that prevent inter-peptide H-bonding. Water breaks the inter-peptide bonds in dried collagen due to its higher H-bonding capacity, plasticizing the collagen fibrils and filling the inter-fibrillar spaces. This causes matrix to expand to its full extent [35]. The removal of associated water through chemical dehydration, i.e. replacement with different polar solvents such as methanol, acetone and ethanol, causes shrinkage of collagen matrix in demineralized dentin [36-37]. Although it is stated that this shrinkage can lead to higher tensile modulus and ultimate tensile strength [25], it will also reduce the inter-fibrillar spaces that serve as diffusion channels for resin infiltration and ultimately compromises the bonding of adhesive systems to dentin [38]. The bond strength of 10 min and 1h GSE treated dentin restored with Single bond has already been assessed [27, 39], showing that pretreatment with GSE increases tensile bond strength. In the present study the shear bond strength of Single Bond to dentin was evaluated at 1 min, 1h and 24h immersion times. The rational for choosing 1 min immersion period was its clinical relevance.

Since there are no researches with exactly the same design as the present study, precise comparisons cannot be made. Srinivasulu et al. [23] reported that treatment of dentin with GSE significantly improves bond strength of resin composite to deep dentin. The primary contrast between this study and the present study goes back to different GSE used. Srinivasulu et al. used Puritans Pride GSE but we produced GSE in pharmacognostics laboratory. Apparently the produced GSE could not enhance the cross-links of collagen matrix, not able to improve the bond strength.

The majority of recent studies on GSE products reported improved characteristics of dentin including stiffness [20, 25], tensile strength of bond between adhesive and dentin [3, 26-27, 30], stabilization of collagen matrix [16, 28] and nano-hardness of the dentin [28, 40] after pretreatment with GSE. The main difference between these studies and the present study, as mentioned before, is the different GSE product used. Moreover, different selected tooth,

different composite and adhesive materials, different storage times etc. could have affected the results.

PAs are polyphenolic phytoconstituents with chemical structures based on flavolol-3-ol monomers [41]. In addition to different linkages the monomeric matrices, the flavones skeleton can bear various substitutions and patterns and differ in stereochemistry. As a result, PAs are typically very complex products to analyze and provide a phytochemical challenge [40], as a result, we did not determine the PA content of the produced GSE which is one of the limitations of the present study. We may suggest further researches into mechanical properties of demineralized dentin and shear bond strength of adhesive to dentin after treatment with GSEs, with various methods of extraction. With the limitations of this study we concluded that the produced GSE material could not improve the bond strength between adhesive and dentin.

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