ORIGINAL ARTICLE

EFFECT OF GRAPE SEED EXTRACT ON SHEAR BOND STRENGTH AT RESIN-DENTIN INTERFACE

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ABSTRACT

This study was done to determine the effect of experimental primer containing collagen cross linker in improving the shear bond strength of tooth coloured resin restorations.

Mid coronal dentin of 40 extracted human teeth was achieved and conditioned with 10% phosphoric acid. After thorough rinsing and drying, teeth were randomly divided into two groups. Teeth in experimental group were treated with surface application of 6.5% Proanthocyanidin primer. Surfaces of all teeth were treated with bonding agent (Adper single bond 2, 3M ESPE fifth generation etch and rinse system) and restored with composite resin. Restored teeth were stored in distilled water for 24 hours followed by shear bond strength test. Adhesive, cohesive and mixed failures were identified. Data was statistically analyzed using t test and Chi square test.

Primer B (containing Proanthocyanidin cross linker) significantly increased shear bond strength and resin penetration at resin-dentin interface.

In the light of results of current study, Proanthocyanidin seem to be a promising cross linker that can improve the quality and durability of tooth coloured resin restorations.

Key Words: *Collagen cross linkers, shear bond strength, resin-dentin interface, Proanthocyanidin, Fourier transmission infrared spectroscopy (FTIR).*

INTRODUCTION

With the emergence of tooth coloured restorative materials, composite resin became popular in terms of aesthetics and micromechanical bonding to tooth structure.[2] Bonding of composites to enamel is very effective due to its high mineral content. Whereas, bond durability with dentin poses challenges.[29] As Dentin is hydrophilic in nature and posses low mineral content and high organic content as compared to enamel. Bonding of composite resin to tooth involve series of different steps. The first step is to produce microporosities through acid etching/conditioning. 10 to 37% phosphoric acid is most commonly used acid conditioner/etchant.[6] This step is followed by application of low viscosity bonding agent that serves as a glue between composite resin and enamel/dentin. The intermediate layer of union formed between tooth and resin is known as hybrid layer. For a long term bonding the hybrid layer must be very effective and gap free.[10]

Different strategies have been taken to improve the durability of resin-dentin bonded interface, such as introduction of bi-functional molecules which are known as primers. Primer is an important constituent of modern bonding agents. The most widely used primer is 2-hydroxymethylemethacrylate (HEMA).[31] It enhances resin penetration by preventing collagen collapse.[33] Introduction of wet bonding technique by Kanka in 1990 also improved resin-dentin bond strength.[26] Recently, role of collagen crosslinkers have been found to strengthen collagen based biomaterials. They serve to produce new cross links between and within the delicate collagen mesh. They have also been found to increase stability of connective tissue like dentin and interface formed between dentin and composite resin.[4] Among different available cross linkers, grape seed Proanthocyanidin (PA) gained much popularity regarding different its beneficial effects which includes antibacterial, antiviral, anticarcinogenic, anti-inflammatory, antiallergic and

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antioxidant properties.[25,30] PA is a natural cross linking agent. It is found as secondary metabolite present in certain fruit, vegetable, nuts, seeds, flowers, leaves and bark. Seeds of grapes belonging to family "Vitis Venifera" are richest source of PA.[7,8,11,21] A lot of work has been done and still continuing to assess effect of PA on stability and mechanical properties of dentin and quality of resin-dentin bonded interface.[27,32,35] PA interact with proteins by forming hydrogen, covalent or ionic bonds. Association between dentin collagen and PA helps to stabilize the collagen which otherwise does not maintain its triple helix structure in isolated conditions.[14]

PA has found to resist enzymatic breakdown at resin-dentin interface.[23] It seems to be a promising agent that can elevate the longevity of adhesive restorations.[8,10,12] The aim of this study was to evaluate the effect of PA based experimental primer on shear bond strength of etch and rinse system to dentin surface in a clinically relevant time of one minute following the clinical protocol of bonding or resin composite to tooth.

It was hypothesized that the use of PA will raise the shear bond strength at composite resin-dentin interface in time as short as one minute when the etchant used is also very mild. The quality of hybrid layer produced by PA primer will be more pronounced as compared to no treatment group.

METHODOLOGY

Fourty extracted sound human premolar teeth were collected from exodontia department of de'Montmorency College of Dentistry, Lahore. Ethical approval was obtained from Post Graduate Medical Institute, Lahore. Extracted teeth were cleaned from debris by scrubbing and stored in distilled water for not more than six months. It was followed by freezing at -4ºC them to avoid unwanted changes in the teeth that can happen after extraction. After the sample size was achieved, all the teeth were brought back to room temperature at 24ºC for sample preparation.

The teeth were embedded in self cure acrylic resin to make custom made acrylic blocks for easy handling in electric lathe machine for tooth reduction. Tooth reduction was carried out using slow speed carbide saw (Jig saw blade, type: U Length: 2-1/4, China) fixed in an electrical lathe machine (Bilal Lathe Machine, Masha Allah Machinery Store, Ahmad Engineering Services PECO Road, Lahore) under constant water supply. After achieving a flat mid coronal occlusal dentin surface, the occlusal and axial surfaces were reduced simultaneously until an occlusal diameter of 5mm and a height of 4mm for each tooth was achieved. A surface smear layer was created by grinding occlusal surfaces with 600 grit silicon-carbide abrasive papers (3M wetordry).

After the sample preparation, the dentin surfaces of sample teeth were conditioned with 10% phosphoric acid gel (Universal dental supplies, Lahore) for 15 seconds. Teeth were rinsed thoroughly with running tap water and dried using absorbent papers (Gapadent, Lot # 201115) according to Kanca's wet bonding technique.[16] Teeth were randomly divided into two groups A and B with 20 teeth in each group respectively.

Before making the experimental primer, the powdered extract (Italo Biological Technology Co., Limited. China) was tested for competency and verification through FTIR spectroscopy. Following verification using the principle of % W/V, Primer B was made by dissolving 6.5 grams of 95% grape seed extract powder in 100 ml of the solvent (acetone-water 30:70). pH of primer B was adjusted to 7 by adding 1M NaOH drop by drop.

Teeth in group A did not receive application of experimental primer whereas surface of teeth in group B received 60 seconds application of 6.5% proanthocyanidin primer (primer B). Teeth in group B were thoroughly rinsed with running tap water after 60 seconds and properly blot dried using absorbent papers. The reason for thorough rinsing is because PA is an antioxidant and it interferes with free radical activity of photoinitiator camphoroquinone.[11]

After blot drying, all the teeth in control and experimental groups entered the bonding phase which was carried out by applying two consecutive coats of bonding agent (Adper Single Bond 2, 3M ESPE, fifth generation bonding agent) using a micro brush with gentle agitation to improve penetration Excess bonding agent was gently air dried for 3 seconds and light cured for 20 seconds with a LED curing unit (Light Emitting Diode) (Woodpecker Medical Instruments, Co. Ltd.). For the application of composite resin (Z250 XT 3M ESPE, shade A1), a plastic ring with an internal diameter of 5mm and height of 2.5mm was used to build round cylinder of composite resin on the surfaces of prepared teeth in the same dimensions as that of plastic rings that is 5x2.5 mm according to British standards for polymer based crown and bridge materials (BS EN 10477). Composite resin was placed in increments. Each increment was light cured for 20 seconds.

The restored samples were then stored in distilled water at 37oC for 24 hours. Shear bond strength was determined with Universal Testing Machine (Shimadzu Corporation, Tokyo, Japan). The specimens were positioned one by one in a custom made metal jig. The restored surface was positioned perpendicular to the 0.9mm thick loading shear blade having a chisel like configuration. The shear blade was placed at the junction of the tooth and composite resin interface following ISO standards for bond strength test proto-

cols-TR 11405. Cross head speed was kept at 0.5mm/min (TR 11405) until the debonding occured. Shear bond strength was recorded in MPa. The premature failures were not included in analysis. Means and standard deviations were calculated for shear bond strength and expressed in MPa. Independent sample t test was used to determine the significant difference in shear bond strength in relation to different proposed study groups. P -value less than or equal to 0.05 was taken as significant.

After testing, specimens were examined at 8-10 times under electronic zoom microscope (Olympus SZ x 7, Model SZ2-ILST Japan) and modes of failure were classified as adhesive (at the interface), cohesive (within the composite or tooth substrate) and mixed (combination of adhesive and cohesive failures). Chi square test was used to compare the fracture pattern between study groups.

RESULTS

The mean and standard deviation were calculated and are shown in Table 1. One minute post etch application of 6.5% PA primer increased the mean shear bond strength of group B (10.37 MPa) which was statistically higher ($p < 0.001$) than shear bond strength of control group A (7.78MPa). The mode of fracture was assessed with electronic zoom microscope for every specimen. The majority of the fractures (75%) occured at the interface for group A as mentioned in Table 2 and shown in Fig 1A. Whereas the percentage of cohesive failure within the tooth was highest (80%) in Group-B as mentioned in Table 2 and shown in Fig 1B.

Results of optical microscopy revealed that resin penetration was greater in group B than group A. Formation of long, thick and continuous resin tags were observed in group B as shown in Fig 2. FTIR spectrograph of grape seed extract (Fig 3) showed bending peaks of O-H between 3100-3400 cm-1 which are associated with the glycosidic groups in PA. C=C were identified at wavelength of around 1700cm-1. Peaks arising in the region of 1500 and 777cm-1 are associated with aromatic ring breathing mode and CH out of plane deformation with two adjacent free hydrogen atoms.

TABLE 1: DESCRIPTIVE STATISTICS FOR SHEAR BOND STRENGTH (MPA)

		Group A	Group B
N		20	20
Mean		7.78	10.37
SD		1.00	0.71
95%		7.31	10.03
Confidence interval	Lower	8.25	10.70
	Upper bound		
Minimum		6.05	9.27
Maximum		9.79	11.87
p-value		< 0.001 (significant difference)	

TABLE 2: TYPES OF FRACTURE PATTERNS

Fracture pattern	Study groups		Total samples	p-value
	A	B		
Adhesive fracture	15	03	18	< 0.001
Cohesive fracture	02	16	18	< 0.001
Mixed fracture	03	01	04	0.282
Total	20	20	40	

DISCUSSION

Collagen Cross Linkers are agents that forms inter and intramolecular crosslinks with type I collagen fibers in dentin and helps to stabilize the matrix. The present study observed the effect of experimental primer containing grape seed PA. One minute post etch application of PA improved the shear bond strength significantly ($p < 0.001$) at composite resin-dentin interface. PA was used as a separate primer that was thoroughly rinsed after one minute so that it does not act as an accessory layer between resin and tooth. PA was not added into the bonding agent as the antioxidant behavior of PA interferes with the photoinitiator camphoroquionone which can result in reduced degree of conversion and incomplete polymerization of resins in the bonding agent. A decrease in micro tensile bond strength was observed when PA was added to bonding agent.[11] Therefore, it is suggested to use it as separate primer.[18,22,23]

The application time for the PA primer was kept one minute after acid etching of dentin surface to increase the clinical relevance. A number of studies comparing PA with other cross linking agents observed improvement in bond strength using longer treatment durations and immersion techniques.[3,5,8,24] However to enhance clinical usage, shorter exposure time was studied and still found to be effective.[18-20,35] Glutaraldehyde (GDL) was found to raise resin-dentin tensile bond strength in an application time of one minute. PA is more biocompatible as compared to GDL. PA readily form crosslinks with collagen type I of dentin as compared to GDL which forms only covalent bonds with amide groups in collagen.[28] The mechanism of cross linking of PA is by multiple mechanisms which involve covalent, ionic, hydrogen and hydrophobic interactions.[1] Therefore PA seem to form more stable

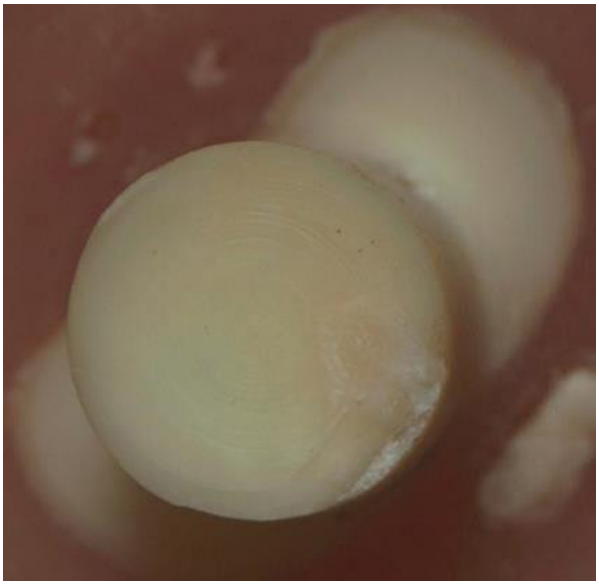

Fig 1A: Representative electronic zoom microscopic image of adhesive fracture

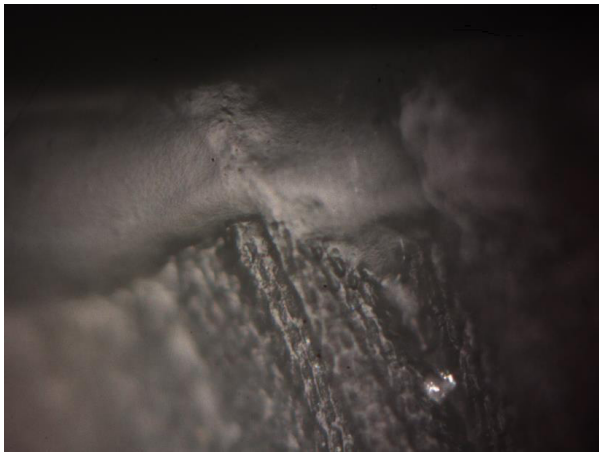

Fig 2A: Representative optical microscopic image of Group-A treated with Adper Single bond 2 adhesive system. Arrow showing shallow resin tags.

Fig 1B: Representative electronic zoom microscopic image showin cohesive failure (within the tooth).

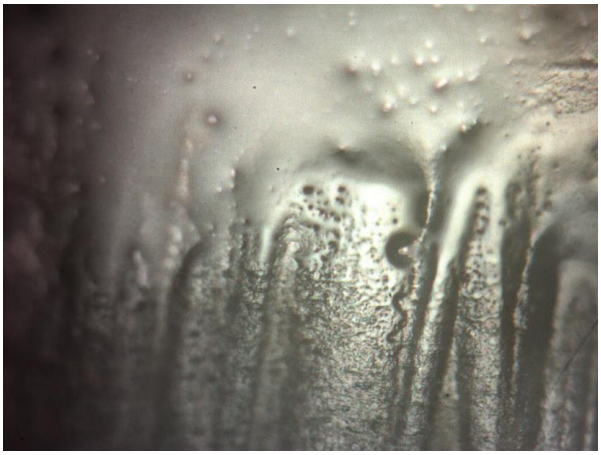

Fig 2B: Representative optical microscopic image of Group-B treated with primer B and Adper Single bond 2 adhesive system. Arrow showing long thick & continuous resin tags.

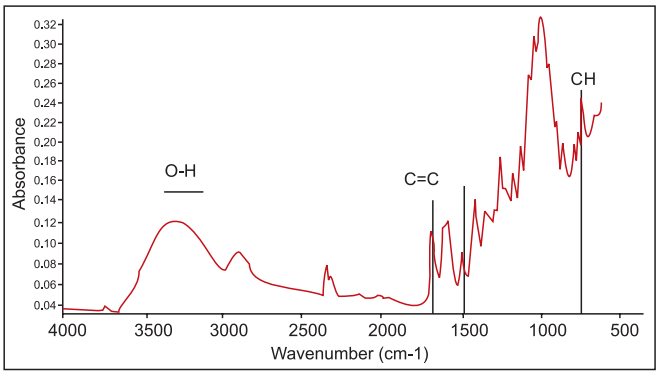

Fig 3: FTIR spectrograph of Grape seed extract (GSE) powder

bond with amino acids of collagen in an application time of as short as one minute.

FTIR spectroscopy of grape seed PA in the present study revealed presence of plenty of hydroxyl groups characteristic of GSE polyphenol.[9] The hydroxyl groups readily form hydrogen bonds with carbonyl groups of aminoacids of collagen type I. The acidity of hydroxyl groups result in formation of negatively charged phenoxide ion which is capable of forming ionic and covalent bonds with aminoacids.[13] PA strengthens collagen by making it insoluble through its bonding mechanisms. Different spectroscopic analysis revealed hydrogen bond formation between side chains of collagen and phenolic hydroxyl groups of PA.[14]

Study of fracture pattern in the present study revealed that group B had maximum number of significant cohesive failure within the tooth substrate (Table 2). This might indicate that the resin-dentin interface was stronger than dentin substrate itself. Whereas, 75% of bonded interface in group A failed at the interface ($p < 0.001$).

In the present study, 6.5% concentration of PA was

used that has been previously found to be effective in raising bond strength at resin-dentin interface.[1,7,3,10] 10% phosphoric acid was used in the study to demineralize dentin surfaces. Dentin is porous and delicate in nature. Use of higher concentrations of etchant might disturb the delicate collagen mesh of dentin. The stability and expanded network of dentin collagen is important for a good bonding with adhesive resin.[17]

Optical microscopy revealed that resin penetration was greater in PA treated samples as shown in Fig 2. PA produced long, thick and continuous resin tags whereas shallow resin penetration was observed in control group. PA has a great affinity with collagen type I present in dentin which resulted in good penetration and encapsulation of collagen fibers.

Isolation of demineralized dentin surfaces was achieved by Kanka's wet bonding technique[16] in order to protect the delicate matrix of dentin collagen from collapsing. When the bound water in the inter tubular spaces is lost in air jet drying, the dentinal tubules lose their patency for infiltration of monomers which produce defective resin tags and presence of voids. Moreover, wet bonding technique results in better resin penetration in combination with hydrophilic resins like HEMA.[15]

The study under observation was done on extracted teeth whereas there are different confounding factors inside the oral cavity like temperature fluctuations due to hot and cold food, composition of saliva and forces of mastication. Hence, in vitro testing is though a prerequisite for clinical trials however it might not reproduce same finding in clinical studies. The results of this study may be helpful in highlighting the role of Grape seed extract PA for promoting efficient and stable bonding at resin-dentin interface.

CONCLUSION

Within the limitations of this in vitro study it is concluded that collagen cross-linkers improve shear bond strength at composite resin dentin interface. PA is an effective natural collagen cross linker which is suggested for use as an adjunct to dentin adhesives.

In future, emphasis must be made on scanning electron microscopy of interface formed between PA treated dentin surfaces. Compatibility and comparative analysis of bond strength with different commercially available etchant and bonding agents are worth for further clinical investigations.

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4	**Hira Khalid:**	Data collection and assistance in write up of article.