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Shear Bond Strength of Composite to Deep Dentin After Treatment With Two Different Collagen Cross-linking Agents at Varying Time Intervals

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Clinical Relevance

Deep dentin does not serve as a suitable bonding substrate for contemporary bonding agents. The use of natural collagen cross-linkers like sodium ascorbate and proanthocyanidin as dentin pretreatment agents greatly improves bond strength to deep dentin.

SUMMARY

Objective: This in vitro study evaluated the shear bond strength of composite resin to deep dentin using a total etch adhesive after treatment with two collagen cross-linking agents at varying time intervals.

Materials and Methods: Thirty freshly extracted human maxillary central incisors were sectioned longitudinally into equal mesial and distal halves (n=60). The proximal deep dentin was exposed, maintaining a remaining dentin thickness (RDT) of approximately 1 mm. The specimens were randomly divided into three groups based on the surface treatment of dentin prior to bonding as follows: group I (n=12, control): no prior dentin surface treatment; group II (n=24): dentin surface pretreated with 10% sodium ascorbate; and group III (n=24): dentin surface pretreated with 6.5% proanthocyanidin. Groups II and III were further subdivided into two subgroups of 12 specimens each, based on the pretreatment time of five minutes (subgroup A) and 10 minutes (subgroup B). Shear bond strength of the specimens was tested with a

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universal testing machine, and the data were statistically analyzed.

Results: Significantly higher shear bond strength to deep dentin was observed in teeth treated with 10% sodium ascorbate (group II) and 6.5% proanthocyanidin (group III) compared to the control group (group I). Among the collagen cross-linkers used, specimens treated with proanthocyanidin showed significantly higher shear bond strength values than those treated with sodium ascorbate. No significant difference was observed between the five-minute and 10-minute pretreatment times in groups II and III.

Conclusion: It can be concluded that dentin surface pretreatment with both 10% sodium ascorbate and 6.5% proanthocyanidin resulted in significant improvement in bond strength of resin composite to deep dentin.

INTRODUCTION

Continuous developments in dentin bonding systems have led to their widespread use in daily dental practice.[1] Total etch adhesive systems, which remove the smear layer with phosphoric acid and combine the function of primer and adhesive in one bottle, have been widely used because of their documented long-term clinical success.[2,3] Although contemporary dentin bonding systems show improvement in better handling and bonding characteristics, the achievement of a strong and stable bond to deep dentin remains a challenge in restorative dentistry.

Dentin is a complex hydrated biological composite material with structural components and properties that vary with location.[4] Various studies have shown that a reduction in bond strength occurs when resin composite is bonded to deep dentin. This significant influence of dentin depth on the bond strength of dentin bonding systems can be attributed to the complexities in the structure of deep dentin, such as an increase in the number of tubules and their diameters with much less intertubular dentin matrix, as compared to superficial dentin.[5-7] In addition, the higher water content in deep dentin (22 volume % as compared to 1 volume % in superficial dentin) may dilute the organic solvents of some bonding systems, resulting in separation of monomers from the soluble phase and leading to the formation of resin globules in water.[8]

Successful bonding to dentin depends largely on proper resin infiltration of the demineralized microporous collagen fibril scaffold (hybrid layer) and the formation of resin tags.[9,10] Since the durability of the bond between dentin and adhesive system depends largely on the structural integrity and mechanical properties of acid-demineralized collagen fibers, any attempt to stabilize collagen will help enhance the bonding.[11] Mechanical properties of collagen can be improved by an increase in the formation of intra- and intermolecular and intermicrofibrillar cross-links. This can be achieved by the use of various collagen cross-linkers, both synthetic and natural, on the dentin substrate prior to the bonding procedure.[12,13] Naturally occurring collagen cross-linkers such as sodium ascorbate and proanthocyanidin have been reported to increase the collagen cross-linking in sound and caries-affected dentin,[12-14] but their effects on bonding to deep dentin have not been reported in the literature. Hence, the aim of this *in vitro* study was to determine the shear bond strength of composite resin to deep dentin using a total etch adhesive after treatment with different collagen cross-linking agents at varying time intervals.

MATERIALS AND METHODS

The materials used in this study and their composition are given in Table 1.

Preparation of Solutions

Two solutions were prepared for this study: 1) 10 g of sodium ascorbate powder (sd fiNE cHEM Ltd, Mumbai, India) were dissolved in 100 mL of distilled water to make 10% sodium ascorbate solution, and 2) 6.5 g of grape seed extract in the form of powder (Puritans Pride Inc, Oakdale, NY, USA) were collected from the capsules and dissolved in 100 mL of distilled water to make 6.5% proanthocyanidin solution.

Specimen Preparation

Thirty freshly extracted human maxillary central incisors were collected for the study, adhering to the protocol of the Institutional Review Board of SRM University (SRMU/M&HS/SRMDC/2010/M.D.S-PG Student/114). The teeth were cleaned of debris and stored in 0.2% thymol until use. Each tooth was sectioned longitudinally, parallel to the long axis of the tooth, into an equal mesial and a distal half by means of a low-speed diamond disc (HI-DI Diamond Precision Tools Ltd, London, UK) under copious water supply (Figure 1a,b). The contents of the pulp chamber were then removed ultrasonically to know its extent.

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Table 1: *Materials Used in This Study and Their Composition*

Study	Materials	Composition
1	Sodium ascorbate (sd fiNE cHEM Ltd, Mumbai, India)	100-g powder containing 99.1% sodium ascorbate
2	Proanthocyanidin Grape Seed Extract (Puritans Pride Inc, Oakdale, NY, USA)	100-mg capsule containing 97.3% proanthocyanidin
3	Total Etch Adhesive System (Dentsply DeTrey GmbH, Konstanz, Germany)	
	Etchant: DeTrey Conditioner 36	36% ortho-phosphoric acid
	Adhesive: Prime & Bond NT	Dipentaerythritol penta acrylate monophosphate (PENTA), di- and tri- methacrylate resins, amorphous silicon dioxide nanofillers, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone
4	Composite resin: Ceram X Nano Ceramic Restorative (Dentsply DeTrey GmbH)	Methacrylate modified polysiloxane, dimethacrylate resin, pigments, stabilizers, camphoroquinone, ethyl-4 (dimethylamino) benzoate, barium-aluminum-borosilicate glass, methacrylate functionalized silicon dioxide nanofiller

Using a diamond disc, the dentin in the proximal wall of each half of the crown incisal to the cemento-enamel junction was removed until the remaining dentin thickness (RDT) was approximately 1 mm (Figure 1c) as measured with a metal caliper (Iwanson Spring metal caliper, I.D-Tech, Sialkot, Pakistan) from the outer surface of the prepared proximal portion of crown to the pulp chamber. All the specimens were immersed in an ultrasonic bath to remove the smear layer. The roots of the specimens were then mounted in self-cure acrylic resin. The number of specimens thus obtained was 60. The prepared proximal surfaces of the specimens were acid-etched with 36% ortho-phosphoric acid (DeTrey Conditioner 36, Dentsply DeTrey GmbH, Konstanz, Germany) for 15 seconds, rinsed with water for 15 seconds, and blot dried. These specimens were randomly divided into three groups based on the surface treatment of dentin as follows:

Group I (n=12, control): No dentin pretreatment was done. Adhesive bonding and composite buildup was done according to the bonding protocol as described here. Two successive coats of the adhesive Prime & Bond NT (Dentsply DeTrey GmbH) were applied on the prepared proximal dentin surface of the specimens according to the manufacturer's instructions, and light curing was done (Astralis 3 light curing unit (530 mW/cm^2), Ivoclar Vivadent, Schaan, Liechtenstein) for 40 seconds. Composite buildup was done (Figure 1d) by placement of two increments of 2-mm-thick composite resin (Ceram X Nano Ceramic Restorative, Dentsply DeTrey GmbH) using a 3-mm-diameter plastic tube as a matrix, with each increment being light cured for 40 seconds.

Group II (n=24): 10% sodium ascorbate pretreatment. This group was further subdivided into two subgroups based on the pretreatment time as follows:

Subgroup IIA (n=12): The etched dentin surface was treated with 10% sodium ascorbate solution for five minutes and rinsed with water, followed by the bonding/buildup procedure as described above.

Subgroup IIB (n=12): The etched dentin surface was treated with 10% sodium ascorbate solution for 10 minutes and rinsed with water. Adhesive bonding and composite buildup followed as described above.

Group III (n=24): 6.5% proanthocyanidin pretreatment. This group was further subdivided into two subgroups based on the pretreatment time as follows:

Subgroup IIIA (n=12): The etched dentin surface was treated with 6.5% proanthocyanidin solu-

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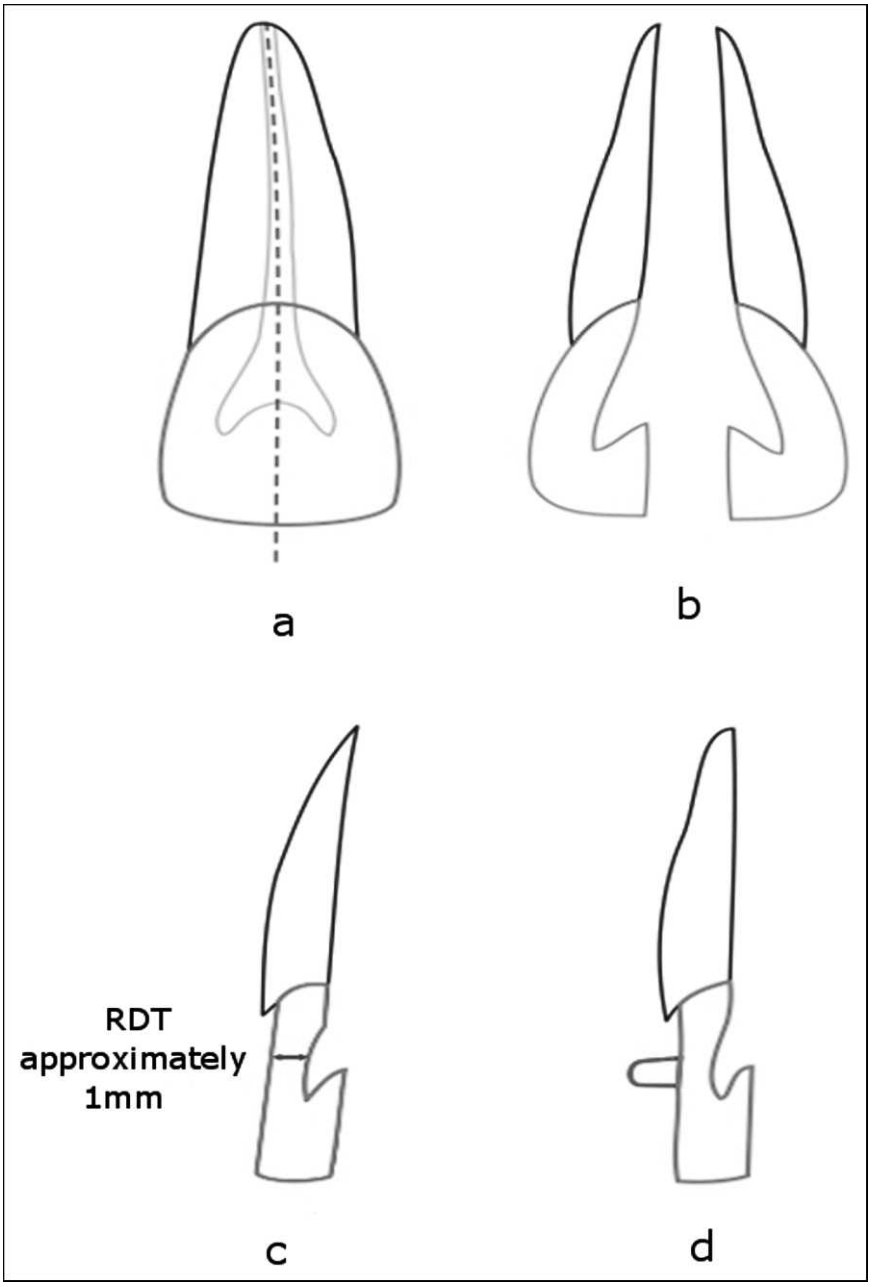

Figure 1. *Specimen preparation for shear bond strength testing. (a and b): Sectioning the tooth labio-palatally to obtain an equal mesial and a distal half. (c): The dentin in the proximal wall of each half of the crown incisal to the cemento-enamel junction was removed until the remaining dentin thickness was approximately 1 mm. (d): Composite resin buildup.*

tion for five minutes and rinsed with water, followed by the bonding/buildup procedure as described above.

Subgroup IIIB (n=12): The etched dentin surface was treated with 6.5% proanthocyanidin solution for 10 minutes and rinsed with water. Adhesive bonding and composite buildup followed as described above.

Shear Bond Strength Testing

All the specimens were then stored in distilled water at 37°C for 24 hours. Shear bond strength was determined using a universal testing machine (LR 100K, Lloyd Instruments, Largo, Florida, USA) at a crosshead speed of 1 mm/min. The results were tabulated and statistically analyzed. Paired *t*-test was used to calculate the *p*-value ($p<0.001$).

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Table 2: *Comparison of Mean Shear Bond Strength of Different Study Groups*

Group	Mean ± SD (Mpa)	Significant groups ($p<0.001$)
I	17.84 ± 0.56[a]	IIIA, IIIB, IIA, IIB vs. I
IIA	22.12 ± 0.90[b]	IIIA vs. IIA
IIB	23.05 ± 0.60[b]	
IIIA	27.57 ± 0.92[c]	IIIB vs. IIB
IIIB	27.85 ± 1.01[c]	

[a] *For each column, same superscript letters indicate no statistically significant difference (p>0.05) between the groups and different superscript letters indicate a statistically significant difference (p<0.001) between the groups.*

RESULTS

The results of this study are shown in Table 2. The results showed that the mean shear bond strength values of subgroup IIA (22.12 ± 0.90), IIB (23.05 ± 0.60), IIIA (27.57 ± 0.92), and IIIB (27.85 ± 1.01) were significantly higher than the mean shear bond strength value in the control group (17.84 ± 0.56; $p<0.001$). Both five-minute and 10-minute pretreatment times of 6.5% proanthocyanidin (IIIA [27.57 ± 0.92] and IIIB [27.85 ± 1.01]) yielded significantly higher shear bond strength values than the corresponding pretreatment times of 10% sodium ascorbate (IIA [22.12 ± 0.90] and IIB [23.05 ± 0.60]; $p<0.001$). No significant difference in shear bond strength values was found between the two pretreatment times in both the 10% sodium ascorbate and the 6.5% proanthocyanidin groups ($p>0.05$).

DISCUSSION

The structure and properties of dentin are the principal determinants of many procedures in restorative dentistry.[15] Adhesive bonding to dentin will be most effective only if the hybrid layer that is formed between resin monomers and collagen fibrils is structurally stable.[14] Dentin contains micrometer-diameter tubules surrounded by highly mineralized peritubular dentin embedded within a partially mineralized intertubular dentin.[4,16] The collagen-rich intertubular matrix area decreases from about 96% at the dentino-enamel junction to nearly 12% at the predentin. The amount of collagen fibrils per unit volume of dentin decreases from superficial to deep dentin. Studies have shown that large dentinal tubules with much less collagen-rich intertubular dentin matrix make bonding to deep dentin unpredictable.[4,7,17]

Fibrillar type I collagen accounts for 90% of the organic matrix of dentin, while the remaining 10% consists of noncollagenous proteins, such as phosphoproteins and proteoglycans.[12,18] Type I collagen serves as a scaffold for the deposition of apatite mineral phase and provides viscoelasticity to the tissue by forming a rigid, strong, space-filling biomaterial.[15] Structural integrity and mechanical properties of the collagen fibrils of acid demineralized superficial and deep dentin play an important role in the determination of bond strength and its durability.[11] Covalent inter- and intramolecular cross-links are the basis for stability, tensile strength, and viscoelasticity of the collagen fibrils. Various chemicals, both synthetic and natural, have the ability to increase these collagen cross-links.[12,15,19]

Sodium ascorbate is an important component in the synthesis of hydroxyproline and hydroxylysine in collagen. Hydroxyproline serves to stabilize the collagen triple helix, and hydroxylysine is necessary for the formation of intermolecular cross-links in collagen.[20]

Proanthocyanidins (PA) are naturally occurring bioflavonoids found in high concentrations in grape seed, pine bark, cranberries, lemon tree bark, and hazel nut tree leaves.[21] Although PA from grape seed extract has been shown to effectively cross-link collagen in *in vitro* studies,[14,19,22,23] their effect on the bond strength of resin composites to deep dentin remains an unexplored area in restorative dentistry.

A novel method of sample preparation was adopted in the current study. Maxillary central incisors were chosen because of greater cervico-incisal length of the crown (10.5 mm).[24] In addition, the pulp chamber is located in the center of the crown, equidistant from the dentinal walls.[25] Since the pulp chamber is broad mesiodistally, sectioning was done labiopalatally to achieve two equal halves. When observing the cross section of maxillary central incisors, mesial and distal outlines of the pulp chamber were nearly straight. Thus, more uniform RDT is available for standardization of deep dentin. Radiographic confirmation of RDT was not required in this study, as it was directly measured from the pulp chamber to the proximal surface of tooth.

In the present study, group I (control) recorded a mean shear bond strength value of 17.84 ± 0.56

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MPa to deep dentin, which is less than the optimal bond strength of resin composite to superficial dentin (20–23 MPa).[26,27] This is in accordance with previous studies done by Suzuki and others,[28] Olsson and others,[29] and Yazici and others,[30] who showed that bond strength of resin composite to deep dentin can be as low as 10.3-16.7 MPa.

Results of this *in vitro* study showed an increase in shear bond strength after pretreatment with 10% sodium ascorbate and 6.5% proanthocyanidin as compared to control group. This can be attributed to improved dentin collagen stability, obtained from an increase in the number of collagen cross-links, achieved by the use of these collagen cross-linkers.

Group III (6.5% proanthocyanidin) showed a significantly higher bond strength to deep dentin compared to group II (10% sodium ascorbate) in both the subgroups ($p<0.001$). This is in accordance with the findings of Macedo and others,[14] who showed that the application of 6.5% grape seed extract and 5% gluteraldehyde to caries-affected and sound dentin significantly improved the microtensile bond strength of composite to dentin. Similarly, Walter and others[31] showed that 0.5% proanthocyanidin efficiently stabilized collagen and increased its resistance to caries compared to 0.625% genipin and 5% gluteraldehyde. Bedran-Russo and others[12] found that naturally occurring cross-linking agents such as 0.5% proanthocyanidin and 0.625% genipin are capable of stabilizing demineralized dentin collagen more effectively compared to 5% gluteraldehyde.

This could be attributed to the specificity of proanthocyanidin to facilitate the enzyme proline hydroxylase, which catalyzes the hydroxylation of proline, an essential step in collagen biosynthesis. Proanthocyanidins and proteins have been shown to interact in four different ways: 1) covalent interactions, 2) ionic interactions, 3) hydrogen bonding interactions, or 4) hydrophobic interactions.[14,22,23] Castellan and others[19] showed that when demineralized dentin is treated with PA, it results in improved mechanical properties and reduced water absorption as a result of the dense collagen network formed by the use of exogenous cross-linkers.

Macedo and others[14] showed that treatment of dentin surface with 6.5% proanthocyanidin for 1 hour resulted in an increase in the microtensile bond strength of the specimens. Hence, in this study, in order to establish more clinically relevant and feasible application times, lesser application times of five and 10 minutes were chosen. The present study showed no statistical significant difference in the shear bond strength values between the two pretreatment times in both 10% sodium ascorbate and 6.5% PA groups.

In the present study, when deep dentin was treated with sodium ascorbate, the bond strength reached values (22.12–23.05 MPa), comparable with the optimal bond strength values of superficial dentin. But after dentin surface treatment with proanthocyanidin, the deep dentin bond strength significantly increased to levels (27.57-27.85 MPa) that are higher than the optimal bond strength values of superficial dentin. Hence, it can be safely recommended that the use of collagen cross-linkers be employed as an effective chairside procedure to overcome the disadvantage of reduced bond strength of composite resin to deep dentin. Although this research allowed for an effective evaluation of the potential effect of these cross-linkers on deep dentin, the time used is still excessive from a clinical point of view. Hence, other application times in the range of 30–60 seconds should be taken into consideration in future experiments to develop a clinically relevant strategy for improving deep dentin bond strength.

CONCLUSIONS

Under the limitations of this *in vitro* study, the following conclusions can be drawn:

1. Dentin surface pretreatment with both 10% sodium ascorbate and 6.5% proanthocyanidin resulted in significant improvement in the bond strength of resin composite to deep dentin compared to the control group.
2. The use of 6.5% proanthocyanidin as a collagen cross-linker on deep dentin significantly improved the shear bond strength values compared to the use of 10% sodium ascorbate.
3. There was no significant difference in the shear bond strength values between the five-minute and 10-minute pretreatment times in both the 10% sodium ascorbate and the 6.5% proanthocyanidin groups.

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