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RESEARCH ARTICLE

EFFECT OF GRAPE SEED EXTRACT (PROANTHOCYANIDIN) AND SODIUM ASCORBATE; NATURAL CROSS LINKERS ON THE PUSH OUT BOND STRENGTH OF FIBRE POST TO ROOT CANAL DENTIN – AN IN VITRO STUDY

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ABSTRACT

The aim of this study is to investigate the effects of 5% proanthocyanidin-rich extract (grape seed extract [GSE]) and 10% sodium ascorbate on dentin and thereby to evaluate the bond strength and stability of the adhesion of fiber posts to the root canal dentin. Selected thirty extracted human single rooted teeth were decoronated below the cemento-enamel junction perpendicular to the longitudinal axis. The roots were cut to a uniform length of 14 mm from the apical end. Pulp tissue and pre-dentin were removed and the root canals were obturated upto achieved working length. Post space was prepared 5 mm short of working length. Then specimens were divided based on the surface treatment of dentin prior to bonding as follows: Group I (n = 10, control): No prior dentin surface treatment; group II (n = 10): Dentin surface pretreated with 5% proanthocyanidin; and group III (n = 10): Dentin surface pretreated 10% sodium ascorbate. Push out bond strength of the specimens was tested using universal testing machine and the data were statistically analyzed. The results showed that the mean push out bond strength values of experimental Group I (GSE) (8.30 ± 2.33), Experimental Group (SA) (7.56 ± 2.33) were significantly higher than the mean push out bond strength value in the control group (6.42 ± 2.60). It can be concluded that dentin surface pretreatment with both 10% sodium ascorbate and 5% proanthocyanidin resulted in significant improvement in bond strength of resin composite to deep dentin.

INTRODUCTION

Dentin is a complex hydrated biological composite material with structural components and properties that vary with location. There is a reduction in bond strength occurs when resin composite is bonded to deep dentin (dentinal tubules within pulp chamber) (Marshall, 1997). It is very well understood that the density of dentinal tubules varies with dentinal depth and as well as the water content of dentin is lowest in superficial dentin and highest in deep dentin. This can be attributed to the complexities in structure of deep dentin, such as an increase in the number of tubules and their diameters with much less intertubular dentin matrix as compared with superficial dentin (Singh, 2015). Mechanical properties of collagen and its resistance to enzymatic degradation can be improved by an increase in the formation of intra and inter-molecular 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 (Bedran-Russo, 2007).

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. Cross-linking agents, such as tannic acid, decrease the enzymatic degradation rate of collagen and increase dentin mechanical properties.⁴ In addition, sodium ascorbate and grape seed extract (GSE), as cross-linking agents, increase collagen stability and bond strength.⁵ This in vitro study evaluated the push out bond strength of composite resin to deep dentin after treatment with two collagen cross-linking agents.

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:

- 10 g of sodium ascorbate powder (sdfiNECHEM Ltd, Mumbai, India) were dissolved in 100 mL of distilled water to make 10% sodium ascorbate solution, and
- 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 5% proanthocyanidin solution.

Specimen Preparation: Thirty single-rooted human teeth with anatomically similar root segments and fully developed apices were selected.(Fig.1) Each tooth was decoronated below the cement enamel junction perpendicular to the longitudinal axis. The roots were cut to a uniform length of 14 mm from the apical end.To remove pulp tissue and pre-dentin, the root canals were enlarged using files #15, #20, #25, and #30 (Dentsply Maillefer, Ballaigues, Switzerland) and #1, #2, and #3 burs (Dentsply Maillefer). The apical 1 mm end was left unprepared to prevent the apical extrusion of solutions and luting cement. Roots were rinsed with 5 mL 0.9% sodium chloride (NaCl) solution to remove the remaining debris. Roots were obturated upto working length and post space was prepared 5 mm short of working length. Each group was etched with 37% phosphoric acid. Before application of primer, teeth are rinsed with 5% Proanthocyanidin solution, 10% Sodium Ascorbate and NaCl according to division of group as described earlier, teeth. The intracanal restoration was performed using fiber posts no. 3. Dual core resin adhesives was applied to the post surface and immediately polymerized for 20 seconds on each side. The dual-polymerizing resin luting material Para Core dual curing core & resin cement (COLTENE) was mixed and injected into the prepared root canal. The cement was light polymerized for 30 seconds on each surface (buccal, palatal, mesial, and distal), resulting in a 2-minute light polymerization cycle (Fig.2)

Push-out Test: Specimen Preparation, Post Dislodgment, and Failure Pattern Analysis: Each root was cut horizontally with a slow-speed, water-cooled diamond bur to produce 5 mm thick slices.(Fig.3) The push-out test was performed by applying a load at 0.5 mm/min to the apex in the direction of the crown until the fiber post relined segment was dislodged from the root slice (Fig.4).

RESULTS

The mean push-out bond strength values (MPa) and standard deviation are shown in Table 2. The results showed that the mean push out bond strength values of experimental Group 1 (GSE)(8.30 ± 2.33), Experimental Group(SA) (7.56 ± 2.33) were significantly higher than the mean push out bond strength value in the control group (6.42 ± 2.60).

Table. 1

Sr. No	Study Material
1.	5g grape seed extract powder
2.	10g of sodium ascorbate powder
3.	Coltene Dual Core Composite resin
4.	Easy Post from Densply
5.	d-Tec 37% Orthophosphoric Acid
6.	Coltene One Coat 5 th Gen Bonding Agent

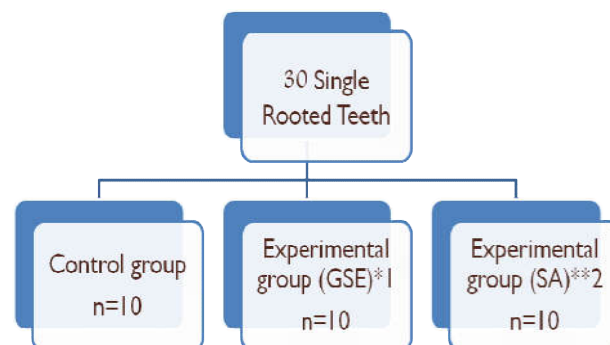
DISCUSSION

Endodontically treated teeth are often severely damaged by decay, excessive wear, or previous restorations, resulting in a lack of coronal tooth structure (Sadek, 2007).



Fig. 1.

Division of teeth into groups



*5%Grape Seed Extract (Proanthocyanidin)** 10%SA Sodium Ascorbate

Coronal restoration quality is an important factor in the success of endodontically treated teeth. Therefore, bonding of the post to the root dentin should aim to achieve gap-free long-lasting interfaces, which are indicative of a coronal seal and adequate retention (Cecchin *et al.*, 2014). The major shortcomings of dentin as a bonding substrate are its heterogeneous composition and hydrophilic nature (Swift, 1995).

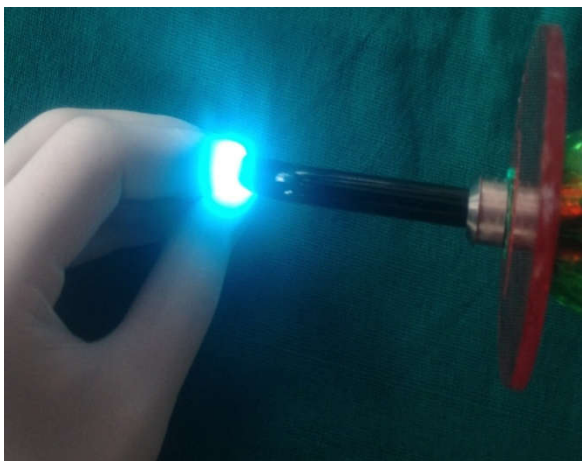


Fig. 2.

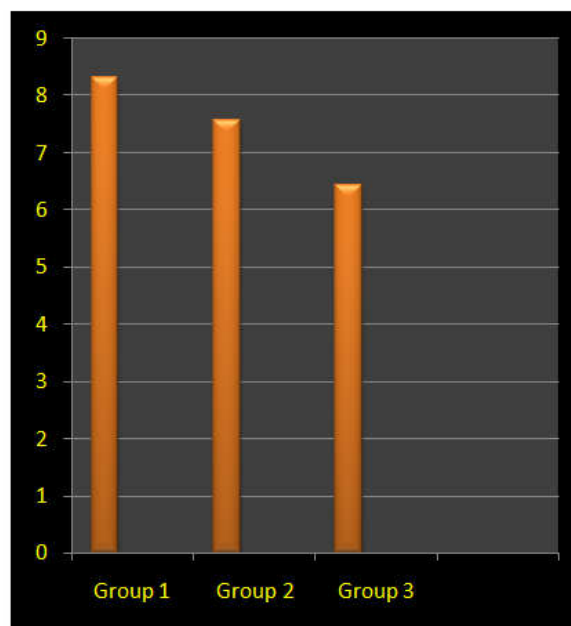


Fig. 3.



Fig. 4.

It presents an intricate composition comprised of 70% by weight of an inorganic phase of carbonate-rich hydroxyapatite crystals, 20% by weight of an organic phase of predominantly type I collagen and 10% water. Covalent inter- and intra-molecular cross-links are the basis for stability, tensile strength and viscoelasticity of the collagen fibrils (Bedran-Russo, 2009).



Graph

Table 2.

	1	2	3	4	5	6	7	8	9	10
Group1 GSE	11.3	8.3	7.1	4.9	6.8	10.2	12.2	9	6.4	6.9
Group2 SA	6.1	9.8	11.1	6.4	7.2	6.9	10.4	3.9	7.6	6.2
Group3 Control	4.6	8.2	10.4	4.9	6.4	5.2	8.9	1.6	5.4	8.6

ANOVA

bond	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	17.939	2	8.969	1.559	
Within Groups	155.340	27	5.753		
Total	173.279	29			

Various chemicals, both synthetic and natural, have the ability to increase these collagen cross-links in biological tissues. 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 (Regulation of collagen synthesis by ascorbic acid, 1981). Proanthocyanidins (PA) are naturally occurring bioflavonoids found in high concentrations in grape seed, pine bark, cranberries, lemon tree bark and hazel nut tree leaves. However, the specific mechanism of inhibition of proteolytic enzymes by PACs is unknown. Changes in the conformation of bacterial collagenase promoted by PACs through hydrogen and hydrophobic interactions have been suggested. For MMPs, cross-linkage of the catalytic sites of these enzymes and inhibition of other noncollagenous proteins that activate these enzymes also have been hypothesized (Chaussain, 2013). It also could be speculated that the collagen cross-linking mediated by PACs might change the type I collagen molecular and fibrillary arrangement in such a way that the cleavage sites of proteolytic enzymes such as MMPs and other collagenases are blocked or hidden (Macedo *et al.*, 2009).

Possibly, the combined cross-linking and anticollagenolytic effects of PACs are responsible for preventing degradation of dentin collagen within the hybrid layer. Results of this in vitro study showed that when deep dentin was treated with sodium ascorbate and proanthocyanidin, the bond strength reached values comparable with the optimal bond strength values of dentin. The mean push out bond strength values of experimental Group 1 (GSE) and Experimental Group (SA) were statistically significantly higher than the mean push out bond strength value in the 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. The specificity of proanthocyanidin facilitates 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 (Kurozumi *et al.*, 2016; Xie, 2008; Castellan *et al.*, 2010):

- Covalent interactions,
- Ionic interactions,
- Hydrogen bonding interactions, or
- Hydrophobic interactions.

In the present study, group 3 (control) recorded a mean push out bond strength value of 6.42 ± 2.60 Mega Pascals (MPa) to deep dentin, which is lesser than the optimal bond strength of adhesive resins to superficial dentin.

Conclusion

Group 1 (5% proanthocyanidin) showed a significantly higher bond strength to deep dentin compared with group 2 (10% sodium ascorbate). Despite the limitations of the present study, retreatment with Proanthocyanidin and Sodium Ascorbate increases the bond strength of fiber posts to root dentin. However, the study evaluate the effects of enzyme inhibition and collagen cross-linking on the durability of adhesive restorations and stability of dentin matrix; therefore, it is concluded that natural cross linkers can be used as dentin conditioner to enhance the bond strength and adhesive durability of posts to root dentin.

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