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ORIGINAL ARTICLE

Push Out Bond Strength of a Glass Fibre Post to Root Dentine Pretreated with Proanthocyanidin and Phytosphingosine – An *In Vitro* Study

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ABSTRACT

Objective: To evaluate the push out bond strength of a glass fibre post to root dentine pretreated with 6.5% proanthocyanidin (PAC) and 0.02% phytosphingosine (PHS).

Methods: Thirty-three freshly extracted single rooted human teeth were decoronated to a length of 14 mm. Root canals were prepared using rotary NiTi files and obturated with gutta percha and resin sealer. Post space was prepared using peeso reamers, retaining 5 mm of apical gutta percha. Following smear layer removal and acid etching of the post space, samples were randomly assigned to 3 groups based on the dentine pretreatment, namely the control (no pretreatment) group, 6.5% PAC group, and 0.02% PHS group. A glass fibre post was luted using a dual cure adhesive and luting cement. 1 mm thick root slices were sectioned from coronal, middle and apical levels of the post and their push out bond strength was evaluated using a universal testing machine. Data was analysed with one-way ANOVA and Games-Howell post hoc test ($P<0.05$).

Results: At all levels, PHS showed higher push out bond strength than PAC and control groups, with a significant difference between the experimental groups at the middle and apical thirds ($P<0.05$). The push out bond strength of PAC group was significantly higher than the control group in the coronal and apical thirds ($P<0.05$).

Conclusion: Both PAC and PHS improved the push out bond strength of a glass fibre post to dentine.

Keywords: Collagen, dentin, glass fibre post, phytosphingosine, proanthocyanidin, push-out bond strength

HIGHLIGHTS

- The use of irrigation solutions have a deleterious effect on the physicochemical properties of root dentine.
- This is the first study to evaluate the effect of phytosphingosine on root dentine bonding.
- Both 6.5% proanthocyanidin and 0.02% phytosphingosine improved the stability of the resin-dentine interface.
- Phytosphingosine could be a potential colourless alternative to proanthocyanidin in improving the adhesion of glass fibre posts to dentine.

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INTRODUCTION

Endodontically treated teeth can be functionalised using direct or indirect restorations. If such teeth are further compromised because of extensive loss of tooth structure, an intracanal post is preferred by the clinicians to achieve anchorage for the final restoration (1). Hence, long-term retention of the post becomes pivotal for the stability and retention of the final restoration (2).

Among the commercially available fibre posts, glass fibre posts are predominantly used for the restoration of endodontically treated teeth. These posts have the advantage of possessing an elastic modulus value, which is near identical to that of human root dentine, enabling uniform distribution of stresses along the post-cement-dentine interface and to the remaining tooth structure, thereby reducing the risk of vertical root fracture (3).

The post is luted to the root canal using resin cement in combination with a total-etch or self-etch bonding system (4). Resin-dentine adhesion is a technique sensitive procedure that encounters problems associated with long-term stability, owing to the structural and chemical complexities of the resin-dentine interface. The combined effect of hydrolytic deterioration of resinous components and the host-derived enzymatic degradation of collagen fibrils leads to loss of integrity of

resin-dentine bonds over time (5,6). Matrix metalloproteinases present in dentine, which are activated from their proactive forms by etching and bonding procedures, comprise the predominant endogenous proteolytic mechanism (7). An acidic environment and active matrix metalloproteinases are capable of activating another group of proteases found in dentine, the cysteine cathepsins, which further potentiates activation of matrix metalloproteinases. Thus the interplay between cysteine cathepsins and matrix metalloproteinases results in collagen degradation within the hybrid layer (8).

The stability of dentin collagen can be improved by inhibiting enzymatic degradation or by augmenting the collagen structure of dentine by treating it with natural or synthetic crosslinking agents. Proanthocyanidin (PACs) available in abundance in fruits and nuts have shown to improve the biomechanical properties and biostability of the demineralized dentine matrix by stimulating interfibrillar, intrafibrillar, and inter-microfibrillar cross-links in the collagen matrix (9,10). Studies have shown that PACs are as effective as chlorhexidine in reducing dentine matrix degradation by inhibiting matrix metalloproteinases and cysteine cathepsins (10). PAC pretreatment of dentine has shown to improve the immediate bond strength of resin to dentine (4).

Phytosphingosine (PHS) is a sphingoid base, a fundamental building block of more complex sphingolipids. It is present abundantly in epidermis and is also the major lipid in the brain. It is also present in plants and fungi and has shown to possess bactericidal and fungicidal activity (11). Previous studies have demonstrated that PHS exhibits matrix metalloproteinases inhibition and collagen-stabilising ability (12).

A recent study by Shriram et al. (2020) (13) evaluated the influence of dentine pretreatment with PHS on the flexural strength of dentine and the depth of penetration of bonding agent into the dentine. A thorough search of the literature shows that there are no studies evaluating the effect of dentine pretreatment with PHS on the push-out bond strength of glass fibre posts to dentine. PHS, with the property of collagen synthesis, could be a potential agent to improve and maintain the stability of the dentin-adhesive interface. Hence, the aim of this in vitro study was to comparatively evaluate the push-out bond strength of glass fibre posts bonded to root dentine pretreated with 6.5% proanthocyanidin or 0.02% phytosphingosine. The null hypothesis was that dentine pretreatment with PAC or PHS will not influence the bond strength of fibre posts to dentine.

MATERIALS AND METHODS

Preparation of 6.5% PAC solution

6.5 g of grape seed-derived PAC powder (HealthyHey foods LLP, Mumbai, India) was weighed using a digital weighing balance and dissolved in 100 mL of distilled water to make a 6.5% PAC solution.

Preparation of 0.02% phytosphingosine solution

500 mg of PHS powder (Evolution Life Science, Chennai, India) was weighed using a digital weighing balance and dissolved in 100 ml of ethanol (Merck, Mumbai, India). 20 mM Tris supplemented with 0.1% Tween 20 was added to dilute the solution to obtain a concentration of 0.02%. The experimental solution was then centrifuged at 10,000 rpm for 10 min.

Sample preparation

Based upon the pilot study, sample size was calculated using G*Power software version 3.1.9.2, which indicated that 11 samples per group would provide more than 90% power in determining statistically significant difference in the push-out bond strength values among the three groups. The alpha was set at 5% and the effect size was 0.458.

Thirty-three intact single rooted human teeth, extracted for orthodontic or periodontal reasons, were collected and stored in 0.1% thymol solution until use. The collection of tooth samples was in conformation with the principles of Declaration of Helsinki. The methodology was presented to the Institutional Review Board and approval was obtained (SRMU/M&HS/SRMDC/2020/S/029). Sample preparation was adopted from Kim et al. (14). Teeth selected for the study were clinically and radiographically examined to ensure that only those with similar mesiodistal and buccolingual widths and similar anatomic segments of the root canal were included in the study. The teeth were decoronated at the cementoenamel junction and the root length was standardised to 14 mm using a diamond disc under water coolant. A size10 K-file (Mani, Inc., Tochigi, Japan) was used to establish working length 1 mm short of the apex. Cleaning and shaping was done at the working length using rotary NiTi files (Protaper gold, Dentsply, Maillefer, Ballaigues, Switzerland) following the sequence S1, S2, F1, F2 and F3. Canals were then irrigated with 5 mL of 3% sodium hypochlorite (NaOCl) (Nimai Dento India, Tamilnadu, India) between each change of instrument. Following instrumentation, the smear layer was removed using 5 mL of 17% EDTA (Endoprep-RC, Anabond Stedman Pharma Research (P) Ltd, Tamilnadu, India). Finally, the canal was irrigated with 5 mL of 3% of NaOCl. Obturation was completed using F3 gutta-percha cones (Dentsply Maillefer, Ballaigues, Switzerland) and AH plus sealer (Dentsply, Konstanz, Germany). Post space preparation was created with up to #3 peeso reamer (Mani Medical Hanoi Co., Ltd., Thai Nguyen Province, Vietnam), retaining the apical 5 mm of gutta-percha. The smear layer was removed using 5 ml of 17% EDTA and the post space was etched with 37% phosphoric acid (Anabond Stedman Pharma Research (P) Ltd, Tamilnadu, India). The etchant was rinsed off with water and the excess water was dried with paper points. The specimens were randomly assigned to three groups (n=11) based on the pretreatment of root canal dentine. Samples in control group did not receive any pretreatment and those in the 6.5% group and 0.02% PHS group were irrigated with 5 mL of the respective experimental solutions using a 23-gauge needle. The solution was left in place for 5 min, with intermittent agitation using a microbrush. At the end of the exposure time, the solutions were rinsed off and the canals were dried with paper points. In all the groups, dual curing adhesive (ExciTE F DSC, Ivoclar Vivadent AG, Schaan, Liechtenstein) was applied using a micro brush and light cured for 20 seconds. Simultaneously, the glass fibre post was silianised and luted using a dual-cure resin cement (Variolink, Ivoclar Vivadent

TABLE 1. Mean±standard deviation of the push out bond strength (MPa) of all the groups at different levels of the post space

Groups	Levels of post space		
	Coronal	Middle	Apical
Control	8.13±2.43[b]	9.25±2.88[b]	7.35±1.92[c]
6.5% PAC	16.74±4.8[a]*	12.15±3.16[b]	11.15±4.30[b]
0.02%PHS	18.2±5.42[a]	16.96±4.06[a]	14.59±3.05[a]

Under each level of post space, different alphabets denote significant difference between the groups. *6.5% PAC showed significantly higher push-out bond strength value at coronal third, compared to middle and apical thirds (P<0.05)

AG, Schaan, Liechtenstein) and light cured for 30 seconds. The samples were later stored in distilled water for 24 hours.

Push out bond strength testing

Horizontal sections were made at the coronal, middle and apical levels of the post space using a diamond disc under water coolant to obtain two 1 mm thick slices from each section. The push-out bond strength of the glass fibre post to the root dentine was measured using a universal testing machine at a crosshead speed of 0.5 mm/min until the fibre post dislodged from the specimen. The average of push-out bond strength values obtained from the two slices of each section was taken as the mean push-out bond strength value of the specimen at the given root level. The mode of failure of all debonded specimens was evaluated using an optical microscope at 40x magnification.

Statistical analysis

The data were tabulated and statistically analysed using SPSS statistics V22.0 (IBM, USA). Normality assessment was calculated using Shapiro-Wilk test and the data were found to be normally distributed. Hence, one-way ANOVA followed by Games-Howell post hoc test was performed. Statistical significance was set at P<0.05.

RESULTS

The mean and standard deviation of the push-out bond strength of all groups at three different levels of the post space is given in Table 1. The push-out bond strength of samples in control group was the least at all the three levels of the post space. Intergroup comparisons revealed that at the coronal third, PAC and PHS showed significantly higher push-out bond strength than the control group (P<0.05), with no significant difference among the PAC and PHS groups (P>0.05). In the middle third, PHS was significantly higher than control and PAC (P<0.05). In the apical third, both PHS and PAC were significantly higher than the control group, with PHS showing significantly greater values than PAC (P<0.05). Intragroup comparison showed no significant differences within the control group and PHS, whereas in PAC, the push-out bond strength values were significantly higher at the coronal third, compared to middle and apical thirds (P<0.05). Five failure modes were identified and classified as adhesive failure either between the dentine and luting cement (A), or between the post and the luting cement (B), combined adhesive failure showing features of both A and B (C), cohesive failure of the luting cement (D) and mixed failure involving combinations of A, B and D (E) (Fig. 1).

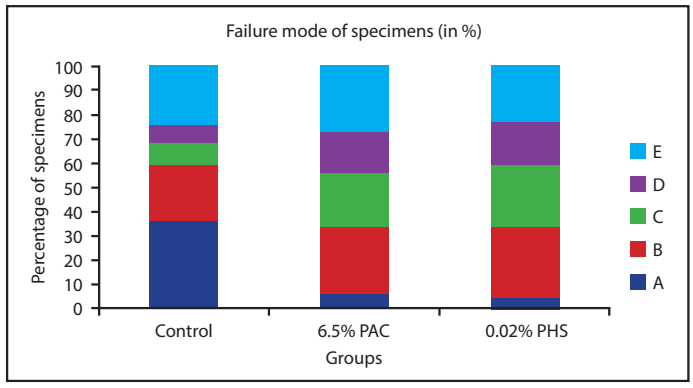

Figure 1. Failure modes of all the groups (in %).
A- Adhesive failure between dentin and luting cement; B- Adhesive failure between post and luting cement; C- Combination adhesive failure showing features of both A and B; D- Cohesive failure of the luting cement; E- Mixed failure involving combinations of A, B and D

DISCUSSION

A successful post-endodontic restoration depends on the integrity of the interface that the luting cement forms with the root dentine and the post. Contemporary resin luting cements are preferred as they bond chemically with both the post and the resin composite core and micromechanically with the dentine. This results in a functionally and mechanically homogenous unit (14). The weak link in this unit is the hybrid layer formed between the resin cement and the dentine. Analysis of failure modes in this study revealed that, compared to the experimental groups, the most predominant type of failure in the control group was an adhesive failure between the dentine and the luting cement. Hydrolytic or enzymatic degradation of the hybrid layer can lead to such a failure of resin-dentine adhesion (5, 6). Numerous studies have focused on improving the stability of this interface for a successful adhesion. Various natural cross-linking agents like tannic acid, genipin, ascorbic acid and PACs have shown to crosslink collagen and resist enzymatic degradation by inactivating the action of matrix metalloproteinases (4, 15-17).

The results of the present study showed that both PAC and PHS improved the push-out bond strength of the fibre post to dentine compared to control. Hence the null hypothesis was rejected. Failure mode analysis of fractured specimens in both the PAC and PHS groups showed the least percentage of adhesive failure at the resin-dentine interface. PACs belong to a class of polyphenolic bioflavonoids, which are the most commonly used natural cross linkers for stabilising collagen matrices (18). Their mechanism of action can be attributed to four monomer molecules present in their structure namely, catechin, ent-catechin, epicatechin, and ent-epicatechin, which have affinity towards proline-rich protein such as collagen (19). PACs form an insoluble complex with carbohydrates and proteins and facilitate the enzyme proline hydroxylase activity that is essential for collagen biosynthesis (20). Pretreatment with PAC significantly improved the immediate bond strength compared to no pretreatment.

The increase in immediate push-out bond strength seen in this study could be attributed to the cross linking between PAC and the collagen brought about by covalent-, ionic-, hy-

drogen bonding- or hydrophobic interactions between the two. The amino acid proline in collagen is a good hydrogen acceptor and ensures a strong hydrogen bond with PAC (19). Thus, the PAC-collagen complex is stabilised predominantly by hydrogen bonding between the protein amide carbonyl and the phenolic hydroxyl groups of polyphenols in addition to covalent and hydrophobic bonds (21).

In addition to its cross-linking effect, PAC downregulates the enzymatic degradation by inhibiting the matrix metalloproteinases and cysteine cathepsins activity. Epasinghe et al (2013) demonstrated that PAC could inactivate more than 90% of soluble recombinant matrix metalloproteinases -2, -8 and -9 and approximately 70-80% of cysteine cathepsins B and K (10). The other mechanism of downregulating enzymatic degradation is increasing the density of the collagen network by inducing exogenous cross links and decreasing collagenase absorption (21). It could also be speculated that the collagen cross-linking mediated by PACs might change the type I collagen molecular and fibrillar arrangement in such a way that the cleavage sites of proteolytic enzymes such as matrix metalloproteinases and other collagenases are blocked or hidden (22). Hence, the combined cross-linking activity and anti-collagenolytic effects of PACs are responsible for preventing degradation of dentin collagen within the hybrid layer. Cecchin et al. (2015) studied the immediate and long-term (12 months) effects of PAC-rich grape seed extract pretreatment on the adhesion of fibre posts to root dentine using total-etch and self-etch adhesive systems. They however observed that the use of PAC proved beneficial in improving only the long-term bond strength of the post to dentine (4).

A novel material, PHS, a natural sphingoid base belonging to the sphingosine family of lipids was tried as an alternative to PAC in this study. PHS is found abundantly in fruits, vegetables, fungi, yeast and in the human body, it is found in epidermis, oral cavity and mucosal surface. It has been reported to have anti-inflammatory, antibacterial and antifungal properties (11,12,23). The structure of PHS and sphingosine are similar, except that PHS has a hydroxyl group at C-4 of the sphingoid long-chain base whereas, sphingosine consists of a trans double bond between C-4 and C-5 carbon chains (24). Cukkemane et al (2015) studied the potential of sphingolipids as an anti-biofilm agent by evaluating the adherence of Streptococcus mutans on sphingolipid-pretreated hydroxyapatite surfaces. They concluded that sphingolipids including PHS could serve as potential anti-biofilm agents against cariogenic oral biofilms (11).

Kim et al (2006) showed that PHS was a peroxisome proliferator–activated receptor ligand and it increased the transcriptional activity of the latter. It is evidenced that spingosine regulates deposition of collagen through this pathway (24). Sphingolipids increase the synthesis of procollagen-1 by adult human fibroblasts under in vitro conditions. Procollagen-1 is an important factor in the formation of extra cellular matrix. Under in vivo conditions, an increased deposition of fibrillin-1 and procollagen-1 along with matrix metalloproteinase-1 inhibition was evidenced in photo-aged skin treated with PHS (25). The upregulation of collagen and matrix metalloproteinase inhibitory activity of PHS could have resulted in an increased push-out bond strength in the present study. PHS has the tendency to assemble into highly positively charged aggregates that would in effect produce a high affinity for negatively charged phosphate-rich surfaces like hydroxyapatite (26). This in turn enables a uniform coating of this material on the dnetine surface. PHS being a colourless liquid could also offer an additional advantage over PAC, which is known to stain dentin reddish-brown to brown (27). A recent study by Shriram et al (2020) has proven that dentine pretreatment with PHS increased the flexural strength of dentine and also improved the depth of penetration of the adhesive into the demineralized dentin compared to pretreatment with PAC. They concluded that PHS could be a potential collagen cross-linking agent and an alternative to PAC (13). Our study has put forth the role of PHS as a natural collagen-stabilising agent in root dentine. Bond stability of aesthetically glass fiber posts to dentine could be compromised due to chemicals used during endodontic treatment or during adhesive cementation procedures. PHS could serve as a promising, non-staining natural agent in improving the adhesion of glass fibre posts to dentine.

Only one adhesive system was tested in this study. With a plethora of bonding agents commercially available, further studies should assess the effect of other bonding strategies and bonding agents in this experimental set up. Various other concentrations of PAC and PHS, with shorter application times need to be tested. Their effect on matrix metalloproteinases and cysteine cathepsins also need to be further explored. The present study evaluated only the short-term effect of PAC and PHS under in vitro conditions in root dentine. Future studies should focus on long-term effects of these agents and clinical trials of their usage in root dentine bonding.

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that the use of 0.02% PHS and 6.5% PAC improved the push-out bond strength of glass fiber post to root dentine. 0.02% PHS was equally effective as 6.5% PAC in improving the adhesion of the fiber post to dentine.

Disclosures

Conflict of interest: The authors declare no potential conflict of interest related to the study.

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