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Effect of root dentin pretreatment with different concentrations of chitosan on the push-out bond strength of fiber post using a self-adhesive resin cement

Elham Ahmadi[1], Seyedeh Mahsa Sheikh-Al-Eslamian[2], Sara Valizadeh[3], Mohammad Javad Kharazifard[4] and Faezeh Aghajani[5*]

Abstract

Background This study aimed to investigate the effect of root dentin pretreatment with different concentrations of chitosan on the push-out bond strength (PBS) of fiber post using a self-adhesive resin cement.

Methods After post-space preparation in 56 maxillary central incisors that underwent endodontic treatment and were filled with gutta-percha (Spident, Korea) and AH Plus resin sealer (Dentsply, USA), the teeth were randomly divided into four groups ($n=14$) for pretreatment with 2.5% chitosan, 1% chitosan, 17% ethylenediamine tetra-acetic acid (EDTA), and saline. Fiber posts were cemented into the root canals using Panavia SA resin cement. The teeth were then thermocycled (5°C-55°C, 5,000 cycles), and the roots were sectioned into coronal, middle, and apical thirds. The PBS was measured in a universal testing machine. The mode of failure was also determined under a stereomicroscope and a scanning electron microscope (SEM). The PBS data were analyzed using two-way ANOVA and Tukey's post hoc test.

Results The mean PBS of 2.5% chitosan group was significantly higher than that of control group at the coronal, middle, and apical thirds of the root in the post-placement region. However, 2.5% chitosan group had a significant difference in PBS with the EDTA group only in the middle and coronal thirds ($P<0.05$). Pretreatment with 1% chitosan and 17% EDTA did not significantly increase the PBS at any level from the root in the post-placement region. Cohesive failure was dominant in 2.5% chitosan group, while mixed failure had the highest frequency in other groups.

Conclusion Root dentin pretreatment with 2.5% chitosan improved the PBS of fiber post to root dentin by using a self-adhesive resin cement.

Keywords Chitosan, Bond strength, Fiber post, Resin cement

*Correspondence:
Faezeh Aghajani
Drfaezeh.ai1373@yahoo.com
Full list of author information is available at the end of the article

Background

Application of intracanal fiber posts is highly popular for reconstruction of endodontically treated teeth. However, achieving stable and optimal adhesion to radicular dentin, especially in the apical third, is clinically challenging due to the adverse effects of numerous confounding factors [1]. The cement-dentin bonding interface is the weakest point in intracanal cementation of fiber posts [2]. Demineralization of extrafibrillar dentin is an effective strategy to achieve a strong resin-root dentin bond [3]. In this strategy, high molecular weight chelating agents can selectively remove apatite crystals from the extrafibrillar dentin space, functioning similarly to mild self-etch adhesives. This process results in the formation of a hybrid layer less than 1 µm thick [4]. Importantly, these chelating agents preserve the mineral content of intrafibrillar dentin, preventing washout and protecting exposed collagen fibers from matrix metalloproteinases [5]. As a result, collagen fibers remain intact in extrafibrillar dentin even after prolonged exposure to such materials [6, 7]. Maintaining close proximity of the material to the substrate during chelation is crucial for effective calcium (Ca^{2+}) ion release, which helps retain the smear plug [5]. The presence of this smear plug not only seals the dentin but also alleviates concerns regarding the replacement of resin monomers with water in the intrafibrillar space of demineralized dentin. This reinforces bond strength and contributes to long-term bonding durability [7].

Chitosan is a natural polysaccharide obtained from chitin, which is equally or even more effective than 17% ethylenediaminetetraacetic acid (EDTA) in smear layer removal [8, 9]. In an acidic pH, chitosan serves as a chelating agent due to possession of free hydroxyl and amino groups, which can react with reactive molecules and cause cross-linking of collagen fibers. This interaction strengthens demineralized collagen, stabilizes the collagen network, and enhances resistance to hydrolytic degradation over time [10, 11].

Previous studies used different concentrations of chitosan for pretreatment of coronal dentin [10, 12–14] and root dentin [15], and assessed its effect on the bond strength and durability. The results revealed that 1% chitosan with high molecular weight was effective as a chelating agent for elimination of extrafibrillar apatite crystals when applied with the etch-and-rinse technique [5]. Considering the absence of studies on the effect of root dentin pretreatment with different concentrations of high molecular weight chitosan and a self-adhesive resin cement, this study aimed to assess the effect of root dentin pretreatment with different concentrations of chitosan and 17% EDTA following post space preparation on the pushout bond strength (PBS) of fiber post using Panavia SA self-adhesive resin cement.

Methods

The protocol of this in vitro experimental study was approved by the ethics committee of the Research Council of School of Dentistry, Tehran University of Medical Sciences (IR.TUMS.DENTISTRY.REC.1401.097).

Inclusion and exclusion criteria

The inclusion criterion was central maxillary teeth extracted for periodontal reasons while the exclusion criteria were caries, root cracks, previous endodontic treatment, and previous restorations.

Sample size

The minimum sample size required for each of the four study groups was 14, according to a study by Ahmadi et al. [16] using the one-way ANOVA power analysis feature of PASS 11 software ($\alpha = 0.05$).

Tooth preparation

Remaining soft tissue on the root surfaces was removed using gauze, and any calculus present was eliminated with manual scalers. The teeth were then immersed in a 0.5% chloramine T solution for one week to ensure disinfection.

Each tooth was decoronated 1 mm above the cementoenamel junction using a low-speed diamond disc (Yuanda, China) with air/water coolant. Root canals were instrumented with the Eighteeth rotary system (Eighteeth, Shanghai, China) and ProTaper rotary files (Dentsply, USA), progressing from S1 to F2 at 300 rpm. Final shaping of the root canals was achieved using #1, #2, and #3 Gates-Glidden drills (Mani, Japan). Root canal irrigation involved 5 mL of 5.25% sodium hypochlorite (NaOCl; Morvabon, Iran) during the procedure, followed by a final rinse with 5 mL of 17% EDTA (Morvabon, Iran) and an additional rinse with 5 mL of 5.25% NaOCl for smear layer removal. Finally, the root canals were rinsed with saline, dried with paper points (Spident, Korea) and obturated with gutta-percha and AH Plus resin sealer (Dentsply, USA) using the cold lateral compaction technique. The optimal quality of obturation was ensured radiographically. Any residual gutta-percha in the coronal section was removed with a heated plugger, and the canal orifice was sealed with temporary restorative material (Coltosol; Ariadent, Iran). The roots were incubated in a Scientific™ incubator (Thermo Fisher Scientific, USA) at 37°C and 100% humidity for one week. It should be noted that all phases of root canal therapy were performed by the same operator.

Preparation of chitosan solutions

Two concentrations of chitosan solutions (1% and 2.5%) were prepared following methods described in previous

studies [12, 13]. Commercially available chitosan was procured from Alborz Nano-Tajhiz Ryan Company (Alborz, Iran) and had a molecular weight greater than 40 kD (90% deacetylated). The solutions were prepared as follows:

2.5% chitosan solution: 2.5 g of high molecular weight chitosan was dissolved in 100 mL of 1% acetic acid (pH = 4.21).
1% chitosan: 1 g of high molecular weight chitosan was dissolved in 100 mL of 1% acetic acid (pH = 3.71).

Post space preparation and cementation

Gutta-percha was removed from the root canals using a #2 peeso reamer (Mani, Japan), ensuring at least 4 mm remained in the apical third of the root canal. The post space was prepared to the desired depth using the drill from the intracanal post kit (White post FGM, Brazil). The roots were then randomly assigned to four groups ($n = 15$ each): 2.5% chitosan, 1% chitosan, EDTA, and saline.

- 2.5% Chitosan Group: The dentin surface was treated with 5 mL of 2.5% chitosan for 1 min and rinsed with distilled water for 20 s.
- % Chitosan Group: The dentin surface was treated with 5 mL of 1% chitosan for 1 min and rinsed with distilled water for 20 s.
- EDTA Group: A 5 mL application of 17% EDTA was applied to the root dentin for 1 min, followed by a rinse with distilled water for 20 s and drying.
- Saline Group: A 5 mL injection of 0.9% saline (Samen, Iran) was placed in the canal space and retained for 1 min.

The root canals were dries with paper points immediately after root dentin treatment. Intracanal post #1 (White post FGM, Brazil) was cleaned with alcohol. Panavia SA (Kuraray, NY, USA) self-adhesive resin cement was applied on the post, and it was introduced into the canal. The cement was light-cured by a curing unit (Woodpecker, China) for 40 s. The intensity of the curing light was assessed by a radiometer (LM-1; Woodpecker, China) prior to each time of use. Coltosol temporary restorative material was used for coronal sealing of the canal.

Thermal cycling

The roots underwent 5000 thermal cycles between 5°C and 55°C, simulating approximately six months of in vivo function. Each cycle included a dwell time of 30 s at each temperature and a transfer time of 30 seconds [17].

Push-Out Bond Strength (PBS) test

Roots were mounted in metal molds filled with transparent polyester, ensuring that the longitudinal axis of the post was parallel to the mold walls. Using a Mecatome (Presi, France) equipped with a diamond disc and running water, the roots were sectioned apical to the cementoenamel junction. Three sections were created from each root at the coronal, middle, and apical thirds of the root in the post-placement region, with a thickness of 1 ± 0.4 mm. In this study, three regions related to the post location were examined, and the apical root third was located at least 4 mm coronal from the true apical. The thickness of each section was measured using a digital micrometer (Mitutoyo, USA) with an accuracy of 0.01 mm. To assess the post radius at the coronal and apical surfaces of each section, the sections were photographed alongside a 1 mm indicator under a stereomicroscope. The images were analyzed using Microstructural Image Processing software.

For the PBS test, sections were placed in an acrylic mold with a 2.5 mm diameter. A universal testing machine (Zwick, Germany) was employed for the PBS assessment, with a jig diameter of 0.8 mm. The sections were positioned so that the jig contacted only the fiber post, avoiding contact with the resin cement or surrounding root dentin. A load was applied at a crosshead speed of 0.5 mm/min until fracture occurred.

The PBS was calculated by dividing the load at fracture by the post-root dentin interface area. Given that the post had an incomplete pyramidal shape, its external surface area was computed using the formula:

$$A = \pi(R + r)\sqrt{h^2 + (R - r)^2}$$

Where R is the post radius at the coronal surface, r is the post radius at the apical surface, and h is the thickness of each section. The PBS of each section was calculated in megapascals (MPa) using the following formula:

$$PBS = F/A$$

Where F is the maximum load applied, and A is the external surface area of the post [15].

Microscopic assessment

Each specimen was inspected under a stereomicroscope (Nikon, Japan) at ×40 magnification to determine the mode of failure, which was categorized as adhesive, cohesive, and mixed [16] (Table 1).

For scanning electron microscopic (SEM) assessment, two sections were randomly selected from each group and glued to an aluminum mold from their apical surface. They were then gold sputter-coated under vacuum, and the sections with detached posts and fracture areas

Table 1 Different failure modes in the study groups

Failure mode	Definition of failure mode
Adhesive at the post-cement interface)AP)	Adhesive failure at the cement-post interface (no cement seen on the post)
Adhesive at the cement- dentin interface)AC)	Adhesive failure at the dentin-cement interface (post is completely coated with resin cement)
Cohesive in dentin)CD)	Cohesive failure in dentin
Cohesive in post)CP)	Cohesive failure in post
Mixed)M1-0–50%)	0%-50% of post diameter is covered with resin cement
Mixed)M250-100%)	50%-100% of post diameter is covered with resin cement

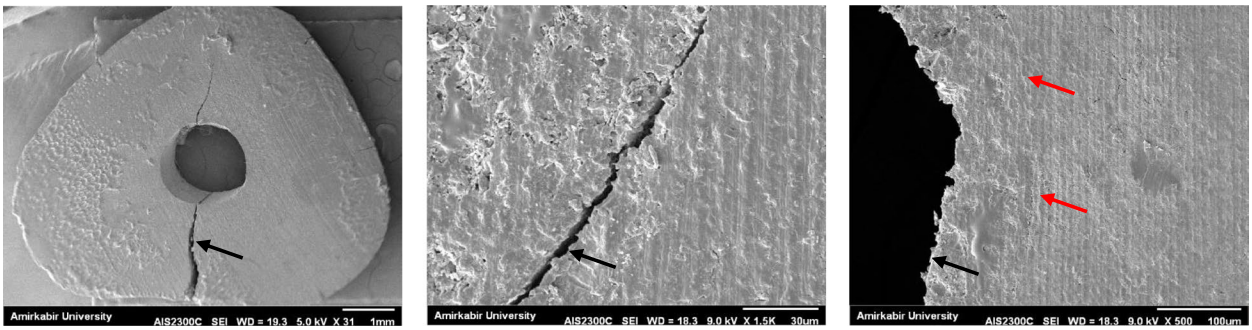

Fig. 1 SEM assessment of the mode of failure; (**a**) Cohesive failure within dentin (CD) (**b**) Adhesive failure at the cement-dentin interface (AC); (**c**) Adhesive failure at the cement-post interface (AP). *The black arrows indicate the location of the fracture. *The red arrows indicate the location of cement-dentin interface

underwent SEM assessment at 50,000X magnification (Fig. 1).

Statistical analysis

Data were analyzed by SPSS version 23 using two-way ANOVA and Tukey's post-hoc test to assess the effect of root dentin pretreatment with different concentrations of chitosan on PBS of fiber post in the apical, middle, and coronal thirds of the root. Level of statistical significance was set at 0.05. Frequency percentage values were reported for the modes of failure.

Results

Using the Shapiro–Wilk test, the data distribution was normal with p values of 0.156, 0.185, and 0.051 for coronal, middle, and apical, respectively. Table 2 and Fig. 2 presents the push-out bond strength (MPa) of fiber posts in the apical, middle, and coronal thirds of the root in the post-placement region in the study groups. As shown, the highest mean PBS was recorded in the coronal third in 2.5% chitosan group; while, the lowest mean PBS was recorded in the apical third in the control group. Two-way ANOVA was applied to compare the mean PBS of the groups, which revealed a significant difference ($P<0.05$) (Table 3).

Table 2 Mean PBS in different parts of the root in the study groups

Location		Mean	Std. deviation
Coronal	**chitoson2.5%**	**9.29**	**3.85**
	chitoson1%	**6.06**	**5.52**
	EDTA17%	**3.68**	**1.73**
	Control	**3.66**	**2.06**
	Total	**5.67**	**4.22**
Middle	**chitoson2.5%**	**7.87**	**2.99**
	chitoson1%	**5.21**	**5.37**
	EDTA17%	**2.85**	**2.64**
	Control	**3.89**	**3.47**
	Total	**4.96**	**4.12**
Apical	**chitoson2.5%**	**5.59**	**2.84**
	chitoson1%	**4.46**	**3.93**
	EDTA17%	**3.64**	**3.71**
	Control	**1.40**	**1.94**
	Total	**3.77**	**3.48**

Pairwise comparisons by the Tukey's post-hoc test revealed significant differences in the mean PBS between 2.5% chitosan and control groups ($P=0.001$), and also between 2.5% chitosan and 17% EDTA groups ($P=0.001$) in the coronal third. The difference between

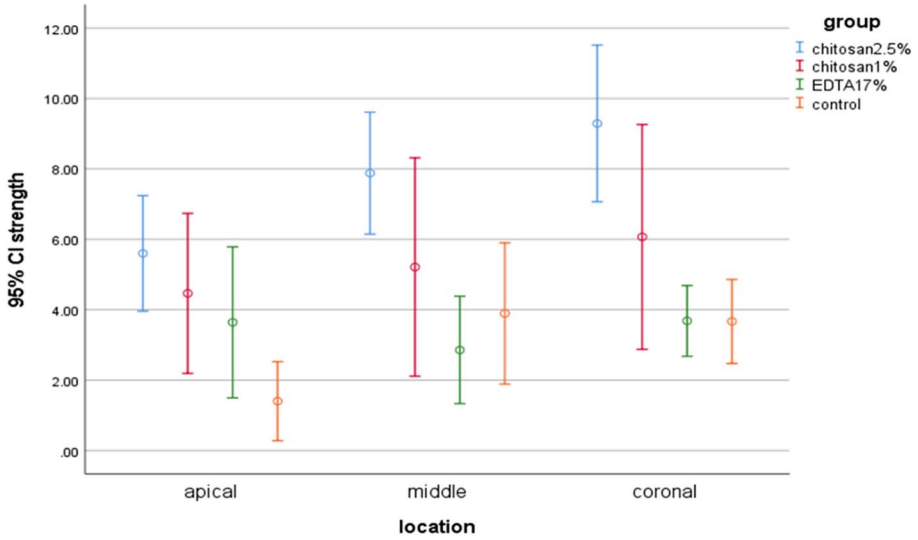

Fig. 2 Bond strength values in different parts of the root in the study groups

Table 3 Statistical comparison of the average bond strength in different preparation groups using Two-way ANOVA test (*= $P < 0.05$)

Location		Sum of squares	df	Mean square	F	Sig.
Coronal	Between Groups	297.165	3	99.055	7.514	.000*
	Within Groups	685.491	52	13.183		
	Total	982.656	55			
Middle	Between Groups	198.058	3	66.019	4.642	.006*
	Within Groups	739.531	52	14.222		
	Total	937.588	55			
Apical	Between Groups	132.289	3	44.096	4.284	.009*
	Within Groups	535.221	52	10.293		
	Total	667.510	55			

2.5% chitosan and control groups ($P=0.035$), and 2.5% chitosan and 17% EDTA groups ($P=0.005$) was also significant in the middle third. In the apical third, 2.5% chitosan and control groups had a significant difference in the mean PBS ($P=0.006$).

Stereomicroscopic results revealed that cohesive failure had the highest frequency in 2.5% chitosan group; while, mixed failure had the highest frequency in other groups such that 50% to 100% of the post diameter was coated with resin cement (Fig. 1).

Discussion

In this study, we found that pretreating root dentin with 2.5% chitosan effectively modified the smear layer and enhanced the push-out bond strength (PBS) of fiber posts to root dentin when using self-adhesive resin cement across the entire root length in the post-placement region. It appears that the high molecular weight chitosan (2.5%) employs a demineralization strategy targeting extrafibrillar dentin. This approach enhances bond strength by preserving the smear plug and ensuring the presence of calcium hydroxyapatite, which facilitates chemical bonding with the 10-MDP self-adhesive resin cement.

The literature search identified only two studies utilizing 2.5% chitosan to enhance bond strength and durability, both focused on coronal dentin [18, 19]. Consistent with the current findings, Paschoini et al. [13] demonstrated that 2.5% chitosan pretreatment improved immediate and 6-month bond strength when combined with Clearfil SE Bond self-etch adhesive. Conversely, Vasei et al. [12] found that a lower concentration of chitosan improved bond strength more effectively than the 2.5% concentration. This discrepancy may stem from differences in the molecular weight of the chitosan used; the 2.5% chitosan in their studies may not have been of high molecular weight, allowing it to penetrate interfibrillar spaces during the

chelation process. Consequently, it could accumulate within the collagen network of demineralized dentin, leading to the opening and elimination of intrafibrillar spaces. This accumulation likely hindered resin penetration and the formation of an effective hybrid layer. Vasei et al. [12] used Clearfil SE Bond two-step self-etch bonding agent and Adper Single Bond 2 two-step etch-and-rinse adhesive. Their findings indicated that applying phosphoric acid after chitosan resulted in lower bond strength compared to etching prior to chitosan application. This reduction may be attributed to chitosan accumulation in fibrillar spaces, which could diminish the efficacy of phosphoric acid etching. Notably, similar to our study, Vasei et al. [12] used 1% and 2.5% concentrations of chitosan with pH values of 3.98 and 4.74, respectively.

In the present study, the application of 1% chitosan did not significantly enhance the push-out bond strength (PBS) compared to the other groups. However, several studies [5, 10, 12, 13] have reported that the use of 1% chitosan on coronal dentin increased bond strength. For instance, Ata et al. [14] demonstrated higher immediate and six-month shear bond strength, while Zidan et al. [10] observed increased micro-tensile bond strength after 12 and 24 months in the chitosan group compared to the control group. These studies also noted a reduction in nano-leakage corresponding to the improved bond strength. In both of these studies [10, 14], chitosan was applied to coronal dentin following phosphoric acid etching and rinsing of the dentin surface. The more acidic pH of lower concentration chitosan may facilitate more effective removal of the smear layer, thereby enhancing bond strength through improved smear layer elimination. Despite these findings, our results do not align with theirs. This discrepancy suggests that this approach may be detrimental to root dentin and the use of self-adhesive resin cements. The bonding mechanism of self-adhesive cements relies on the chemical interactions between acidic monomers, such as 10-MDP, and the calcium hydroxyapatite content in root dentin. Consequently, excessive removal of calcium hydroxyapatite by chelating agents could compromise bond strength [20].

Our findings also contrast with those of Gu et al. [5], who utilized one hydrophobic and one hydrophilic experimentally formulated adhesive with both wet-bonding and dry-bonding techniques. They employed high molecular weight 1% chitosan to selectively remove Ca^{2+} ions from extrafibrillar dentin, thereby enhancing bond strength and durability to coronal dentin. The more acidic pH of 1% chitosan used in their study may have contributed to the significant removal of calcium hydroxyapatite ions, potentially explaining the differences in results between the two studies.

Search of the literature by the authors revealed only three studies regarding the application of chitosan on root dentin, including the studies by Rasendren et al., [21] Waz et al. [15] and Xiong et al. [11]. In the study by Xiong et al [11], nano-chitosan was used along with a cross-linker. The additional cross-linker can enhance the mechanical properties and decrease the risk of collapse of the collagen network, enhancing resin penetration.

In contrast to our findings, Rasendren et al. [21] used a lower concentration of chitosan (0.2%) with a high molecular weight as the final root canal irrigant after post space preparation. Their results indicated an improvement in the bond strength of fiber posts to root dentin following the chelation process, with no access to intrafibrillar dentin. Notably, they observed a greater increase in push-out bond strength (PBS) in the chitosan group compared to the EDTA group, which aligns with our findings. However, their use of nano-particulate chitosan complicates direct comparison with our results, as nanoparticles may achieve greater penetration depth for effective chelation and smear layer removal. Additionally, Rasendren et al. [21] employed an etch-and-rinse technique for bonding fiber posts, which could have contributed to enhanced bond strength following selective chelation by chitosan, as phosphoric acid etching would effectively eliminate the smear layer from root dentin. Furthermore, their study did not incorporate thermocycling, leaving the effects of aging on bond strength unexamined.

Although the exact mechanism of action of chitosan remains poorly understood, it is believed that absorption, ionic exchange, and chelation play crucial roles in its interactions with substrates and metal ions. These reactions are influenced by the specific ions involved, the chemical composition of chitosan, and the pH of the solution [22]. The application of chitosan as a final rinse can effectively remove the smear layer from root dentin, with its efficacy increasing alongside concentration without causing denaturation of collagen fibers [23]. The prevention of collagen denaturation may be attributed to a higher number of residual apatite crystals within the collagen matrix, as high molecular weight chitosan is less likely to penetrate intrafibrillar dentin. Consequently, smear plugs may persist, reducing water permeability in dentin and contributing to long-term bonding durability [5].

Matrix metalloproteinases (MMPs) present in dentin can be activated in the acidic environment created by etch-and-rinse adhesives, and to a lesser extent by self-etch systems, leading to the degradation of collagen fibers and a decrease in bond strength [12]. An increase in chitosan concentration results in greater consumption of $H+$ ions due to the protonation of free amine groups, which raises the pH. This rise in pH helps prevent the

degradation of the organic dentin matrix by MMPs, thereby improving bonding durability [19].

Furthermore, chitosan appears to cross-link with collagen, and this reaction is concentration-dependent. By inhibiting collagenase activity, chitosan slows the degradation of the bonding interface, enhancing bonding durability. Importantly, this interaction does not hinder resin penetration or negatively impact the bonding interface [11]. These factors contribute to the higher bond strength observed with fiber posts following dentin pretreatment with higher concentrations of chitosan.

Chitosan is insoluble at pH values above 7; however, it is highly soluble in acidic conditions, including diluted mild acids such as lactic acid, acetic acid, and formic acid. It is reasonable to hypothesize that these solvents may also influence the dissolution of calcium ions [24]. Previous studies have shown that the efficacy of 5% acetic acid in chelating calcium ions, reducing dentin microhardness, and eliminating the smear layer from root dentin is significantly less than that of 15% EDTA or 1% acetic acid. Therefore, the demineralization of extrafibrillar dentin by chitosan is primarily attributed to its chelating capacity rather than the effects of the solvent [25, 26].

EDTA, as a mild chelating agent, selectively removes hydroxyapatite and non-collagen proteins while minimizing major structural changes in collagen fibrils. Evidence suggests that chitosan exhibits comparable or even superior efficacy in smear layer removal compared to EDTA. The application of chitosan has been associated with lower surface roughness than that observed with EDTA [27, 28].

According to the present results, the application of 17% EDTA did not lead to a significant increase in push-out bond strength (PBS) compared to the control group. These findings are consistent with those reported by Baena et al. [29], who also found that the mean PBS did not increase in any part of the root following pretreatment with 17% EDTA. This suggests that EDTA, as a chelating agent, does not enhance PBS. In contrast, Waz et al. [11] evaluated root dentin and reported an increase in PBS for the 17% EDTA group compared to the saline group; however, their application time was three minutes. In the present study, the application time for high molecular weight chitosan was limited to one minute to focus on the removal of extrafibrillar dentin without excessively reducing bond strength through the over-removal of calcium hydroxyapatite [14]. This approach aligns with relevant previous studies [5, 12, 13].

The current study found a significant difference in push-out bond strength (PBS) among different parts of the root, with the lowest PBS recorded in the apical third across all groups. It is important to highlight that the three regions of the root analyzed in this study are relevant to post placement. This observation may be attributed to the higher density and diameter of dentinal tubules in the coronal and middle thirds compared to the apical third, which facilitates better access for complete polymerization in these regions. Additionally, the difficulty in completely eliminating gutta-percha and sealer residues from narrow and deep post space, particularly in the apical third, likely contributes to the lower PBS observed there. Furthermore, the coronal and middle thirds provide greater volume and more contact with pretreatment materials, resulting in effective modified the smear layer, while less volume reaches the apical third [15, 30].

Scanning electron microscopy (SEM) and stereomicroscopic assessments of failure modes indicated that cohesive failure was most frequent in the 2.5% chitosan group, while mixed failure was predominant in other groups, with 50%-100% of the post diameter coated with resin cement. Another study demonstrated that increasing chitosan concentration led to a higher frequency of cohesive failures within coronal dentin when using self-etch adhesive systems, which supports the present findings and suggests an improvement in bond strength [12]. However, a different study indicated that mixed failure was dominant when chitosan was used alongside self-adhesive systems, although it noted a decrease in adhesive failure frequency within the chitosan group [13]. Similarly, in the current study, adhesive failure at the cement-dentin interface ranked second in frequency within the 17% EDTA group and occurred more frequently than in the chitosan groups.

It is important to draw clinical conclusions from these results with caution, as the design of this in vitro study has limitations and cannot fully replicate all conditions of the oral environment. Factors such as root canal geometry, post design, and resin cement type may also influence outcomes. It is also recommended that the effect of using acetic acid as a control group be investigated in future studies.

Conclusion

Despite study limitations, results suggest that pretreatment with 2.5% chitosan effectively modified the smear layer and improved PBS of fiber posts to root dentin when using self-adhesive resin cement throughout the root length. Conversely, neither 1% chitosan nor 17% EDTA significantly increased PBS in any root section.

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Authors' contributions

E.A, S.V, F.A designed the method of the study. F.A, E.A, M.SH and M.KH collected the data and contributed to analyzing and interpreted data. F.A, M.SH

and E.A contributed to writing manuscript, literature search and manuscript editing. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee of the Research Council of School of Dentistry, Tehran University of Medical Sciences (IR.TUMS.DENTISTRY.REC.1401.097). All samples were collected from extracted teeth for periodontal purposes, and all personal information was anonymity. The informed consent was not required for the participants whose teeth were used in the research.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Dental Research Center, Dentistry Research Institute, Department of Restorative Dentistry, School of Dentistry, Tehran University of Medical Sciences, North Kargar, Tehran 14174, Iran. [2]Department of Operative Dentistry, School of Dentistry, Tehran University of Medical Sciences, North Kargar, Tehran 14174, Iran. [3]Restorative Dentistry Specialist, Tehran, Iran. [4]Dental Research Center, Dentistry Research Institute, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran. [5]Department of Operative Dentistry, School of Dentistry, Zanjan University of Medical Sciences, Zanjan, Iran.

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