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Research Article

Effect of Biomimetic Remineralization of Post Space Root Dentin on Push-Out Bond Strength of Fiber Post Bonded with Self-Adhesive Resin Cement - An *Invitro* Study

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Abstract

Aim: To evaluate the effect of Biomimetic Remineralization (using Casein Phosphopeptide-Amorphous Calcium Phosphate) of post space dentin previously treated with Ethylenediaminetetraacetic acid (EDTA) on push out bond strength of fiber post system with self-adhesive resin cement. Furthermore, to evaluate the remineralization effect on bond strength at different levels of the root.

Methodology: Twenty one extracted human single rooted teeth with single canals were used. All teeth were decoronated at 12mm length from the apex, endodontically treated, and a 7mm post space was prepared. Samples were randomly divided into 3 groups (n = 7 in each) in which the post space is treated as the following: Group (A) irrigation with 17% EDTA followed by CPP-ACP remineralizing solution, Group (B) irrigation with 17% EDTA followed by 2.5% Sodium hypochlorite (NaOCl), and Group (C) irrigation with 17% EDTA alone. Fiber posts were cemented to all teeth using dual cured self-adhesive resin cement. Teeth were cut at 3 levels (coronal, middle, apical) of the post to obtain slices of 1mm thickness, which were subjected to push-out bond strength test using a universal testing machine.

Results: The results showed highest bond strength for the EDTA/CPP-ACP group, followed by EDTA alone group, followed by EDTA/NaOCl. There was a significant difference between EDTA/CPP-ACP and the other groups. However, there was no significant difference between EDTA/NaOCl and EDTA alone groups.

Conclusion: Post space pretreatment with EDTA followed by CPP-ACP remineralizing solution can improve bond strength of self-adhesive resin cements to root dentin. The highest improvement was at the apical third of the post followed by middle.

Keywords: Fiber Post; Self-Adhesive; Post Space; Remineralization; Bond Strength

Introduction

Root filled teeth typically face a number of issues, including insufficient dental tissue due to caries, fractures and root canal access, and non-vitality, which increases fracture risk. This points to the necessity for intra-radicular retentive methods that will permit the restoration to survive functional stresses while also restoring the desired aesthetics [1]. As a result, alternative designs and materials for post systems have been introduced to keep the coronal permanent restoration in place while decreasing the amount of stress imparted to the tooth structure [2].

As a replacement for metal posts, fiber reinforced posts with resin cements have been created. Their key benefit is that their mechanical and optical characteristics are nearly identical to those of dentine, resulting in a homogenous stress distribution and reduced probability of vertical root fracture [3].

At the fiber post-root dentin interface, resin cements have been employed to establish chemical and micromechanical bonding surfaces. Self-adhesive cements work on the attraction between monomeric acidic groups and hydroxyapatite micromechanically and chemically [4].

To improve resin-dentin bonding, various post space conditioning protocols have been examined. They primarily attempt to eliminate the smear layer that forms while post space preparation, leading to greater cement penetration into dentinal tubules [5].

Irrigation with ethylenediaminetetracetic acid (EDTA) followed by sodium hypochlorite (NaOCl) was found to effectively eliminate the smear layer. However, by leaching calcium ions from hydroxyapatite in dentin, employing these chemicals alternately affects the Calcium: Phosphate (Ca: P) ratio [6]. This shift in the proportion of organic to inorganic compartments changes the fracture resistance, texture, modulus of elsticity, and surface roughness of dentin. These alterations affect bonding characteristics of radicular dentin and increase susceptibility to fracture [7].

The process of re-establishing the minerals compartment of dental tissues as a repair procedure is called remineralization, which include re-formation of hydroxyapatite structure [8]. Unfortunately, remineralization of dentin is totally different than enamel since the amount of remaining ions content covering the demineralized collagen fibers is much less than the later. The sophisticated structure of dentin inhibit the traditional remineralization procedure that occur in enamel. Therefore, the development of biomimetic remineralization feature simulates the bio-mineralization mechanism of dentin [9].

A corner stone in the process of biomimetic remineralization is the non-collagenous protein. It act as a middle agent that can guide calcium and phosphate ions to their ideal position on the exposed collagen fibers. These proteins comprise no more than ten percent of the organic compartment. However, their presence is crucial to obtain a functioning remineralization, as they organize the nucleation of minerals in their optimum positions.

Casein phosphopeptide is milk derived protein that has a high binding affinity to calcium and phosphate ions, and at the same time highly attracted to collagen. Nowadays it is used as a topical remineralizing agent that can limit the tissue decalcification and preserve minerals of the tissue [9].

The aim of this *in vitro* investigation is to study the influence of biomimetic remineralization of radicular dentin wall of the post space, after conditioning with EDTA, on the bond strength of fiber post.

Materials and Methods

The protocol of this in-vitro study was reviewed and approved by the ethics committee (EC), Faculty of Dentistry, Cairo university, with respect to scientific content compliance with applicable rehearse and human subjects and regulation. Approval number was (10-10-20).

Twenty-one freshly extracted single rooted teeth were collected from dental clinic of National Diabetes and Endocrinology Institute in Cairo and Oral Surgery Department, Faculty of Dentistry Cairo University.

Specimens' preparation

Teeth were decoronated at a standardized distance of 12mm from the apex with low-speed sectioning disc. Endodontic treatment was performed for all root samples using crown down rotary instrumentation and single cone obturation technique. Post space preparation was done at length 7mm, leaving apical 5mm of root canal filling. The drill used for preparation was low-speed purple colored FibreKleer™ (PENTRON FIBREKLEER™ - USA) fiber post system drill dedicated for tapered fiber post size 1.375.

The roots specimens were assigned into 3 groups randomly according to the post space irrigation protocol as following

- **Intervention 1 group (EDTA/CPP-ACP):** Post space irrigation with 17% EDTA (3ml for 1 minute), followed by remineralizing CPP-ACP solution (10ml for 10 minutes), followed by fiber post cementation with self-adhesive resin cement.
- **Intervention 2 group (EDTA/NaOCl):** Post space irrigation with 17% EDTA (3ml for 1 minute) followed by 2.5% NaOCl (3ml for 1 minute), followed by fiber post cementation with self-adhesive resin cement.
- **Control group (EDTA alone):** Post space irrigation with 17% EDTA alone (3ml for 1 minute), followed by fiber post cementation with self-adhesive resin cement.

Remineralizing solution was prepared by diluting the GC Tooth Mousse (RECALDENT™ GC Corporation, Japan) to 10 folds with distilled water [10]. One ml of Tooth Mouse was added to 10 ml of distilled water and mixed thoroughly with spatula until complete homogeneous solution was obtained.

After treatment of post space in all groups, the post space areas were dried thoroughly. Cementation of pre-silanated tapered fiber post (FibreKleer™ fiber post, PENTRON™ – USA) size 1.375 was done with self-adhesive transparent dual cure resin cement

(BREEZE™ transparent self-adhesive resin cement, PENTRON™ - USA).

Push-out bond strength test

Each root was mounted in acrylic block and sectioned transversely, perpendicular to the long axis of the post, using a water-cooled precision saw into slices. Three (1-mm thick) slices were obtained at certain levels at 2, 4, and 6mm from the decoronated margin to represent coronal, middle and apical parts of the post respectively.

Push-out bond strength was measured by Universal Testing Machine (INSTRON® 2710-113-USA). A stainless-steel plunger with a diameter slightly smaller than the cemented post diameter (three sizes were used: 1, 0.8, 0.6 mm), applied an axial load to the post at a crosshead speed of 1 mm/min. The samples were aligned over a support jig in an apical to coronal direction to avoid any constriction interference (load direction was apico-coronal). The maximum failure load was recorded in Newton (N) and converted into mega-Pascal (MPa).

Scanning electron microscope

Representative samples were sent for scanning electron microscope and mode of failure analysis. The surface and structural morphology of the prepared samples were characterized by using High resolution Scanning Electron Microscopy (SEM). Analysis experiments were carried out on a FEI Quanta FEG 250 instrument. Scanning was done with magnification power of 200x, 500x, and 1000x respectively.

Statistical analysis

Numerical data was represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Homogeneity of variances was tested using Levene's test. Data showed parametric distribution and variance homogeneity so they were presented as mean and standard deviation values and were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and repeated measures ANOVA followed by Bonferroni post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$ for all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.

Results

Push-out bond strength of different groups (Intergroup comparison)

Mean and standard deviation values for push-out bond strength (MPa) for different groups were presented in table 1. There was a significant difference between different groups ($p < 0.001$). The highest value was found in CPP-ACP, followed by EDTA, while the lowest value was found in EDTA/NaOCl. Post hoc pairwise comparisons showed CPP-ACP to have a significantly higher value than other groups ($p < 0.001$).

Root section	Push-out bond strength (MPa) Mean ± SD			p-value
	CPP-ACP	EDTA-NaOCl	EDTA	
Coronal	14.28 ± 1.25^{A}	9.07 ± 1.29^{B}	10.54 ± 2.20^{B}	<0.001*
Middle	16.59 ± 3.44^{A}	8.69 ± 3.15^{B}	10.05 ± 2.64^{B}	<0.001*
Apical	18.02 ± 3.01^{A}	8.42 ± 2.64^{B}	9.64 ± 2.15^{B}	<0.001*

Table 1: Mean and standard deviation values for push-out bond strength (MPa) for different groups.

Means with different superscript letters within the same horizontal row are significantly different*; significant ($p \leq 0.05$).

Push-out bond strength at different root sections (Intragroup comparison)

Mean and standard deviation values for push-out bond strength (MPa) for different root sections were presented in table 2. There was no significant difference between values measured at different root sections in all groups. The highest value was measured at apical section, followed by middle section, while the lowest value was found at coronal section for all roots.

Group	Push-out bond strength (MPa) Mean ± SD			p-value
	Coronal	Middle	Apical	
CPP-ACP	14.28 ± 1.25^{A}	16.59 ± 3.44^{A}	18.02 ± 3.01^{A}	0.070ns
EDTA-NaOCl	9.07 ± 1.29^{A}	8.69 ± 3.15^{A}	8.42 ± 2.64^{A}	0.864ns
EDTA	10.54 ± 2.20^{A}	10.05 ± 2.64^{A}	9.64 ± 2.15^{A}	0.753ns

Table 2: Mean and standard deviation values for push-out bond strength (MPa) for different root sections.

Means with different superscript letters within the same horizontal row are significantly different*; significant ($p \leq 0.05$).

Mode of failure

SEM analysis revealed different types of failure mode. For the CPP-ACP group, there was both cohesive and adhesive failures with the most evident one was adhesive failure at the post-cement interface in all root levels. On the other hand, for the other two groups, cohesive and adhesive failures occurred at all levels, with the most prominent one was adhesive failure at the cement-dentin interface. Figure 1 shows SEM of all groups at different root levels with magnification power of 1000x.

Discussion

Treatment of endodontically treated teeth with compromised remaining tooth structure represents a challenge in restorative dentistry. Excessive loss of tissues, accompanied by alterations in the mechanical and structural properties of remaining tooth structure have crucial effect on treatment planning of durable future restoration [11]. In case of insufficient coronal tooth structure, fiber post system represents one of the ways that utilize the modern adhesive technology to enhance retention of the core, and at the same time provides stress distribution over the length of the root. Despite that, debonding of fiber post is still a noticeable problem that leads to functional failure [12].

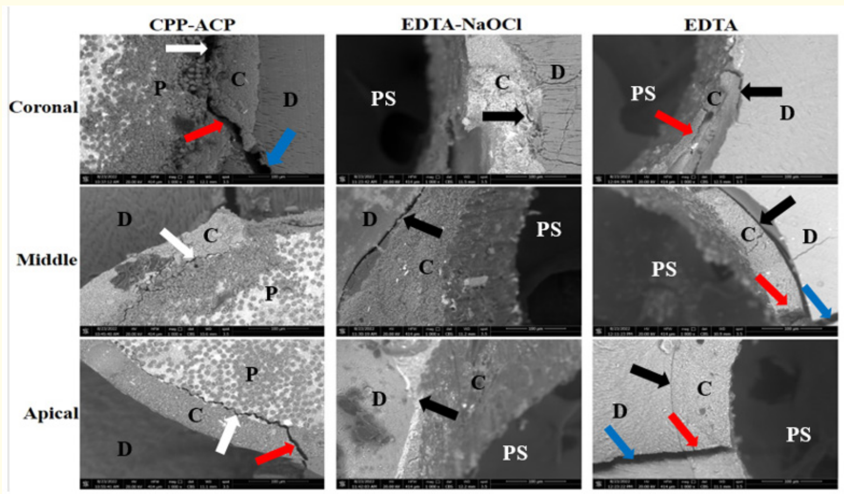

Figure 1: SEM 1000x of representative samples from all groups at three different levels of the root. P: fiber post, PS: post space (the post completely removed) C: resin cement, D: root dentin,

Arrows are areas of failures// White arrows: adhesive failure at post-cement interface, Black arrows: adhesive failure at cement-dentin interface, Red arrows: cohesive failure in the cement. For CPP-ACP group, adhesive failure at the post-cement interface was evident. For EDTA-NaOCl and EDTA groups, adhesive failure at the cement-dentin interface was evident. Blue arrows: dentinal cracks

Some studies investigated the adhesive failure of fiber post, with majority of them agreed that bonding to root dentin was much difficult than bonding to coronal dentin [13]. Subsequently, many protocols were suggested to enhance bonding, including post-space pretreatment and fiber post surface treatment. However, there is no consensus on the most effective protocol to be applied before cementation.

The results in the present study showed highest bond strength for the EDTA/CPP-ACP group, followed by EDTA alone group, followed by EDTA/NaOCl. There was a significant difference between EDTA/CPP-ACP and the other groups. However, there was no significant difference between EDTA/NaOCl and EDTA alone groups.

These results agree with a previous study by Scotti., *et al*. (2016) [13], which found that remineralization of dentin with synthetic hydroxyapatite increased bond strength of fiber post cemented with self-adhesive resin cement, significantly. The relative increase of Ca/P content of dentin following biomimetic remineralization would provide wider range of interaction between the functional monomer of the cement and hydroxyapatite crystals of dentin. The elevation in the mineral content was generalized affecting the whole post space surface area.

Furthermore, the results showed an increased bond strength by moving from coronal to apical levels of the post in the EDTA/CPP-ACP group. Whereas there were almost constant values of bond strength along the post length in the other two groups. These differences in bond strength can be referred to the differences in dentin ultrastructure, mineral content, and dentinal tubules distribution at different areas of the root.

It is well recognized that the density of dentinal tubules decreases by moving apically along the root. Lo Giudice., *et al*. (2015) [14] found that the tubular surface area drops sharply from around 15.47 ± 7.06 μm^2 at coronal third, to around 3.033 ± 2.43 μm^2 apically. Simultaneously, the diameter of each tubule decreases upon moving towards the apical third of the root with narrowing in the tubule lumen. As a result, the amount of peritubular dentin decreases apically, opposed by increase in intertubular dentin [15]. Apical dentin has the least tubule density with some areas showing absence of tubules. It has distinctive mineralized tissue exhibiting high degree of sclerosis [16].

Correlating root dentin ultrastructure to bond strength of resin cement, firstly, Pereira., *et al*. (2021) [17] found that the bond strength of self-adhesive resin cement to root dentin was the highest apically. The increased surface area of intertubular dentin exposed to the cement, would definitely extend the interaction between the monomer of the self-adhesive resin cement and hydroxyapatite. As a result, the bond strength of self-adhesive resin cement increases apically [18].

Secondly, it is suggested that intertubular dentin undergoes higher degrees of remineralization than peritubular dentin. Gu., *et al*. (2010) [19] evaluated remineralized peri- and inter-tubular dentin, and found that remineralized peritubular dentin contained very small nanocrystals of hydroxyapatite (5-10nm), whereas intertubular dentin consisted of larger mineral platelets (50nm). Toledano., *et al.* (2014) [20] stated that demineralized intertubular dentin provides a well preserved and highly organized collagen scaffold that allows deposition of continuous crystalline platelets, unlike peritubular dentin which provides a delicate scaffold that disrupts easily. Moreover, Yao., *et al.* (2022) [21] postulated that intertubular dentin has a critical role in building adhesion, therefore, a good quality of intertubular dentin must be maintained to obtain superior bonding.

Taking in consideration the various distribution of intertubular dentin in the root, the previous studies can indirectly justify the findings of this study, in which the maximum remineralization occurred in the apical third of the post where the amount of intertubular dentin is the highest. On the other hand, the presence of larger amount of peritubular dentin coronally could have a role in limiting remineralization capacity. These differences in remineralization were translated into differences in bond strength.

The results showed that the bond strength decreased when EDTA irrigation was followed by NaOCl, rather than used alone. The demineralizing effect of EDTA followed by the organic dissolution of NaOCl results in dentin erosion and reduction in the mineral content. This would affect the corner stone of the bonding mechanism of self-adhesive resin cements - the interaction between the acidic monomer and hydroxyapatite. In addition, the formation of oxygen residues subsequent to the oxidizing action of NaOCl could interfere with the setting reaction of the resin cement interrupting bond integrity [22]. The uncontrolled deproteinizing activity of NaOCl after demineralization might result in collapse of collagen network, affecting the hybrid layer and compromising the cement dentin interface. Moreover, the high alkalinity of NaOCl (pH around 11) would absolutely influence the acidic monomer of the cement, weakening its activity [23].

Regarding mode of failure analysis by SEM, it was evident that the improvement in bond strength discussed earlier after remineralization altered the interface where the adhesive failure occurred. Despite the cohesive failure that occurred in the resin cement itself, the adhesive failure that used to occur at cement-dentin interface due to relatively compromised bonding has changed to occur at post-cement interface after remineralization, reflecting the improvement of bonding to root dentin.

Conclusion

Within the limitations of this in-vitro study, it can be concluded that

- Remineralization of post space root dentin after removal of smear layer by chelating agents; can improve bonding strength of fiber posts cemented with self-adhesive dual cure resin cements.
- Amorphous calcium phosphate materials could be a good choice for such a remineralization process.
- After remineralization, apical third of the post showed higher bond strength than coronal and middle thirds. While in conventional post space pretreatment without remineralization, the coronal third showed higher bond strength than middle and apical.

Conflict of Interest

The authors declare no conflict of interest.

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