

Effect of Chelating Agents on Push-Out Bond Strength of NeoMTA Plus to Root Canal Dentin

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ABSTRACT

Objective: To evaluate and compare the effect of 17% Ethylenediaminetetraacetic Acid (EDTA), 9% Etidronic acid (HEDP), and 7% Maleic acid (MA) on the push-out bond strength of NeoMTA Plus sealer to the coronal, middle, and apical thirds of root canal dentin. **Material and Methods:** Forty single-rooted human maxillary central incisors were selected and decoronated to 12 mm long root fragments. Working length was established and root canals were then enlarged up to rotary Protaper F3. After each instrumentation, the root canal was irrigated with 2.5% NaOCl. For the final irrigation regimen, the specimens were divided into 4 groups (n=10) and treated with EDTA, HEDP, MA or Saline. Root canals were coated with NeoMTA Plus sealer, and obturation was done with single cone obturation technique. Subsequently, three horizontal sections were taken from the coronal, middle and apical thirds of each specimen, and POBS was measured using a universal testing machine. The type of bond failures was assessed under a stereomicroscope. Statistical analysis was done with One-way ANOVA with Tukey's Post hoc analysis. **Results:** MA and EDTA showed the highest POBS. There was no significant difference in bond strength between MA and EDTA ($p>0.05$). HEDP and Saline showed lower POBS. Among all the four groups, the coronal third showed the highest values, followed by middle and apical thirds. **Conclusion:** The type of chelating agent used during the root canal treatment significantly affects the bond strength of NeoMTA Plus to the root canal dentin.

Keywords: Dental Materials; Endodontics; Tensile Strength; Chelating Agents.

Introduction

Mineral trioxide aggregate (MTA), a tricalcium silicate-based cement, is most widely used as a root canal sealer due to its desirable properties such as biocompatibility, bioactivity, hydrophilicity, radiopacity, good sealing ability, and low solubility [1]. Upon placement, the calcium oxide in the material converts to calcium hydroxide in the presence of moisture leading to a high pH microenvironment that provides a favourable antibacterial effect [2,3]. However, shorter working and longer setting times, less flowability, tooth discoloration are its clinical limitations [4,5]. Newer materials are constantly being developed to address these shortcomings of the materials. Recently, NeoMTA Plus sealer (Avalon Biomed Inc. Bradenton, FL, USA) containing tantalite as radiopacifier instead of bismuth oxide was developed [2]. The use of tantalite reduces tooth discoloration. It also exhibits better handling properties and superior resistance to dislodgement forces. It is reported to have superior push-out bond strength (POBS) compared to Biodentine and ProRoot MTA [6]. Further, it is reported to have a higher calcium phosphate ratio, fast setting, higher crystallinity, and bioactivity compared to MTA Angelus [7].

The outcome of root canal therapy is determined by the quality of instrumentation, irrigation, disinfection and 3D filling. It is reported that after biomechanical preparation of the root canal utilizing manual or rotary instruments, a smear layer is created that covers the dentinal tubules [8]. A study by White et al. [9] concluded that smear layer can compromise the seal of obturating materials since they inhibit sealer adhesion with the dentinal tubules. Chelating agents assist in smear layer removal, improve the chemo-mechanical debridement, and expose an enormous number of dentinal tubules, bringing about increased contact area amongst the sealer and root canal dentin [10]. The efficacy of chelating agents on the smear layer removal and its subsequent effect on the bond strength of root canal sealer vary significantly depending on the type, constituents and concentration of the chelating agent [11,12]. Ever since its introduction by Nygaard-Ostby in 1957, Ethylenediaminetetraacetic Acid (EDTA) has been the most commonly used chelating agent in endodontics. EDTA forms a stable complex with calcium from the dentin, thereby decreasing pH and causing demineralisation. Nevertheless, it has numerous limitations, including cytotoxicity, reduced effectiveness for eliminating the smear layer from the apical third, and reduced dentin microhardness [13-15]. Over the years, various chelating agents have been introduced as an alternative to EDTA with varying success. A previous study [16] have reported that AH Plus and Epiphany exhibited the highest POBS values with 1% NaOCl and 17% EDTA compared to 17% EDTA alone.

Maleic acid (MA), an organic compound with two carboxylic acid groups, is reported to be more efficient in smear layer removal from the apical third of the radicular dentin than EDTA [13]. Similarly, irrigation with 7% MA resulted in higher shear bond strength of AH Plus sealer compared to 17% EDTA [17].

Etidronic acid (otherwise called 1-hydroxyethane 1,1-diphosphonic [HEDP]) is known to be less a aggressive chelator and is used along with sodium hypochlorite without interfering with its antimicrobial property [18,19]. Previous study has reported that AH Plus exhibited significantly higher POBS value with NaOCl & HEDP compared to NaOCl & EDTA [20].

Although the effect of different chelating agents on the bond strength of MTA-based sealer is widely reported, the effect of compositional modifications on the push-out bond strength has not been widely reported. In this regard, it is relevant to investigate the effect of different chelating agents on the bond strength of root canal sealer containing alternative radiopacifier. Hence, the present study aims to evaluate and compare the effect of 17% EDTA, 9% HEDP, and 7% MA on the POBS of NeoMTA plus sealer to coronal, middle and

apical thirds of root canal dentin. The null hypothesis for the current study was that there would be no effect of different chelating agents on POBS of NeoMTA Plus sealer.

Material and Methods

Sample Size Estimation

The sample size was estimated using OpenEpiInfo software, considering the mean difference of 2 and power of 90%, $\alpha=5\%$; the minimum sample size estimated for this study was 40, 10 in each group.

Specimen Preparation

The Institutional Ethical Committee (IEC: 757/2018) approval was obtained prior to the study. A total of 40 single-rooted human maxillary central incisors were selected, and soft tissue fragments along with hard deposits were eliminated with Gracey curette. The selected teeth were preserved in 0.2% sodium azide solution (Sigma-Aldrich, Darmstadt, Germany) until use. Then, the teeth were decoronated using a diamond disc, and standardised 12 mm long root fragments were obtained. Barbed broach (Diadent, Chungcheongbuk-do, Korea) was used to remove pulp tissue. The working length was established by inserting no.15 K file (Mani Inc., Tochigi, Japan) to root canal terminus and then deducting 1 mm. Root canal orifice was enlarged using GG drills (Dentsply Tulsa dental specialties, Baden, Switzerland) up to no.3 size followed by using rotary Protaper system up to a size of F3 (Dentsply, Bensheim, Germany). After each instrument change, the root canal was irrigated with 5 ml of 2.5% NaOCl for 1 minute, using a 27-gauge open-ended needle (Dispovan, Haryana, India). Following instrumentation, according to the final irrigation regimen, specimens were divided into four groups (n=10):

- Group 1 - 5 ml of 17% EDTA (Merck, Darmstadt, Germany) for one minute;
- Group 2 - 5 mL of 9% HEDP (Sigma-Aldrich, Darmstadt, Germany) for one minute;
- Group 3 - 5 mL of 7% MA (KMC Pharmacy, Manipal, India) for one minute;
- Group 4 - 5 mL of 0.9% saline (control) (KMC Pharmacy, Manipal, India) for one minute.

After the irrigation, a final rinse with 10 ml of distilled water (KMC Pharmacy, Manipal, India) was done for each of the groups. Subsequently, the root canals were dried out utilizing paper points (Diadent, Burnaby, Canada).

Following the manufacturer's instructions, NeoMTA plus sealer was mixed and the root canals were coated by using lentulo spiral (Dentsply Malliefer, Ballaigues, Switzerland). Next, F3 Protaper gutta-percha (Dentsply, Bensheim, Germany) was lightly coated with sealer and inserted. The excess cone was removed using a heated instrument and vertically condensed with a plugger. The teeth were stored in PBS at 37 °C and 100% humidity for 24 hours to allow the root canal sealer setting. The teeth were sectioned using a hard tissue microtome to obtain three horizontal sections of 2 mm thickness at the coronal, middle, and apical thirds from each root segment. A total of 30 slices per group were obtained, and the thickness of each slice was recorded using a digital calliper.

Push-out Bond Strength Evaluation

The tooth slice was placed on a custom-designed metallic jig fixed onto the lower half of the universal testing machine. The jig consisted of a central open-ended perforation at the center with rotating screws on either side to position the slice and to hold it in place during the push-out bond strength testing. Customized

plungers made of hardened steel with different diameters (0.9 mm, 0.7 mm, and 0.5 mm for the push-out bond strength of coronal, middle, and apical slices, respectively) were fixed onto the movable upper half of the universal testing machine. Push-out bond strength was measured by loading the obturating material under a compressive mode towards apico-coronal direction at a 1 mm/min crosshead speed until the core material was completely dislodged. The POBS value in MegaPascals (MPa) was calculated by dividing the maximum load observed during the testing in Newtons (N) by the adhesion area of root canal filling (mm^2).

$\text{POBS (MPa)} = \text{Maximum load (N)} / \text{Adhesion area of the root canal filling (mm}^2\text{)} / \text{Adhesion area of root canal filling} = 2\pi rh$; Where “r” represents the radius and “h” represents the slice thickness

Analysis of Failure Modes

The failure mode of debonded specimens was evaluated by means of a stereomicroscope (Olympus SZ61, Olympus Optical Co., Tokyo, Japan). The debonded tooth slices were observed under a stereomicroscope at a magnification of 40x. The mode of failure was categorised as an adhesive (when the debonding occurred at the interface between the sealer and the root canal), cohesive (debonding occurred within the core material, leaving a layer of sealer on the dentin) and mixed (a combination of adhesive and cohesive features).

Statistical Analysis

Data were analyzed using the statistical package SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and level of significance was set at $p < 0.05$. One-way ANOVA with Tukey's Post hoc analysis to find out the variation between the groups at a confidence interval of 95%.

Results

Table 1 and Figure 1 depict the mean and standard deviations of the push-out bond strength values at coronal, middle and apical thirds of the root canal dentin.

7% MA and 17% EDTA showed the highest POBS. No significant difference in bond strength was observed between 7% MA and 17% EDTA (p -value at coronal, middle, apical thirds being 0.891, 0.9925, 0.9995). On the other hand, 9% HEDP and 0.9% Saline showed lower POBS than 7% MA and 17% EDTA.

In Group 1 (EDTA), coronal third showed a higher POBS value followed by middle and apical thirds ($p=0.001$). Group 2 (HEDP), group 3 (MA) and group 4 (saline) showed similar results as in group 1 (EDTA) (p -values were 0.005, 0.0001, 0.001, respectively). A significant difference was seen in all the four groups at coronal, middle and apical thirds.

The mean values at coronal third were 6.554 ± 1.32 , 5.074 ± 1.12 , 6.559 ± 1.50 , and 4.764 ± 0.63 , respectively. Post hoc analysis revealed a significant difference in the mean POBS among group EDTA & HEDP, Group EDTA & saline, Group HEDP & MA, and Group MA & saline.

The mean values at the middle third were 4.949 ± 1.68 , 3.648 ± 0.88 , 4.864 ± 1.21 , and 3.034 ± 0.78 , respectively. Post hoc analysis revealed a significant difference in the mean POBS among Group EDTA & HEDP, Group EDTA & saline, Group HEDP & MA, and Group MA & saline.

The mean values at apical third were 3.639 ± 0.59 , 2.963 ± 1.15 , 3.677 ± 0.83 , and 2.143 ± 1.42 , respectively. Post hoc analysis revealed a significant difference in the mean POBS among Group EDTA & saline and Group MA & saline.

Table 1. Mean and standard deviations of the POBS values of NeoMTA Plus at coronal, middle, and apical thirds of the root canal dentin.

Root Canal	Group (I)	Group (J)	Mean Difference (I-J)	Standard Error	p-value	95% CI	
						Lower Bound	Upper Bound
Coronal	Group 1	Group 2	-1.480	0.002	0.037*	-1.0758	-2.9238
		Group 3	0.0050	0.001	0.891	-0.9618	1.0378
		Group 4	-1.7900	0.09	0.008*	-0.7008	-2.2988
	Group 2	Group 3	1.4850	0.07	0.036*	0.8858	2.1138
		Group 4	-0.3100	0.08	0.934	-0.6248	1.3748
		Group 3	-1.7950	0.004	0.008*	-0.7388	-2.2608
Middle	Group 1	Group 2	-1.3010	0.014	0.0008*	-2.1288	-0.4732
		Group 3	-0.0850	0.023	0.9925	-0.9128	0.7428
		Group 4	-1.9150	0.013	0.0001*	-2.7428	-1.0872
	Group 2	Group 3	1.2160	0.24	0.0019*	0.3882	2.0438
		Group 4	-0.6140	0.12	0.2077	-1.4418	0.2138
		Group 3	-1.8300	0.003	0.0000*	-2.6578	-1.0022
Apical	Group 1	Group 2	-0.6760	0.002	0.2200	-1.6025	0.2505
		Group 3	0.0380	0.15	0.9995	-0.8885	0.9645
		Group 4	-1.4960	0.019	0.0006*	-2.4225	-0.5695
	Group 2	Group 3	0.7140	0.180	0.1805	-0.2125	1.6405
		Group 4	-0.8200	0.26	0.0985	-1.7465	0.1065
		Group 3	-1.5340	0.14	0.0004*	-2.4605	-0.6075

*Indicates a significant difference between the groups.

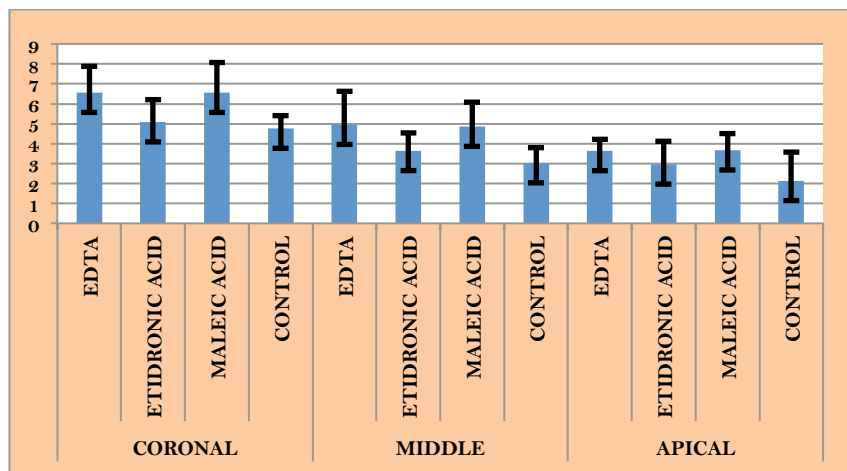


Figure 1. Mean and standard deviations of the POBS values of NeoMTA Plus at coronal, middle, and apical thirds of the root canal dentin.

Analysis of bond failure showed the following results: 60% cohesive, 31.6% mixed, and 8.3% adhesive failure. The count and percentage of bond failure patterns in each group are presented in Table 2. Images obtained by stereomicroscope at 40x magnification for analysis of bond failure are represented in Figure 2.

Table 2. Count and percentage of type of bond failure in each group.

Groups	Adhesive	Cohesive	Mixed
G1	3	18	9
G2	2	21	7
G3	2	16	12
G4	3	17	10
Total	10 (8.3%)	72 (60%)	38 (31.6%)



Figure 2. Stereomicroscopic images of the types of bond failure. (A) Cohesive failure, (B) Mixed failure, (C) Adhesive failure.

Discussion

Root canal irrigation with various chelating agents can alter the structural and chemical composition of radicular dentin and variations in permeability and solubility characteristics [21]. This influences the bonding of root canal filling materials to radicular dentin. Therefore, close contact between the substrate and the adhesive material, either by micromechanical or chemical bonding, is mandatory for optimum adhesion [21,22].

As the chelating agents work on the calcium ions present in the dentin, the ratio of its organic and inorganic components can be substantially modified. This induces changes in the properties of dentin, such as surface roughness, wettability and microhardness. Such induced changes in the dentin may affect the ability of root canal sealers to interact with the dentin and thus its bond strength to dentin [23,24]. It was reported that an increase in the surface roughness of radicular dentin increases the adhesion, which can be attributed to an increase in surface energy and the surface area of the bonding [25]. In addition, type of irrigant, its concentration, and time application may significantly alter the wettability of the dentin [26].

In the present study, POBS was chosen to assess the material's bond strength, as this test is more accurate, reproducible and successful in reflecting the clinical status of the failure of the bond strength. This is less susceptible to small differences among specimens, discrepancies in the distribution of stress during load application, and even with low bond strength values, materials can be evaluated [27].

In the current study, 7% MA and 17% EDTA showed higher POBS values followed by 9% HEDP; thus, the null hypothesis has been rejected. The higher POBS values of 7% MA can be attributed to its better chelating action highly acidic nature, which thereby improves the demineralisation effect [28]. The chelating action of 7% MA has increased the surface roughness and decreased surface tension, thus improving the sealer adhesion to the root canal dentin [24,29]. Among other chelating agents used in the study, 17% EDTA showed superior POBS. The presence of an excessive amount of hydroxyl ion due to alkaline pH of EDTA leads to low dissociation of smear layer. This reduces the chelation of EDTA with calcium ions and slow dissolution of inorganic and organic components, resulting in increased surface roughness and thereby good wettability leading to better POBS value than HEDP [30,31]. The observed POBS of HEDP was lower than the other chelating agents and higher compared to saline (control). The lower value of HEDP can be explained due to its weak chelating action [32]. It could also be attributed to the ability of HEDP to enhance the hydration of NeomTA Plus by the formation of a highly crystalline surface and a high release of calcium from the specimens [33]. The lower POBS obtained in the specimens treated with 2.5% NaOCl and a final rinse with 0.9% Saline could be due to the dissolving effect of NaOCl on the organic components, this results in lower smear layer removal, thus minimising the bonding of NeomTA Plus to the root canal walls [34].





In the present study, POBS of NeoMTA Plus was significantly increased on using chelating agents. To a certain extent, the interaction between sealers and chelating agents can be anticipated as they are chemicals [35]. The physical properties of endodontic cements have been reported to alter after root canal irrigation [36]. The elimination of the smear layer often causes closer contact between cement and root canal dentin, which is needed for optimum adhesion, thus allowing chemical bonding or micromechanical interlocking [37]. The mean POBS values of NeoMTA Plus, mixed with gel or water, were close to those of MTA Plus, and both had greater bond strength values than ProRoot MTA and Biodentine when the root canals were irrigated with 17% EDTA; this have been reported due to its finer particle size [6]. Therefore, in the current study, the increase in the POBS values of NeoMTA Plus with different chelating agents can be attributed to its finer particle size, which can increase cement penetration into the dentinal tubules resulting in improved bond strength.

Another notable finding with respect to POBS was that the value descended from coronal to apical third. This may be because apical dentine has fewer patent tubules than coronal dentine [18,38]. The most common pattern of failure found in our study was cohesive, demonstrating the ability of the materials to bond to the dentine walls. This observation is in line with prior studies [39,40]. Little is known about the perspective of POBS of other tricalcium silicate-based sealers with NeoMTA Plus. Therefore further studies will be needed to compare POBS of NeoMTA Plus sealer with other tricalcium silicate-based sealers. The limitation of the current study is that there was no comparison with AH Plus, which is considered the gold standard root canal sealer.

Conclusion

The results of the present study indicate that using 17% EDTA, 7% MA, 9% HEDP as a chelating agent increases the POBS of NeoMTA Plus. 7% MA and 17% EDTA showed the highest POBS. No significant difference in bond strength was observed between 7% MA and 17% EDTA. 9% HEDP and 0.9% Saline showed lower POBS than 7% MA and 17% EDTA.

Authors' Contributions

PKA		https://orcid.org/0000-0002-3684-4024	Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft and Writing - Review and Editing.
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NVB		https://orcid.org/0000-0002-2208-9443	Conceptualization, Data Curation, Writing - Review and Editing.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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