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Original Article

Impact of 5% pentetic acid on the pushout bond strength of AH Plus sealer to dentin: An *in vitro* study

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Abstract

Introduction: The aim of this study was to compare the effect of pentetic acid (diethylenetriaminepentaacetic acid) with ethylenediaminetetraacetic acid (EDTA), as final chelating agents on the pushout bond strength of AH Plus sealer.

Materials and Methods: Single-rooted mandibular premolars (n = 80) were collected. The canals were instrumented and were randomly divided into four groups (n = 20) according to the final irrigation protocol followed, where Group I: distilled water, Group II: 3% NaOCl and distilled water final rinse; Group III: 3% NaOCl and 17% EDTA as final irrigant, and Group IV: 3% NaOCl and 5% pentetic acid as final irrigant. Canals were then dried and filled with AH Plus sealer. Roots were sectioned transversely at 4 mm from apex, with 1 mm thickness, and tested for pushout bond strength. Results were analyzed using analysis of variance and *post hoc* Tukey's test.

Results: Pushout bond strength of AH Plus was found to be best with 5% pentetic acid final rinse (0.841 ± 0.15 MPa), followed by 17% EDTA (0.83 ± 0.27 MPa), 3% NaOCl (0.68 ± 0.16 MPa), and distilled water (0.52 ± 0.04). However, there was no statistical difference between 5% pentetic acid and 17% EDTA when used as a final rinse ($P < 0.05$). The failure modes in Groups III and IV were mixed, whereas Groups I and II showed adhesive failure.

Conclusion: Within the limits of the study, 5% pentetic acid was as effective as 17% EDTA and hence can be considered as a potential chelating agent in endodontic therapy.

Keywords: AH Plus sealer, chelating agents, ethylenediaminetetraacetic acid, pentetic acid, sodium hypochlorite

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INTRODUCTION

Root canal instrumentation generates a smear layer that plasters the inner walls of the prepared root canal. The smear layer contains an amorphous blend of organic and inorganic debris with microorganisms. Removal of this smear layer is imperative for the diffusion of irrigating solutions and intracanal medicaments into the dentin tubules that harbor bacteria. Moreover, its removal

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improves adhesion of the root canal sealer cement to the dentin walls, by effective bonding of resin-based sealers to dentin, and further helps to seal the root canal core-filling material and dentin.[1-5]

Sodium hypochlorite (NaOCl) is the most widely used irrigant due to its organic tissue dissolving as well as antimicrobial properties, and it aids in removing the organic part of the smear layer. Chelating agents are used to remove the inorganic part of the smear layer that bond with the heavy metal ions and improve the bonding of sealer to dentin. 17% ethylenediaminetetraacetic acid (EDTA) is currently the most commonly used chelating agent, used after biomechanical preparation to remove the smear layer. However, when used in combination with NaOCl, it has shown to suppress the antimicrobial activity of NaOCl.[6] Etidronic acid, which is a weak chelating agent, has been tested and used along with NaOCl so that the antimicrobial properties of NaOCl are unaffected. Pentetic acid also known as diethylenetriaminepentaacetic acid (DTPA) is a chelating agent whose chemical structure is an expanded version of EDTA. Like EDTA, pentetic acid is also an aminopolycarboxylic chelating agent, which forms stable and water-soluble complexes with divalent and trivalent metal ions. Pentetic acid has been granted Food and Drug Administration (FDA) approval and is used in the field of biomedicine as an antidote, after exposure to the transuranic elements such as plutonium, americium, and curium.[7] Calcium and zinc salts of pentetic acid are readily excreted in the urine as they form a chelate.[7] Pentetic acid has also been found to be more biodegradable than EDTA.[8] In an interesting study by Qiu *et al*., it was found that pentetic acid has more antimicrobial activity against Gram-negative bacteria as compared to EDTA.[9] In a study by Gi *et al*., pentetic acid suppressed the elastase-mediated virulence of *Pseudomonas aeruginosa* more efficiently than EDTA.[10] Role of pentetic acid as a chelating agent with antimicrobial properties has not yet been explored in endodontics. Whether pentetic acid has similar efficacy as EDTA in smear layer removal and subsequently facilitates the bonding of resin sealer to dentin needs to be determined.

Pushout bond strength tests have been used in the past to determine the shear bond strength of restorative materials, intraradicular posts, and root-filling materials applied to dentin.[11] Epoxy resin-based sealers bond to dentin, which provides resistance to microleakage and provides some reinforcement of roots against fracture.[12-17] Eradication of the smear layer using chelating agents such as 17% EDTA, 7% maleic acid, and 18% etidronic acid has shown to enhance the bonding of AH Plus sealer.[6,18] Based on the above observations, one needs to study if pentetic acid can also be used as a chelating agent in endodontics. Furthermore, whether it facilitates adequate bonding of sealer to dentin needs to be investigated. Hence, the specific objective of the study was to evaluate the effect of pentetic acid as a chelating agent in endodontics in comparison to EDTA on the pushout bond strength of epoxy-based resin sealer to the root dentin. Additional objective of this study was to evaluate the failure modes on the debonded surfaces.

MATERIALS AND METHODS

The Institutional Ethics Committee of Sinhgad Dental College and Hospital, Pune, approved the present study under the reference number: SDCH/IEC/2017-18/OUT/69. The experimental chelating agent, pentetic acid, was freshly prepared at Sinhgad Pharmacy College Laboratory, Pune. All the irrigants were tested for their pH before being used, using a calibrated digital pH meter (Equip-Tronics, model no. EQ 610, Mumbai, India). The pH of pentetic acid was adjusted to 7.4 by the addition of sodium hydroxide (NaOH) and that of commercial EDTA (DeSmear*,* Ahmedabad, Gujarat, India) was found to be 8.

Sample preparation

Eighty freshly extracted intact single-rooted human mandibular premolars satisfying the inclusion criteria were selected and cleaned off its hard deposits with a scaler. The inclusion and exclusion criteria were as follows:

Inclusion criteria

- Freshly extracted mandibular premolar teeth for periodontal or orthodontic purposes (age group: 25–50 years)
- Mandibular premolar with a single root and single root canal.

Exclusion criteria

- Single-rooted teeth with root caries
- Roots with severe curvature (more than ±10° curvature)
- Multirooted teeth
- Single-rooted teeth with calcified canals
- Single-rooted teeth with multiple canals
- Single-rooted teeth with cracks and defects.

The samples were radiographed mesiodistally and labiolingually at different angulations (Kodak Carestream RVG 5200) to ensure the presence of a single root canal system before selection. The samples were decoronated at the cementoenamel junction using a diamond disc under water cooling and their length was standardized to 13 mm. Each tooth was preflared cervically with a Gates Glidden

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drill #3 (Mani Inc., Utsunomiya, Japan). Using a size 10-K file (Mani Inc., Utsunomiya, Japan), the patency of the canal was verified and total length of the canal was determined by the file just being visible at the apex. The working length was established to the root canal terminus and 0.5 mm was subtracted from this measurement. The root canal preparation was done using ProTaper Universal rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) sequentially up to size F3. A 5-ml disposable plastic syringe (Ultradent Products Inc., South Jordan, UT, USA) with a 30-gauge side-vented irrigating needle was used for irrigation during instrumentation. It was placed passively into the canal up to 2 mm from the apical foramen without binding. Each tooth sample was coded from 1 to 80, and out of them, 20 teeth were randomly allotted to Group I using computer randomization. In this group, only distilled water was used for irrigation. For the remaining 60 samples, 12 ml of 3% NaOCl (2 ml after changing each file) was used during instrumentation over a period of 10 min. The 60 tooth samples were then assigned to the following three groups (*n* = 20) (Groups II, III, and IV) using computer randomization to test the effect of different chelating irrigation regimen:

- Group I: Distilled water→Distilled water
- Group II: 3% NaOCl→Distilled water
- Group III: 3% NaOCl →17% EDTA
- Group IV: 3% NaOCl →5% DTPA.

The tooth samples were subjected to a final rinse with the respective chelating agent of the assigned group for 1 min, and subsequently, 5 ml of distilled water was used for 2 min to flush the canals. The root canals were then dried using ProTaper paper points (Dentsply-Maillefer, Rio de Janeiro, RJ, Brazil) and filled with epoxy resin sealer (AH Plus; Dentsply DeTrey, Konstanz, Germany) using a lentulospiral. The samples were radiographed at three different angulations to verify voids and accurate filling of the sealer. To ensure complete setting of the sealer, the samples were then placed at 37°C in 100% humidity for 7 days in a humidifier.

Evaluation of pushout bond strength

Each root sample was horizontally sectioned using a slow-speed water-cooled diamond saw approximately 4 mm from the apex, and a 2-mm thickness disc was obtained. The discs were coded again with the same code number as assigned previously, and the coronal and apical diameter was measured using a stereomicroscope. The coronal surface was marked with indelible ink for easy reference. A pushout force was applied onto the cross-section of each sample in an apico-coronal direction using the universal testing machine (UNITEST-10, ACME Engineers, India.). A 0.5-mm-diameter stainless steel cylindrical plunger with a crosshead speed of 0.5 mm/min was used until bond failure occurred (accuracy of the machine: ±1%). Pushout bond strength in Megapascal (MPa) was calculated using the values calculated during debonding (maximum failure load) according to the following formula:[19]

$$\text{Push out bond strength }(\text{MPa}) = \frac{\text{Maximum Push out Load }(\text{N})}{\text{Area of bonded interface }(\text{sq. / mm})}$$

where area was calculated using the formula: $\pi\ (R + r)\ ([h^2 + (R-r)]^2)^{0.5}$

Where π =3.14, *R* is the coronal radius, *r* is the apical radius, and h is the slice thickness.

Analysis of failure modes

The failure modes of debonded specimens were analyzed using a stereomicroscope (Olympus SZ61, Olympus Optical Co., Tokyo, Japan) at ×40 magnification, and each sample was categorized as follows:

1. Adhesive failure between sealer and dentin
2. Cohesive failure within sealer
3. Mixed failure.

Statistical analysis

The data were tabulated and analyzed using the Statistical Package for the Social Sciences V.11.5 software (SPSS, IBM, New York, USA) for Windows 2007 (Microsoft, New Mexico, USA). One-way analysis of variance (ANOVA) and *post hoc* Tukey's honestly significant difference tests were used to determine whether significant differences in pushout bond strength values existed between groups. The level of significance was set at a $P < 0.05$.

RESULTS

All specimens showed measurable adhesive properties. The mean and standard deviation values of pushout bond strength (MPa) for the groups are presented in Table 1. A statistical ranking for the bond strength values was obtained as follows ($P < 0.05$): Group IV > Group III > Group II > Group I. Group IV showed the highest pushout bond strength values (0.841 ± 0.15 MPa). Among all the groups, the lowest values were seen for Group I, i.e., the control group (0.52 ± 0.04 MPa). *Post hoc* Tukey's intergroup test showed that there was a statistically significant difference between Group I and all the other three groups. Group II also showed a statistically significant difference with the remaining three groups. However, the

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difference in pushout bond strength values of Groups III and IV was statistically insignificant.

The failure modes of the test samples are listed in Table 2. No premature adhesive failure took place. Posttest failure analysis showed that mixed failure mode was predominant among Groups III and IV, whereas adhesive failure mode was predominantly seen in the Group I and Group II.

DISCUSSION

Adhesion of sealers to intraradicular dentin is essential to maintain a good seal at the sealer–dentin interface to entomb bacteria, prevent bacterial ingress as well as to overcome mechanical stress caused by the flexure of the roots or consequent restorative procedures on the tooth. Bond strength of root canal sealers to dentin is compared using pushout bond strength test.[20-24] Irrigants aid in removing the smear layer, facilitating their diffusion through the dentinal tubules, and increasing the sealing ability of the root canal-filling material.[4,6,23,25] The present study introduced pentetic acid as an alternative to EDTA for use as a final chelating irrigant in endodontics. The study comparatively evaluated the pushout bond strength of epoxy resin-based sealer to dentin using pentetic acid and EDTA as chelating agents along with controls. The null hypothesis was rejected. The results of this study exhibited that the experimental chelating agent, pentetic acid, had equal efficacy as EDTA in terms of pushout bond strength.

In the current study, a lentulospiral was used to fill in the sealer. Radiographic evaluation was done to ensure that there were no voids in the sealer fill. The treatment methods and variables tested were chosen to reflect standard and commonly used protocols in studies for pushout bond strength tests. Pentetic acid was the variable tested to understand what impact it has on pushout bond strength of AH Plus sealer as compared to standard protocols of EDTA. EDTA has four carboxylic groups and two amine groups, whereas pentetic acid has five carboxylic groups and three amine groups to bond with the metal ions to form an octadentate ligand.[26] Thus, pentetic acid has more complexing sites than EDTA for its chelating action. Moreover, the formation constants of pentetic acid for its complexes are 100 times greater than those of EDTA.[26] However, the pushout bond strength values between pentetic acid and EDTA obtained in this study were almost similar, and they were not statistically significant. EDTA had a pH of 8 as compared to pentetic acid, which had a pH of 7.4. The concentration of the freshly prepared pentetic acid was 5%, whereas that of EDTA was 17%. Slightly higher pH of EDTA may have allowed for more comparing sites in the chelate molecule to theoretically improve the chelation. In addition, 5% pentetic acid solution in this study was freshly prepared, whereas a commercially prepared 17% EDTA solution was used in this study and tested *in vitro* on radicular dentin for its effect on the pushout bond strength of epoxy resin sealer. This could be another reason for better pushout bond strength values obtained with pentetic acid. Hence, the results of the current study may have shown slightly better result with 5% pentetic acid as compared to 17% EDTA although the difference between these groups was statistically insignificant. It remains to be investigated whether an increase in concentration of pentetic acid can provide more chelating activity of pentetic acid than EDTA and consequently better pushout bond strength values.

Table 1: Pushout bond strength (mean±standard deviation) in MPa

Groups	Bond strength in MPa (mean±SD)
Group I[a]*: Distilled water→Distilled water	0.52±0.04
Group II[a]*: 3% NaOCl→Distilled water	0.68±0.16
Group III: 3% NaOCl→ 17% EDTA	0.83±0.27
Group IV: 3% NaOCl→5% DTPA	0.84±0.15

*Statistically significant difference with other three groups. NaOCl: Sodium hypochlorite, EDTA: Ethylenediaminetetraacetic acid, DTPA: Diethylenetriaminepentaacetic acid, SD: Standard deviation, [a]: ($P<0.05$)

Table 2: Failure patterns of the experimental groups

Groups	Failure pattern		
	Adhesive	Cohesive	Mixed
Group I: Distilled water→Distilled water	15	3	2
Group II: 3% NaOCl→Distilled water	10	7	3
Group III: 3% NaOCl→ 17% EDTA	4	2	14
Group IV: 3% NaOCl→5% DTPA	5	4	11

NaOCl: Sodium hypochlorite, EDTA: Ethylenediaminetetraacetic acid, DTPA: Diethylenetriaminepentaacetic acid

Epoxy resin-based sealers have consistently shown greater bond strength values when compared to methacrylate-based sealers.[13,27] Besides this, epoxy resin sealers have lower volume shrinkage than methacrylate-based sealers.[28] As a result of this, epoxy resin sealers perform well as root canal sealers. Past studies by Jain *et al.* and Nunes *et al.* have revealed that AH Plus sealer has the highest pushout bond strength.[29,30] This is due to the covalent bonds that are formed due to opening up of the epoxide rings in AH Plus bringing about homogeneous polymerization.[31] This process is slow which allows for the adequate shrinkage stress relaxation.[32] Being most efficacious and commonly used, AH Plus sealer cement was used to evaluate the pushout bond strength of EDTA and pentetic acid in this study.

Clinically, sealer cements are not used to fill the root canal as it sets to a hard consistency and makes retreatment difficult.

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However, in this study, the sealer was used alone to fill the root canal without gutta-percha core with an objective to permit measurement of the dentin–sealer interface bond strength alone, so other confounding factors such as gutta-percha do not interfere with the results. Jainaen *et al.* evaluated the mechanical properties of the interfaces and suggested that thin slice pushout test could prove to be an important experimental tool.[33] Hence, dentin discs were made keeping a uniform thickness of 2 mm so that uniform stress could be applied. The pushout bond strength values obtained using EDTA in our study had values comparable to those by Neelakantan *et al.*, Nunes *et al.*, and Vilanova *et al.* who have also used sealer alone to fill the root canal.[18,30,34]

AH Plus bonds covalently with partially demineralized collagen in dentin necessary through the open epoxide rings on surface. NaOCl can negatively affect the adhesion of sealers in two possible ways.[35,36] The first reason could be due to the removal of organic part of the dentin and the second could be due to the oxygen formed by the dissociation into oxygen and sodium chloride. This oxygen interferes and inhibits the interfacial polymerization reaction of the methacrylate. In a study by De Assis *et al.*, it was concluded that NaOCl deproteinizes dentinal substrate resulting in a hydrophilic surface which interferes with the hydrophobic nature of AH Plus.[35] In another study done by Eldeniz *et al.*, it was seen that elimination of the smear layer showed superior bonding ability of the AH Plus sealer after chelation.[36] Hence, a suitable chelating agent is required after the use of NaOCl. Group I (distilled water) showed significantly lowest bond strength values because the smear layer was left intact.

In this study, the final rinse was administered for 1 min. *In vitro* study outcomes have shown that the use of EDTA for more than 1-min results in greater demineralization and erosion of the dentin.[31,37,38] It is recommended that prolonged exposure to strong chelators such as EDTA may weaken root dentin, and hence, all the tooth samples in this study were subjected to a final rinse of the chelating agent for 1 min, followed by a rinse of distilled water to nullify its prolonged effect over the dentinal walls. It has been proved that different irrigant activation protocols also enhance the action of chelating agents.[4,6,39] However, the objective of our investigation was only to assess the chemical action of chelating agent as final rinse. Hence, none of the irrigant activation protocols were used in this study.

The present study results show that Group IV with pentetic acid showed the highest pushout bond strength (0.84 ± 0.15 MPa) as compared to the other groups. Group III and Group IV showed comparable values. This could be attributed toward pentetic acid having a similar structural composition as that of EDTA. In order to aid in comparison of these agents, the pH of the experimental irrigant, pentetic acid, was adjusted at 7.4, to rule out higher demineralization due to its low pH. The pH of blood and body fluids is 7.4. Furthermore, the concentration used in this preliminary investigation is just 5% pentetic acid. The pH of EDTA used in this study was 8.2 which was more alkaline than 7.4. Treatment with irrigants may cause variations in the chemical and structural composition of root canal dentin, consequently affecting its permeability and solubility characteristics.[32] This also has an impact over the adhesion of material to the dentinal surface.[40] Hence, all the four different irrigation regimens influenced the pushout bond strength values obtained in this study.

Stereomicroscope was used in this study to examine the root discs to analyze the type of failure. Stereomicroscope is a commonly used noninvasive method used to observe mode of failures with an advantage of examining the mode of failure throughout the sample.[41,42] Scanning electron microscopy (SEM) could also have been used to observe morphological changes in the interfaces between dentin and sealer for this study. However, with SEM, only representative parts of the tooth disc sample can be examined and evaluated. Evaluation of failure pattern under stereomicroscope showed that the control groups (Group I and II) revealed adhesive type of bond failure comparable to the results obtained by Baldissera *et al.* indicating reduced bond strength on irrigation with saline.[43] Group III (EDTA) and Group IV (pentetic acid) revealed predominance of mixed failure, thus exhibiting adhesive bonding of the sealer to the dentin. This study had some limitations. Pentetic acid's potential needs to be tested for its efficacy in smear layer removal, dentin erosion, dentin microhardness, antimicrobial activity, and its interaction with NaOCl. Furthermore, other studies could be undertaken with different concentrations of pentetic acid to obtain optimal efficacy with minimal damage to dentin. Nevertheless, pentetic acid may have good potential for its use in endodontics, with its various applications in the field of medicine. Pentetic acid is a chelating agent with a similar structure to EDTA, and hence, it was used in this study with a potential application in endodontic therapy. 5% pentetic acid gave comparable pushout bond strength with AH Plus sealer as 17% EDTA. This agent being FDA approved, it can be safely used as an additional or alternative chelating agent in endodontics. Further studies should be carried out using pentetic acid at various concentrations as a chelating agent to explore its biocompatibility with NaOCl, its smear layer removal efficacy, and pushout

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bond strengths of sealers in comparison to EDTA so as to establish it as an alternative to EDTA in endodontics.

CONCLUSION

The present study emphasizes the fact that the adhesion of epoxy-based sealers to root canal dentin is directly influenced by chemical treatment. Moreover, under the present laboratory conditions, the overall results showed that irrigation using a chelating agent had a positive impact on the bond strength of the epoxy-based sealer to root dentin. Within the limits of this preliminary investigation, a final rinse with 5% pentetic acid improved the bond strength values, which were comparable to those of 17% EDTA.

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Conflicts of interest

There are no conflicts of interest.

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