An *in vitro* comparative evaluation of the effect of three endodontic chelating agents (17% ethylenediamine tetraacetic acid, 1% peracetic acid, 0.2% chitosan) on the push out bond strength of gutta percha with a new bioceramic sealer (BioRoot RCS)

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**Abstract**

**Aim:** This study aims to evaluate the effect of three endodontic chelating agents (17% ethylenediamine tetra-acetic acid [EDTA], 1% peracetic acid [PAA], 0.2% Chitosan) on the push out bond strength of gutta percha with a new bioceramic sealer (BioRoot RCS).

**Materials and Methods:** Forty-eight single-rooted mandibular premolars were selected and decoronated to obtain standardized root length of 15 mm. The root canals were prepared up to #30 files (Hyflex CM) and copious irrigation was done with 3 ml of 5.25% of NaOCl. According to the final irrigation, specimens were divided into three groups: Group 1 (n = 16) 0.2% chitosan, Group 2 (n = 16) 17% EDTA, and Group 3 (n = 16) 1% PAA for 1 min. Samples from each group were obturated with bioceramic sealer and gutta percha and sealed with temporary filling material. Two horizontal slices of 2 mm was obtained from the middle third of each sample (n = 32). Push out bond strength and failure modes were assessed. Kruskal–Wallis test followed by Mann–Whitney post hoc analysis was used for push out analysis. Chi square test was used to compare the modes of failure. The statistical significance level was set at *P* = 0.05.

**Results:** The highest push out bond strength was obtained with Group 1 (0.2% chitosan) followed by Group 3 (1% PAA). Group 2 (17% EDTA) showed least push out bond strength when compared to Group 1 (0.2% chitosan) and Group 3 (1% PAA). The mode of failure was mainly cohesive for all groups.

**Conclusions:** The highest push-out bond strength of BioRoot RCS was seen after treatment with 0.2% chitosan while the least was after EDTA treatment. 1% PAA treatment also favorably affected push-out bond strength of BioRoot RCS.

**Keywords:** BioRoot RCS; chitosan; ethylenediamine tetra-acetic acid; peracetic acid; push-out bond strength

**INTRODUCTION**

Three-dimensional obturation of the root canal is one of the key factors for success in endodontics. The use of a sealer in conjunction with gutta percha is the most widely accepted obturation technique. The sealer establishes a strong and long lasting link between the root canal wall and the gutta percha thus preventing root canal re-infection due to microleakage. 1,2 Various types of sealers are used in endodontics. Most recently, bioceramic sealers have been introduced. Bioceramic sealers are based on calcium silicate formulations and have the advantage of being biocompatible and bioactive. They

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act by releasing calcium and hydroxyl ions which in contact with tissue fluids form an apatite layer and chemically bond to the dentin. Micromechanical interlocking of the sealer to the root dentin helps to maintain the integrity of the sealer-dentin interface during function. BioRoot Root Canal Sealer (Septodont, Saint-Maur-des-Fosses, France) is a recently introduced bioceramic sealer, made from pure calcium silicate with good flow characteristics.

Contemporary methods of root canal shaping produce a layer of organic and inorganic material called the “Smear layer” that may also contain bacteria and their byproducts. The smear layer clogs the dentinal tubules and interferes with sealer adaptation during obturation. Therefore, it is recommended to remove the smear layer before obturation.

Current methods of removing smear layer include chemical, ultrasonic, and laser techniques. However, none of them are totally effective. The most common method in clinical practice is the final rinse with 17% ethylenediamine tetra-acetic acid (EDTA) after canal preparation is completed.

EDTA is the most widely used chelating agent for smear layer removal and compliments the cleaning of root canals by acting on inorganic material. It reacts with the calcium ions in dentin and forms soluble calcium chelates and promotes decalcification of dentin. EDTA is biocompatible and has a pH of 7; however, it lacks anti-bacterial effect. The use of EDTA for extended periods may lower the modulus of elasticity and flexural strength of dentin. This adversely affects the physical and mechanical properties of dentin which eventually increases the risk for root fracture.

Therefore, milder chelating agents such as peracetic acid (PAA), chitosan, phytic acid, and maleic acid have been recently suggested to dissolve smear layer disintegrate the root canal system. PAA is a peroxygen, that is, sporicidal, bactericidal, virucidal, and fungicidal at low concentrations of <0.5% even in the presence of protein. Chitosan is a natural amino polysaccharide which is biodegradable. Adsorption, ionic exchange, and chelation are proposed mechanisms for the chelating effect of chitosan.

Push-out bond strength testing is a relevant approach to assess the adhesion of a root canal sealer to the canal wall and core material. Currently, there is limited information regarding the dislodgment resistance of BioRoot RCS after smear layer removal using newer chelating agents.

Therefore, the aim of this study was to compare the effect of three endodontic chelating agents (17% EDTA, 1% PAA, and 0.2% Chitosan) on the push out bond strength of gutta percha with BioRoot RCS.

The null hypothesis was:

- There is no difference in bond strength of BioRoot RCS to root dentin after using different chelating agents (EDTA, PAA, and Chitosan).

**MATERIALS AND METHODS**

Forty-eight extracted, single-rooted human mandibular premolar teeth were obtained and stored in thymol solution for 2 weeks. The teeth were decoronated to a standardized root length of 15 mm using diamond disc with water spray. After removal of the crown, the initial patency of each root canals was obtained with 10, 15, and 20 K-file (Mani, Japan) and in between irrigation was done with 3 ml of 5.25% sodium hypochlorite (NaOCl). Hyflex CM (Coltene Whaledent, Allstetten, Switzerland) orifice shaper 25.08% was used to enlarge the coronal aspect of each canal. Apical preparation of the canal was done with 20.04% followed by 25.04%, 20.06%, and finally with 30.04% Hyflex CM. Between each file canals were recapitulated with size 20 K-file and were irrigated with 3 ml of 5.25% NaOCl. Then, the specimens were randomly divided into three groups (n = 16). According to final irrigation method as follows: Group 1 – After the completion of instrumentation, the root canals were irrigated with 5 ml of 0.2% Chitosan for 1 min. Group 2 – After canal preparation, the root canals were irrigated with 5 ml of 17% EDTA for 1 min. Group 3 – After canal preparation, the root canals were irrigated with 5 ml of 1% PAA for 1 min.

Specimens in each group were obturated with gutta percha and BioRoot Root Canal Sealer. Radiographs of the specimens were taken to confirm the quality of obturation. The root canal entrances were sealed with a temporary filling material (TMP-RS). The roots were then stored in phosphate buffered saline solution (pH = 7.4) at 37°C and incubated for 7 days.

**Push out strength**

The middle portion of each root was sectioned perpendicular to its long axis into 2.00 ± 0.05 mm thick serial slices using a water-cooled diamond blade on a precision cut off machine. Thirty-two slices were achieved per group. The filling material was loaded with a 0.7 mm diameter cylindrical stainless steel plunger. Loading was performed on a universal testing machine at a speed of 0.5 mm/min in an apical-coronal direction. The maximum load applied to the filling material before debonding was recorded in newtons which expressed the bond strength in megapascals.

**Analysis of failure modes**

The slices were then examined under a stereomicroscope at 40× magnification to determine the failure mode. Modes of failure were considered as follows: adhesive - at filling material-dentin interface, Cohesive - within filling material, and mixed failure.

**Statistical analysis**

Kruskal–Wallis Test followed by Mann–Whitney post hoc analysis was used to compare the mean Push out bond...
strength (in MPa) between three groups. Chi-square test was used to compare the modes of failure between three groups. The level of significance ($P$ value) was set at $P < 0.05$.

**RESULTS**

The highest push out bond strength was obtained with Group 1 (0.2% chitosan) followed by Group 3 (1% PAA). Group 2 (17% EDTA) showed least push out bond strength when compared to Group 1 (0.2% chitosan) and Group 3 (1% PAA). The mode of failure was mainly cohesive for all groups [Tables 1-3].

**DISCUSSION**

Adhesive root canal sealers enhance resistance to fracture as well as minimize microleakage of irritants and pathogens into the periradicular tissues. BioRoot RCS (Septodont, Saint-Maur-des-Fosses, France) is a recently introduced bioceramic sealer that possesses a number of advantageous properties such as biocompatibility, bioactivity, good adhesion to dentin, and gutta percha. The powder primarily consists of tricalcium silicate, povidone and zirconium oxide as radiopacifier, and liquid contains calcium chloride with a hydrophilic biocompatible polymer for enhancing adhesion.

The smear layer formed during canal preparation may inhibit the penetration of irrigants, medicaments and sealers into the dentinal tubules. The presence of the smear layer interferes with sealer penetration during obturation. Therefore, its removal prior to obturation is highly recommended. This is routinely done using chelating agents. While using bioceramic sealers for obturation, final rinsing of root canals using chelating agents is a matter of concern, because the chelating agent also affects the dentinal walls, changing the proportion of Ca: P available. Since calcium silicate sealers react with the calcium of the dentin for the biomineralization process, these changes may affect their adhesion.

In the present study, three chelating agents were employed for removal of smear layer. 17% EDTA has been the gold standard irrigant for smear layer removal. Hence, this was used as the control group. 0.2% Chitosan and 1% PAA have been recently recommended as viable alternatives to EDTA. Hence, these were the test irrigants employed. The present study is one of the few studies to evaluate the effect of Chitosan and PAA on the bond strength of BioRoot RCS.

The final irrigation with the chelating agents was done for 1 min as this is the ideal recommended time for satisfactory chelating effects. To mimic the clinical situation, the samples were irrigated in a vertical direction to enable contact with all aspects of the root canal walls.

Many methods such as push out bond strength test, tensile test, and shear test can be used to assess the bond strength of materials to dentin. The present study used push out bond strength test to evaluate the adhesion of BioRoot RCS as it is reportedly efficient, practical, and reliable, reproducible, and easy to interpret. It is more effective as it allows even root canal sealers with low bond strengths to be evaluated. Various studies have shown that the presence of gutta percha does not significantly affect the POBS of the sealer. Hence, in the present study, obturation was done using BioRoot RCS along with gutta percha.

According to the results, treatment with 0.2% Chitosan showed better results when compared with 17% EDTA and 1% PAA. Chitosan polymer is hydrophilic and adsorbs to the root dentin which helps it to penetrate deeper into the dentinal tubules. In addition, it has a large number of free hydroxyl and amino groups that make it cationic in nature enabling ionic interaction with the dentin calcium ions.

A study by Darrag et al. has shown that a lower concentration of 0.2% chitosan has the advantage of removing smear layer without decalciﬁying effect of 17% EDTA. This has been substantiated by another study done by Silva et al. which demonstrated that the moderate decalciﬁying effect of chitosan removed the smear layer efﬁciently with minimal erosion of intra-radicular dentin.

1% PAA was one of the final irrigants used in this study because in low concentrations it retains its antimicrobial activity and promotes removal of smear layer without causing signiﬁcant changes in dentinal structure. At higher concentrations, it can lead to considerable reduction in dentinal hardness. Hence,
1% PAA was used for the present study. 1% PAA is a weaker chelating agent than 17% EDTA. However, due to its acidity the calcium stays in solution and does not reprecipitate. This explains why its chelating action is comparable to that of 17% EDTA. The bond strength of BioRoot RCS was comparatively higher when 1% PAA was used for smear layer removal as compared to 17% EDTA. However, overall 0.2% chitosan was more effective than 1% PAA in maintaining the bond strength of BioRoot RCS. Based on the present bond strength data, PAA may be considered a suitable alternative to EDTA.

In the present study, push out bond strength of BioRoot RCS was significantly reduced after smear layer removal using 17% EDTA. Donnerneyer et al. attributed this to reduction of calcium at the sealer-dentine interface or a degradation of the calcium silicate fraction in the sealer.[2,3]

A recent SEM-EDS and XRD study by Harik et al. has shown that using EDTA as a final irrigant led to reduction of calcium releasing ability of BioRoot RCS by half. Furthermore, there was depletion of calcium phosphate deposits on the material in contact with dentine after irrigation with EDTA. This validates the findings of the present study.[22]

In the present study, the mode of failure was mainly cohesive for all the groups. This finding is in accordance with Huffman et al. who showed that the failure mode for a calcium-based sealer was cohesive after a 7-day storage period in phosphate-buffered solution. A limitation of the present study could be that roots of premolar teeth are not perfectly straight, curvatures are usually present. Therefore, when we attempt to cut horizontal sections, it will not be perfectly perpendicular to the long axis. This may affect the push out bond strength readings.

From this study, it can be inferred that milder chelating agents do not interfere with the adhesion of BioRoot RCS to radicular dentin. Further studies are necessary to check their effect on other bioceramic sealers as well. The results of the present study cannot be directly extrapolated to the in vivo situation as several variables can influence the clinical outcome. Therefore, further in vivo studies are also necessary to evaluate the performance of bioceramic sealers.

CONCLUSIONS

Among the tested chelating agents 0.2% chitosan, a milder chelating agent, demonstrated the highest push-out bond strength of BioRoot RCS and gutta percha followed by 1% PAA. The least push out bond strength was seen when 17% EDTA was used as the final irrigant. It may be worthwhile to explore the role of milder chelating agents in smear layer removal when bioceramic sealers are employed for obturation.

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Conflicts of interest
There are no conflicts of interest.

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