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Comparative Evaluation of Chitosan and Nano-chitosan Final Irrigant Solution on The Push Out Bond Strength of Bio-ceramic Sealer

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ORIGINAL STUDY

Comparative Evaluation of Chitosan and Nanochitosan Final Irrigant Solution on the Push-Out Bond Strength of Bioceramic Sealer

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Abstract

Purpose: EndoSeal MTA sealer push-out bond strength (POBS) was evaluated after using chitosan and nanochitosan as the final rinse. **Materials and methods:** Universal ProTaper rotary device was utilized to instrument 28 natural human single-rooted lower bicuspid teeth, followed by 1-min irrigation by 2.6% sodium hypochlorite. All samples were classified randomly into four main groups ($n = 7$) as per the utilized final rinse: Group S: normal saline, Group E: 17% EDTA, Group CH: 0.2% chitosan, and Group CNP: 0.2% nanochitosan. The final rinse was standardized to be 5 ml/3 min for each solution. Obturation of all samples was carried out with ProTaper GP and EndoSeal MTA sealer, which was then placed in a humidifier for 1 week. All roots were sectioned into horizontal slices of 2-mm thickness and immersed in self-curing acrylic resin. The samples were put through a push-out test with a 0.5-mm/min loading speed. Data were statistically analyzed. **Results:** Chitosan at the coronal level (Group CH) showed the greatest POBS, followed by groups CNP, EDTA, and S with a statically significant difference ($P < 0.05$) among all tested groups versus the control one. While at the apical level, the highest POBS was observed in the EDTA group followed by groups CH, CNP, and S with a statistically significant difference between all the tested groups versus the control one. **Conclusion:** Chitosan and its nanocounterpart have a comparable result to EDTA irrigant solution. Different chelating agents can greatly influence EndoSeal sealer POBS.

Keywords: EndoSeal MTA, Nanochitosan, Push out

1. Introduction

Eradication of microorganisms as well as prevention of either infection or reinfection are the cornerstones for the success of endodontic treatment [1]. Accordingly, mechanical instrumentation combined with chemical irrigation followed by three-dimensional root canal obturation are crucial steps in endodontic treatment. Technically, the objective of cleaning and shaping is complete debridement and removal of all infected tissues and remains from the root canal system, creating a homogeneously tapered canal that facilitates insertion of medicaments along with hermetic obturation [2]. Solid core material composed of gutta-percha (GP) with sealer is the mostly used obturation material that can easily offer a three-dimensional hermetic seal protecting the periapical tissues from any upcoming infection [3].

On one hand, sodium hypochloride (NaOCl) is considered the principal irrigant solution that has the advantage of organic tissue dissolution and broad antimicrobial activity. On the other hand, it has no chelating action that will aid in smear layer removal that in turn prevents sealer and root canal-filling material to enter inside the dentine orifices and tubular system and in turn adversely influence all the attempts to seal the root canal system. Thus,

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using a chelating agent along with NaOCl is mandatory in the cleaning process of the root canal [4].

The most-used chelating agent is EDTA that is used as a final rinse to eradicate the smear layer that was formed throughout root canal shaping. EDTA forms soluble calcium chelates when it reacts with the calcium ions (Ca^{2+}) existing in the dentin. Unfortunately, otherwise, its beneficial use as a final rinse can harmfully affect the dentinal structure if it is used for a long time as well as the periapical tissues if it is extruded beyond the apex. The purpose of having chelating agents without any harmful effects searching for a natural chelating agent was a priority in the last few years [5].

Chitosan (CH) is a polysaccharide derived from chitin that is found in crustacean shells of shrimps and crabs. Considering chitosan as a chelating agent is due to its ability to bind with metal ions, cholesterol, fats, and proteins. It is advantageous over EDTA as it has antibacterial and antifungal activities, mucoadhesive, analgesic, and hemostatic properties in addition to being biodegradable into nontoxic residues. Chitosan can function in many forms, so it is used in many areas within the medical industry, including tissue engineering, wound healing, periodontal applications and bone healing treatment, dental plaque and tumor inhibition, drug delivery, antiviral, and anti-inflammatory [6].

Natural materials like nanochitosan (CNPs) have outstanding physical, chemical, biological, and antibacterial capabilities, making them superior environmentally friendly materials [7]. Several techniques, including ionotropic gelation, microemulsion, emulsification solvent diffusion, and emulsion-based solvent evaporation, can be used to create chitosan nanoparticles [8]. In comparison to 17% EDTA, 0.2% CNP final irrigation had the same impact on smear layer elimination. However, 0.2% CH results in root dentin that is more microhard and has a smoother surface than 17% EDTA [9]. Great apical sealing ability is produced by the 0.2% final irrigation of chitosan nanoparticles [10].

As three-dimensional hermetic obturation is one of the success factors in endodontic treatment, so careful selection of obturating material is very critical [3]. The typical root canal-filling material is GP, but due to its poor dentin adherence and sealing capabilities, a sealer is required. The main role of the sealer is formation of monoblock by fusing the core material to the root canal wall [11,12]. Recently, biocompatible bioceramic sealants have been available on the market since they are believed to lessen the inflammatory reaction in the event of an overfill during obturation, it does not shrink upon setting, otherwise, it expands slightly on completion of the setting process. Finally, being alkaline (pH 12.8) increases its mineralization process and its bactericidal properties [13].

So, with chitosan and its nanocounterpart serving as the final irrigant solutions, the study's objective was to assess EndoSeal MTA push-out bond strength (POBS) to the root canal wall using a POBS test. After employing chitosan and its nanocounterpart as the final irrigants, the null hypothesis stated that there was no difference in the bond strength of EndoSeal MTA to the root canal wall.

2. Materials and methods

The current study was performed in the Endodontic Department, Faculty of Dental Medicine for Girls, Al-Azhar University. Ethical approval for the use of extracted human teeth was obtained in accordance with guidelines from Research Ethics Committee (REC), Faculty of Dental Medicine for Girls, Al-Azhar University Code (REC-EN-23-01).

2.1. Sample size calculation

The following formula was used to calculate sample size: sample size $(N)=(Z\alpha/2 + 1-\beta)$ $2 \times 2 \times SD2/\Delta2$, where SD = standard deviation of POBS value of the apical section estimated from previous study [14], which was 1.2, and $\Delta2$ = effect size estimated between the samples (suggested to be at least 1.8), α = level of significance (0.05), and $1-\beta$ = power.

Accordingly, $N=(1.96 + 0.84)^2 \times 2 \times (1.2)^2/(1.8)^2 = 6.96$.

So, we conducted our study on a total sample size of 28 (seven in each group).

2.2. Sample selection and preparation

For this investigation, 28 completely developed, single-canal, single-rooted mandibular premolars with single roots were employed. CRIS: Checklist for Reporting In-vitro Studies, guidelines and regulations was used to report the methods. Teeth with root caries, external resorption, fracture, or cracks were excluded. The associated blood, debris, and soft tissue were washed off the teeth with tap water before storing them in distilled water until use. An Isomet-4000 linear precision saw with water cooling was the tool used to standardize root length to be ~16 mm plus or minus 1 mm, by decapitating all teeth's crowns [15].

2.3. Root canal instrumentation

K-file # 10 was used to check the canal patency as well as measuring the working length by deducting 1 mm from the length of the file placed at the apical foramen. Then, with a reduction handpiece powered by a torque-controlled electric motor and rotating speed regulated in accordance with the manufacturer's instructions for each file used, root canals were mechanically instrumented with ProTaper universal rotary NiTi files up to size #F3. A 27-gauge side-vented needle inserted 2 mm shorter than the working length was used to irrigate the canal with 2.6% NaOCl 2 ml^1 min after each instrument use. After mechanical cleaning, 5 ml of distilled water was used to flush the canals [16].

2.4. Sample grouping

According to the assigned final rinse, all selected samples were randomly classified into four experimental groups ($n = 7$ samples each).

(1) Group S: samples were irrigated with normal saline.
(2) Group E: samples were irrigated with 17% EDTA.
(3) Group CH: samples were irrigated with 0.2% chitosan.
(4) Group CNP: samples were irrigated with 0.2% nanochitosan solution.

2.5. Preparation of 0.2% chitosan solution

In Al-Azhar Technology Incubator (ATI), chitosan solution was formulated. Chitosan that had been deacetylated to 90% was diluted in 100 ml of 1% acetic acid, and the mixture was then agitated for 2 h using a magnetic stirrer. The produced solution should be used within a week of preparation, while storing it in the fridge [14].

2.6. Preparation of nanochitosan solution

Chitosan nanoparticle solution was prepared by using ionic gelation process by adding tripolyphosphate aqueous solution drop by drop to chitosan solution and homogenizing the mixture with a polytron homogenizer at 5000 rpm. The CH-tripolyphosphate solutions were stirred, and then centrifuged for 4 min at 13 000 rpm to collect nanoparticles. The supernatant was discarded after the nanoparticles had been properly washed with distilled water. When the nanoparticles formed, the zone of opalescent suspension became apparent [17].

A standardized volume of 5 ml/3 min of each solution was used in each group. Finally, the canals were flushed with 5 ml of saline solution and dried with paper points before obturation.

2.7. Root canal obturation

After completion of the instrumentation step, rinsing of the root canals with 5 ml of distilled water and then drying using sterile #30 paper points was done. All samples were obturated using #F3 master GP cone (#30/0.09). The sealer was introduced to the canal using master GP cone. After being covered with the sealant, the cone was pumped into the canal, introduced, and then extended to its maximum working length. Using a finger spreader (#30) and auxiliary cones (#30/0.02), the root canal was filled. Once the spreader can no longer be seen extending more than 2 mm beyond the root canal orifice, obturation has been pronounced complete. Excess GP was torn off using a cherry red heated instrument and then the warm mass in the canal coronal third was compressed vertically by hand plugger. Finally, the root canal entrance was sealed with temporary filling. To assure appropriate obturation quality, all samples were radiographed. To allow the sealer to properly set, samples were kept at 37 °C with 100% humidity for a week.

2.8. Push-out bond strength test

Samples were implanted in acrylic resin blocks, and then blocks were horizontally sectioned using an Isomet saw mounted with a diamond disc of 0.6-mm thickness (Buehler Lake Bluff, Illinois, USA), spinning at 2500 rpm and feeding at 10 mm/min. Getting one piece from each root third gives each sample a total of three sections. An electric digital caliper (Avenger Products, North Plains, USA) was used to verify the thickness of sections. A compressive loading was placed on each root section via the universal testing machine (Instron, Norwood, Massachusetts, USA) at a crosshead speed of 0.5–1 mm/min using a 0.9-mm-diameter stainless-steel plunger. After that, the samples were positioned over a support jig and loaded until bond failure was experienced in an apicocoronal direction. The POBS was determined in megapascals (MPa) by dividing the highest weight required to dislodge the filler material by the bonded area: POBS (MPa) = maximum failure load (N)/adhesion surface area of root canal filling (mm^2).

The adhesion surface area of each section was calculated as $\pi(r1 + r2) \times L$.

$$L = \sqrt{(r1 - r2)2 + h}$$

where π is the constant 3.14, r1 is the coronal radius, r2 apical radius, and h is the thickness of the section in mm (Fig. 1).

To evaluate the bond failure type, specimens were examined using a stereomicroscope (Olympus SZX7; Olympus Corp., Tokyo, Japan) at × 40 magnification. Each specimen was classified into one of the three failure modes: adhesive failure where 100% of the root canal sealer completely separates from the dentin, cohesive failure where 100% of the material fails within the material and the remaining dentin is covered by sealer, and mixed failure where adhesive and cohesive failure modes are combined.

2.9. Statistical analysis

Statistical analysis of POBS data was conducted using version 16 of the SPPS software (SPSS Inc., Chicago, Illinois, USA). Data were presented as mean values and SD and then analyzed using the analysis of variance test. Post-hoc Tukey tests were used to establish the significance between each pair of groups where a significant difference existed. *P* values less than 0.05 were used to determine significance.

3. Results

The SD and mean values of the POBS for samples from all groups are recorded in MPa (Table 1).

Regarding the comparison between all groups at each root canal level, it was observed that the greatest (POBS) mean values ± SD were in group CH at coronal level followed by groups CNP, E, and S, respectively, with a statically significant difference (0.004) between all the tested solutions in relation to the control one. While the difference was statistically insignificant between the investigated groups. At the middle level, the results showed that maximum (POBS) mean values ± SD were in the CH group followed by groups E, CNP, and S with a statistically insignificant difference between all groups. On the other hand, at the apical level, the highest (POBS) mean values ± SD was in the EDTA group followed by groups CH, CNP, and S with a statistically significant difference (0.033) between all the investigated final rinses compared with the control one. While the difference between the tested groups was statistically insignificant (Table 1).

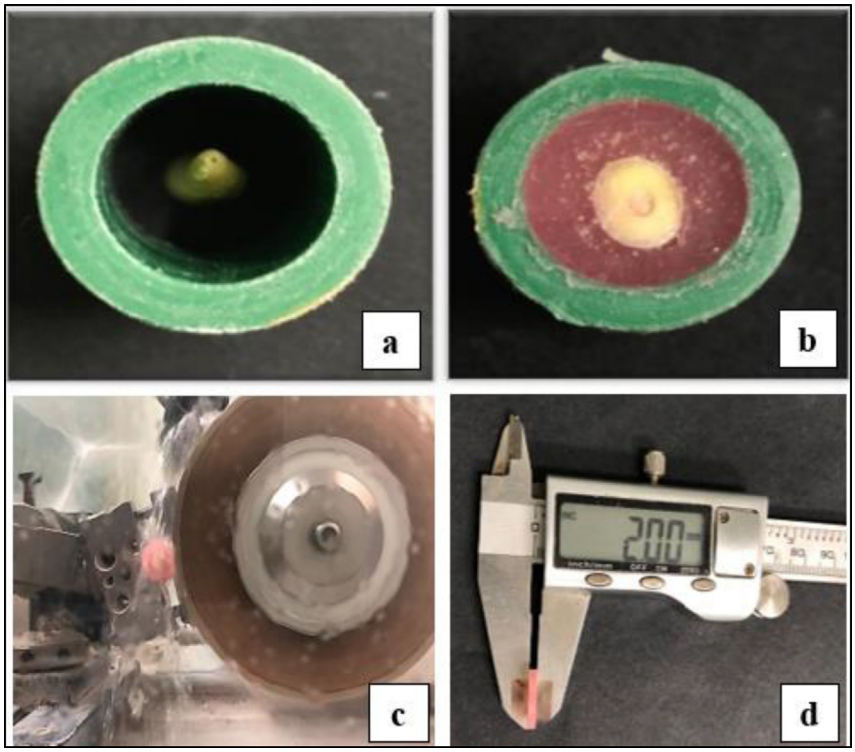

Fig. 1. Photograph showing (a) root sample suspended in brass, (b) root sample embedded inside a self-cured acrylic resin, (c) Isomet working under water coolant, and (d) digital caliper with 2-mm thickness of slice.

Table 1. The means values and SD for the comparison between push-out bond strength of the investigated final irrigating solutions.

Push-out strength (POBS/MPa)	Group S (saline)	Group E (EDTA)	Group CH (chitosan)	Group CNP (nanochitosan)	*P* value
Apical					
Mean	0.17[a]	1.97[b]	1.85[b]	0.55[bA]	0.033*
SD	0.10	1.60	1.27	0.43	
Middle					
Mean	0.33	1.16	2.39	0.74[A]	0.227
SD	0.22	1.13	2.65	1.22	
Coronal					
Mean	0.28[a]	3.27[b]	3.68[b]	3.36[bB]	0.004*
SD	0.15	1.11	2.39	0.66	
P value	0.365	0.071	0.426	0.000*	

*Significant difference (*P*-value ≤0.05).
[a] and [b]: lowercase different superscripts denote significant differences within each row.
[A] and [B]: uppercase different superscripts denote significant differences within each column.

Regarding the comparison between the root canal level within each group, in all groups, the coronal third revealed the maximum (POBS) mean values ± SD. The results of Group CNP showed that the difference between the coronal third compared with middle and apical thirds was statistically significant. While the difference in groups S, E, and CH was statistically insignificant among all levels (Table 1).

Following the POBS, specimens were examined under a stereomicroscope, and the results indicated that the largest failure rate was adhesive (63.44%), followed by cohesive failure (21.5%), and the lowest was mixed failure (15%) (Table 2, Fig. 2).

Table 2. Failure mode percentage following the push-out bond strength test between radicular dentin and obturating material.

Final rinse	Adhesive (%)	Cohesive (%)	Mixed (%)
Group S (saline)	56.6	20	23.3
Group E (EDTA)	62.5	25	12.5
Group CH (chitosan)	73.33	13.33	13.33
Group CNP (nanochitosan)	66.6	25	8.33
Total	63.44	21.5	15

4. Discussion

Monoblock concept in endodontic treatment can be created by the filling materials along with correct cleaning and shaping of the radicular part, which are prerequisites for an effective endodontic practice. The use of sealers has been mandated to intimately adhere the root canal-filling material to the dentinal canal walls in attempts to overcome the well-known shortcoming of GP's poor bonding ability to root canal walls [18].

That is why there is a continuous improvement in root canal-filling materials and bioceramic materials are now becoming widely used. The EndoSeal MTA sealer is one of the bioceramic sealants that have been recently introduced into the market.

Standardization of samples in all groups was the goal of this investigation. This was accomplished by employing newly harvested human single roots that were ~16-mm long, completely formed the apex to allow instrumentation in the canal, as a well-developed apical constriction to prevent the intracanal irrigation from being withdrawn from the canal and allow room for easier insertion of root canal sealer. Teeth with straight canals were chosen

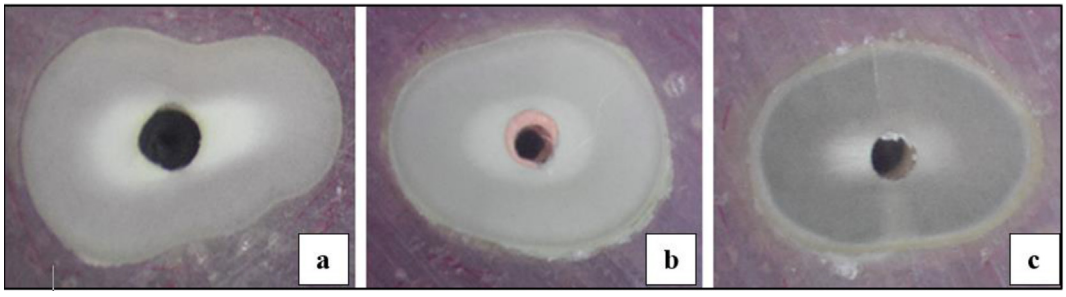

Fig. 2. The representative specimens were examined under a stereomicroscope at a magnification of × 40 and the results showed: (a) adhesive failure demonstrated that the radicular dentin was devoid of sealer attachment; (b) cohesive failure demonstrated that almost the entire radicular dentin was still shielded with sealer; and (c) mixed failure (mixed between adhesive and cohesive modes), which demonstrated that some areas of the radicular dentin were shielded.

to provide specimens that allowed the load to be vertically aligned along the vertical axis of the root to yield pure forces during a testing procedure [19]. Using a nonstop machine and diamond disc with copious amounts of water as coolant, the anatomical crowns were decoronated at the level of CEJ perpendicular to the root long axis in order to eliminate any variables existing between samples [20].

Utilizing an irrigating needle with a 27-diameter vent at its sides to be closer to the apex has the greatest impact along the entire canal wall's length [16].

In this study, 2.5% NaOCl (2-ml) irrigation was used between each file size throughout the chemo-mechanical process because of its antibacterial qualities, capacity to dissolve tissue, and elimination of the organic particles of the smear layer, in addition to being the most straightforward and frequently utilized solution for irrigation [21].

EDTA solution can eradicate the dentinal smear layer out from radicular walls in all root canal thirds. However, the antibacterial action of EDTA is quite moderate. Additionally, continuous exposure to EDTA has a deleterious impact on the microhardness of dentin and may cause peritubular and intertubular dentin to erode. Conversely, the smear layer was successfully eliminated with limited erosion of dentine when 0.2% CH solution (5 ml/3 min) was used as a final irrigant. Additionally, it strengthens the collagen structure, increasing the resilience of the dentinal surface to collagenase breakdown [22].

The hydrophilic characteristic of CH advances its close contact and adsorption with radicular dentin, which may account for its effectiveness as a chelating agent. Additionally, it is cationic in nature as a result of the numerous free amino acids (NH_2) as well as hydroxyl groups (OH), promoting the ionic bonds across Ca^{2+} ions in the chelating agent and dentinal tissue [23].

Due to the ultrasmall size of nanoparticles, and high surface area to mass ratio as well as elevated chemical reactivity, nanoparticles exhibit more advanced physical and chemical properties than their bulk counterparts. Studies have shown that CNPs can be utilized as a substitute for EDTA in endodontic treatment as they are efficient in smear layer eradication and bacterial recolonization prevention in root dentin [21].

In this study, EndoSeal MTA sealer, a recently developed, premixed, and preloaded material, was used to obturate the teeth. It has outstanding sealing properties as well as antimicrobial effects, acceptable cytocompatibility, is simple to remove with NiTi files when retreatment is required, improved biomineralization of the dentinal tubules, and reasonable bond strength to root dentin in addition to significantly higher flow values and higher radiopacity [24,25].

The most common methodology for determination of the efficiency of root canal obturating materials' adherence to the dentine walls of the canals is bond strength testing. As well, the POBS test permits the obturating material to adapt to the canal's structure and get through the orifices of dentine tubules, that improves replication of the intraoral circumstances. Additionally, it has the benefit of monitoring the sealer bond strength at all root canal levels, even when the bond strength is weak, it enables evaluation of the sealers [26,27].

Regarding the comparison between all groups at coronal and middle levels, it was observed that the maximum POBS mean values ± SD were in group CH. This may be explained by the hydrophilic properties of CH polymer, which allow it to absorb to the root dentine and enter further into the dentinal tubule. Additionally, it is cationic in nature and can interact ionically with the Ca^{2+} in dentine, thanks to its abundance of free NH_2 and OH groups [8,28]. According to a different study, low concentrations of 0.2% CH had the benefit of eliminating smear layers without having the decalcifying effects of 17% EDTA [29].

On the other hand, at the apical level, the greatest POBS mean values ± SD were in the EDTA group followed by groups CH, CNP, and S. Since it is well-known that chelating agent effectiveness varies on the length of application, pH, concentration, and volume of the solution, irrigant solutions used for an extended length of time might produce dentin surface roughness [29]. Our findings might be attributed to the fact that all groups had standardized 3-min interaction times, with the EDTA group receiving longer contact time, giving it a chance to eliminate the smear layer from the canal walls in all three levels of the canals with this 3-min contact time. Besides, erosion can result at the dentin wall that may have helped EndoSeal MTA penetrate the dentinal tubules [30].

However, there was an insignificant difference between the investigated groups at all levels, the CNP group has lower findings in relation to CH and EDTA groups. CNPs have the additional benefit of being antibacterial besides bonding effectively to dentin and promoting sealer entrance into the dentinal tubules, which results in mechanical interlocking to the canal wall by debridement of the root canal dentin from smear layer [31]. Numerous earlier investigations concurred with our findings,

reporting that EDTA had superior bond strength and more sealer penetration as compared with 0.2% CH and its nanoequivalent, with also insignificant results [23,29–33].

Finally, the coronal level showed the greatest effect for all experimented solutions followed by middle and then the apical third root canal [34]. It may be attributed to the average number of dentinal tubules decreasing from coronal to apical [35], greater intratubular dentin increasing the dentin surface area reachable for demineralization influence of the irrigants, which in turn can influence the root canal sealer adhesion, and it was conveyed that there is a direct relation between dentin surface area and enhanced bond strength [8,28].

The results of Group CNP revealed that the variance between the coronal level in relation to middle and apical levels was statically significant. In comparison to EDTA, CNPs had a smaller effect on dental hard tissue. Their size influences how deeply this final rinse solutions can reach the tubules of the radicular system. Since weak chemicals demineralize less dentine surface, CNP solutions could eradicate the smear layer deprived of demineralizing the dentine [9].

Because the smear layer was kept unaltered in the control group, the POBS in this group was the least, demonstrating the negative impact of the undisturbed smear layer on the POBS value [15].

4.1. Conclusion

Chitosan and its nanocounterpart have a comparable result to EDTA irrigant solution. However, nanochitosan showed the least bond strength. EndoSeal bioceramic sealer POBS was enhanced using different chelating agents.

4.2. Recommendation

Future research should be directed toward the determination of the dislocation resistance in teeth irrigated by chitosan, and its nanocounterpart then obturated with EndoSeal MTA sealer.

Conflicts of interest

There are no conflicts of interest.

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