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Research Article

Evaluation of the Effect of Final Rinse with Chitosan Nanoparticles and EDTA on the Push-out Bond Strength of Resin-based and Bioceramic-based Root Canal Sealers (A Comparative *In Vitro* Study)

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Abstract

Aim: This study was conducted to evaluate the effect of chitosan nanoparticles (CNPs), CNPs/EDTA (1:1) and EDTA as final irrigants on the push-out bond strength of different root canal sealers.

Methodology: Thirty extracted human, single-rooted mandibular premolars were decoronated and prepared using ProTaper Next rotary system with 2.5% sodium hypochlorite (NaOCl) irrigation. The specimens were randomly distributed into three groups according to the final irrigation protocol; Group 1: 0.2% CNPs, Group 2: 0.2% CNPs/17% EDTA (1:1), and Group 3: 17% EDTA. A standardized volume of 5 ml of each chelating solution was used for 3 min. Each group was further divided into two subgroups based on the root canal sealer used for obturation; AH Plus resin sealer and Sure-Seal Root bioceramic sealer. For assessment of the push-out bond strength, roots were sectioned horizontally to obtain 2 mm-thick discs from the coronal, middle and apical thirds, then discs were subjected to a compressive load via the universal testing machine followed by assessment of the failure pattern using stereomicroscope.

Results: AH Plus showed a significantly higher push-out bond strength compared to Sure-Seal bioceramic sealer within the three experimental groups (p < 0.05). There was no significant difference in AH Plus bond strength among the three groups, while Sure-Seal bioceramic sealer showed a significantly higher bond strength in the CNPs group (p = 0.02). AH Plus showed a significantly higher bond strength at the coronal third compared to Sure-Seal bioceramic sealer (p \leq 0.001), however there was no significant difference between them in the middle third. In the apical third, Sure-Seal recorded significantly higher bond strength (p \leq 0.05) except for the EDTA group. For AH Plus, the highest push-out bond strength values were recorded in the coronal third, while Sure-Seal bioceramic sealer showed significantly higher bond strength at the apical third. The failure mode was predominately cohesive for both sealers.

Conclusion: Removal of the smear layer improves the bond strength of AH Plus to root dentin, but negatively affects that of Sure-Seal bioceramic sealer. CNPs had less adverse effects on the bond strength and adherence of Sure-Seal bioceramic sealer compared to EDTA.

Keywords: Chitosan; EDTA; Nanoparticles; Smear Layer; Bond Strength; AH Plus; Sure-Seal

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Introduction

Successful root canal treatment depends on the combination of thorough chemo-mechanical debridement and three-dimensional obturation of the root canal system. The main objective of three-dimensional obturation is to provide a gap-free interface between the canal walls and different root canal filling materials. The most accepted root canal obturation techniques involve the use of guttapercha cones and root canal sealers. Over the past century, various materials and techniques have been introduced aiming to improve the stability and sealing ability of the root canal filling materials [1].

The sealing ability of root canal filling materials depends mainly on the adhesion properties of root canal sealers and their ability to penetrate deeply into the dentinal tubules [2]. During chemo-mechanical preparation, the cutting action of hand or rotary files results in the formation of a smear layer on the instrumented surfaces of the canal walls. The smear layer is a thin layer of organic and inorganic remnants, which act as a barrier hindering penetration of antibacterial irrigants, intracanal medicaments and root canal sealers into the dentinal tubules [3]. EDTA is the most commonly used chelating agent for removal of the smear layer. However, prolonged exposure to EDTA results in dentin erosion and causes marked reduction in dentin microhardness [4].

Chitosan is a natural polysaccharide obtained by deacetylation of chitin, one of the most abundant natural biopolymers found in crustacean exoskeleton as crabs and shrimps [5]. Over the past few years, it has gained a great interest among researchers because of its biocompatibility, biodegradability, bio-adhesion and lack of toxicity. In addition, chitosan presents with high chelating capacity and antimicrobial activity against a wide range of gram-positive and gram-negative bacteria as well as fungi [6,7]. Furthermore, chitosan structure is similar to that of the extracellular matrix components which reinforces the collagen constructs and improves dentin resistance to degradation by collagenase [8]. Chitosan root canal irrigation was developed for removal of the smear layer after complete mechanical debridement of the root canals. Compared to EDTA, it can effectively remove the smear layer with less adverse effects on dentin structure [9].

Chitosan nanoparticles (CNPs) are biocompatible, polymeric nanoparticles with high antimicrobial activity. In the field of end-

odontics, these nanoparticles were mainly used as antimicrobial agents and drug carriers. Kishen., *et al.* found that there was a significant decrease in the adherence of *Enterococcus faecalis* to dentin after treatment with chitosan nanoparticles [10]. Shrestha., *et al.* demonstrated the efficacy of chitosan nanoparticles in eliminating and disrupting *E. faecalis* biofilm [11]. Recently, it was reported that chitosan nanoparticles based irrigation can serve as an alternative chelating agent for EDTA as it removes the smear layer effectively when used as a final irrigant [12,13].

Many studies have assessed the smear layer removal efficacy of different chelating agents, however data regarding their effect on the adhesive potential of different root canal sealers to radicular dentin is still lacking in the literature. Therefore, the present study was conducted to evaluate the effect of chitosan nanoparticles and EDTA on the bond strength of epoxy-resin and bioceramic-based root canal sealers.

Materials and Methods Sample size

Based on the results of a previous study by Yap., *et al.* 2017 [14] and using power 80% and 5% significance level, a total sample size of 30 roots were included in the study. Thus, the samples were randomly distributed among 3 groups: each containing 10 samples.

Sample selection

Thirty human single-rooted mandibular premolar teeth extracted for periodontal reasons or due to orthodontic treatment, were collected from the outpatient clinic of Oral Surgery Department, Faculty of Dentistry, Cairo University. Criteria for teeth selection included straight roots with single root canals and completely formed apices. Teeth with open apices, visible cracks and root resorption were excluded. The selected teeth were thoroughly washed under running water and immersed in 5.25% NaOCl solution for 15 minutes to disinfect the teeth and remove any soft deposits on the root surface. The remaining hard deposits were removed from the root surface using curettes and finally teeth were stored in saline solution till the time of use.

Sample preparation

Teeth were decoronated at the cemento-enamel junction (CEJ) using a low-speed, water-cooled diamond disc to obtain a standardized root length of approximately 16 mm. The working length and

canal patency were determined by inserting a # 10 K-file (Mani Inc., Tochigi, Japan) until it reaches the apical foramen, then subtracting 1mm from this measurement. Apices of all roots were sealed using sticky wax. All samples were instrumented with a crown down technique using ProTaper Next rotary Ni-Ti system (Dentsply Maillefer, Ballaigues, Switzerland) starting with X1 (#17/.04), X2 (#25/.06), X3 (#30/.07) and finally X4 (#40/.06), using X-Smart Plus Endo Motor (Dentsply Maillefer, Ballaigues, Switzerland) at a rotational speed of 300 RPM and 2.5 Ncm torque for all files. During instrumentation, the canals were irrigated with 3 ml of 2.5% NaOCl using a disposable plastic syringe with a 30-G needle (Sung-Shim Medical Co., Bucheon, Gyeonggi, South Korea) between each file size to reach 1 - 2 mm from the apex without binding. At the end of mechanical preparation, the canals were flushed with 5 ml saline solution.

Experimental groups distribution

Samples were randomly classified into three groups (n = 10) according to the final irrigation protocol as follows:

- Group 1: 0.2% Chitosan Nanoparticles (CNPs).
- Group 2: 0.2% CNPs/17% EDTA (CNPs/EDTA) (1:1).
- Group 3: 17% EDTA.

A standardized volume of 5 ml of each solution was used for 3 min in each of the three groups. For the CNPs/EDTA group, 2.5 ml of CNPs was used for 1.5 min, followed by 5 ml sterile saline, then 2.5 ml of EDTA (Meta biomed, Cheongju-si, Chungbuk, Korea) for another 1.5 min. Finally, the canals were flushed with 5 ml saline solution and dried with paper points before obturation. Each group was further divided into two subgroups (n = 5) based on the root canal sealer used for obturation:

- AH Plus subgroup: Canals were obturated using Gutta-percha/AH Plus resin sealer.
- Sure-Seal subgroup: Canals were obturated using Gutta-percha/Sure-Seal Root bioceramic sealer.

Preparation of CNPs irrigation

The CNPs were prepared at the Central Nanotechnology Characterization Lab, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. The ionotropic gelation method was used for synthesis of the CNPs as described in a previous study [15] with

some modifications. First, $0.2~\mathrm{gm}$ of low molecular weight chitosan powder (Acros Organics, Belgium) was dissolved in 80 ml of 1% (v/v) acetic acid (Sigma Aldrich, USA) under magnetic stirring (Cimarec, Thermo Scientific, USA) for 30 minutes. After that, 20 ml of $0.7~\mathrm{mg/ml}$ sodium tripoly-phosphate (TPP) (Sigma Aldrich, USA) was slowly added into the chitosan solution using drop-wise addition under stirring at 800 rpm to form the nanoparticles, thus achieving a final concentration of $2~\mathrm{mg/ml}$ CNPs (0.2%). Finally, the CNPs were stored in the refrigerator and ultra-sonication of the suspension was done immediately before its usage.

Root canal obturation

All samples were obturated using single-cone technique with X4 master gutta-percha cone (#40/0.06) (Dentsply Maillefer, Ballaigues, Switzerland) that matches the master file. Each subgroup was obturated by one of the tested sealers.

In AH Plus subgroup, the canals were obturated using guttapercha cones and AH Plus resin sealer (Dentsply DeTrey GmbH, Konstanz, Germany). AH Plus was prepared by mixing two equal amounts of paste A and B using a metal spatula till a homogenous, creamy mix was obtained. The master gutta-percha cone was coated with the sealer and introduced into the canal in a pumping motion. After that, the master cone was withdrawn from the canal, recoated one more time with the sealer, and then finally introduced into the canal to the full working length.

In Sure-Seal subgroup, the canals were obturated using gutta-percha cones and Sure-Seal bioceramic sealer (Sure-Dent Co., Seongnam-si, Gyeonggi-do, Korea). Sure-Seal bioceramic sealer is supplied in the form of a pre-loaded syringe with disposable intracanal tips. The tip of the syringe was inserted into the root canal no deeper than the coronal one third, then the sealer was gently and smoothly dispensed into the canal while withdrawing the disposable tip. The master gutta-percha cone was coated with a thin layer of the sealer and introduced into the canal to the full working length.

Excess gutta-percha was removed and the warm mass in the coronal third of the canal was vertically condensed with a hand plugger, then the root canal entrance was sealed with a temporary filling material. All samples were kept on gauze pads at 37°C and 100% relative humidity for one week to allow proper setting of the sealers.

Assessment of the push-out bond strength

Samples were embedded in acrylic resin blocks and sectioned horizontally using a water-cooled precision saw (Buehler USA, Lake Bluff, Illinois, USA) to obtain sections of 2 mm thickness. One section was obtained from each root third making a total of 3 sections per each sample and 15 sections per each subgroup. Each root section was subjected to a compressive loading via the universal testing machine (Instron, High Wycombe, Buckinghamshire, UK) at a crosshead speed of 0.5 mm/min using a (0.5, 0.7 and 1.0 mm) diameter stainless steel cylindrical plunger for apical, middle and coronal sections respectively. The samples were aligned over a support jig and load was applied in an apicocoronal direction until bond failure occurred. The maximum load needed to dislodge the filling material was recorded in Newtons (N) and the push-out bond strength was calculated in Megapascals (MPa) by dividing the load by the bonded area:

Push-out bond strength (MPa) = Maximum failure load (N)/Adhesion surface area (mm^2)

The adhesion surface area of root canal filling in each section was calculated as follows: $\pi (r_1 + r_2) \times L$

$$L = \sqrt{(r_1 - r_2)^2 + h^2}$$

Where π is the constant 3.14, r_1 is the coronal radius, r_2 is the apical radius, and h is the thickness of each section in mm.

Assessment of the failure mode

After the push-out test, each root section was examined and photographed using stereomicroscope (Leica Microsystems, Wetzlar, Germany) at 50X magnification to determine the mode of failure. The failure modes expected to be present are: adhesive failure either at the sealer-dentin interface or between the sealer and core material, cohesive failure within the filling material (sealer or core material) or mixed failure (both adhesive and cohesive).

Statistical analysis

Statistical analysis was performed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL). Data were presented as mean and standard deviation (SD) values and examined for normality using Shapiro Wilk test. One-way analysis of variance (ANOVA) was used for inter-

group comparison and for comparison between different root canal sections, followed by a Tukey's post-hoc test for pairwise comparisons whenever a statistical significant difference was recorded by ANOVA test. Independent t test was used for intra-group comparison. A p-value less than or equal to 0.05 ($p \le 0.05$) was considered statistically significant.

Results

The results of the push-out bond strength test showed that AH Plus had a significantly higher bond strength compared to Sure-Seal bioceramic sealer in the three groups (p < 0.05). There was no significant difference in AH Plus bond strength among the three groups (p = 0.478). On the other hand, Sure-Seal bioceramic sealer displayed a significantly higher bond strength in the CNPs group (p = 0.02) (Table 1).

	AH Plus		Sure-S		
Group	Mean	SD	Mean	SD	P-Value
Group 1 (CNPs)	1.77	0.15	1.44 ^A	0.08	0.005*
Group 2 (CNPs/ EDTA)	1.93	0.26	1.29 ^{AB}	0.05	0.001*
Group 3 (EDTA)	1.83	0.18	1.26 ^B	0.12	< 0.001*
P-Value	0.478		0.020*		

Table 1: Mean, Standard deviation (SD) for push-out bond strength, and p- value for the tested sealers in the three groups.

*: Different superscript letters in the same column indicate statistically significant values.

AH Plus showed a significantly higher bond strength at the coronal third compared to Sure-Seal bioceramic sealer (p \leq 0.001) with no significant difference between them in the middle third (p > 0.05). In the apical third, Sure-Seal recorded significantly higher bond strength (p < 0.05) except for the EDTA group where there is no difference between the two sealers (Table 2).

For AH Plus, the highest push-out bond strength values were recorded in the coronal third with a statistically significant difference from the middle and apical thirds. While Sure-Seal bioceramic sealer showed significantly higher push-out bond strength values at the apical third followed by the middle and coronal thirds. However, there was no statistically significant difference in AH Plus and

		AH Plus		Sure-Seal			
Group	Root level	Mean	SD	Mean	SD	P-Value	
Group 1 (CNPs)	Coronal	2.60	0.38	0.85	0.26	<0.001*	
	Middle	1.14	0.14	1.22	0.34	0.638	
	Apical	1.57	0.30	2.23	0.23	0.004*	
Group 2 (CNPs/ EDTA)	Coronal	2.56	0.45	0.69	0.04	0.001*	
	Middle	1.64	0.53	1.05	0.18	0.065	
	Apical	1.58	0.43	2.13	0.13	0.025*	
Group 3 (EDTA)	Coronal	2.57	0.21	0.66	0.01	< 0.001*	
	Middle	1.27	0.32	1.14	0.16	0.435	
	Apical	1.64	0.43	1.98	0.38	0.215	

Table 2: Mean, Standard deviation (SD) for push-out bond strength, and p-value at different root levels for the tested sealers in the three groups.

Sure-Seal bond strength at different root levels among the three groups (p > 0.05) (Table 3 and 4).

Group	Group 1 (CNPs)/AH Plus		Group 2 (CNPS/ EDTA)/AH Plus		Group 3 (EDTA)/AH Plus		P-Value
Root level	Mean	SD	Mean	SD	Mean	SD	
Coronal	2.60 ^A	0.38	2.56 ^A	0.45	2.57 ^A	0.21	0.985
Middle	1.14 ^B	0.14	1.64 ^B	0.53	1.27 ^B	0.32	0.119
Apical	1.57 ^B	0.30	1.58 ^B	0.43	1.64 ^B	0.43	0.961
P - Value	< 0.001*		0.011*		< 0.001*		

Table 3: Mean, standard deviation (SD) for push-out bond strength, and p-value at different root levels for AH Plus sealer in the three groups.

AH Plus and Sure-Seal bioceramic sealer showed predominantly cohesive failure modes within the tested groups.

Discussion

The long-term success of endodontic treatment greatly depends on the ability of root canal filling materials to form a fluid tight seal

Group	Group 1 (CNPs)/ Sure- Seal		Group 2 (CNPS/EDTA)/ Sure-Seal		Group 3 (EDTA)/ Sure- Seal		P-Value
Root level	Mean	SD	Mean	SD	Mean	SD	
Coronal	0.85 ^B	0.26	0.69 ^c	0.04	0.66 ^c	0.01	0.147
Middle	1.22 ^B	0.34	1.05 ^B	0.18	1.14 ^B	0.16	0.553
Apical	2.23 ^A	0.23	2.13 ^A	0.13	1.98 ^A	0.38	0.368
P - Value	< 0.001*		< 0.001*		< 0.001*		

Table 4: Mean, Standard deviation (SD) for push-out bond strength, and p-value at different root levels for Sure-Seal bioceramic sealer in the three groups.

*: Different superscript letters in the same column indicate statistically significant values.

with the canal walls. In a static situation, a strong bond between root canal sealers and radicular dentin is important to eliminate any space at the sealer-dentin interface, prevent leakage of periapical tissue fluids into the canal space and potential re-infection which may result in the development of a periapical disease [16]. In a dynamic situation, it is also essential to maintain the integrity of the sealer-dentin interface during tooth flexure, post-space preparation and other operative procedures [17]. In both static and dynamic situations, the integrity of the root canal seal is affected mainly by the adhesion properties of endodontic sealers, area of contact to root dentin, and their ability to penetrate into the dentinal tubules [2].

Although the question of keeping or removing the smear layer remains controversial for many years, this bacteria-loaded layer may hinder penetration of disinfecting agents into the dentinal tubules and act as a barrier between the canal walls and root canal filling materials compromising the formation of a satisfactory seal [3]. Moreover, the smear layer is a loosely adherent structure and a potential avenue for leakage between root canal filling and dentinal walls [18].

Bond strength testing is considered to be the most popular method used to determine the effectiveness of adhesion of different root canal filling materials to root dentin [19]. Although several tests are available for measuring the adhesion of endodontic sealers, none of them has been widely accepted. However, the push-out

^{*:} Different superscript letters in the same column indicate statistically significant values.

test was proven to be better than the conventional shear test for evaluating the bond strength, since the sealer was placed in direct contact to the root canal dentinal walls instead of a flat coronal dentin surface which has a different tubular pattern [20]. Additionally, the push-out test allows the material to accommodate to the canal shape and penetrate into the dentinal tubules, which provides better simulation of the clinical conditions [21]. It also has the advantage of measuring the sealer bond strength at different root levels. Another benefit of this test is that it allows the sealers to be evaluated even when the bond strength is low [19]. Furthermore, the push-out bond strength test is less sensitive unlike the tensile test in which small alterations in the specimen or in stress distribution during load application have a major effect on the results [22].

Two root canal sealers with different adhesion properties were tested in this study. AH Plus root canal sealer belongs to the resinbased materials that has the ability to bond to radicular dentin and penetrate deeply into the dentinal tubules [23]. However, the polymerization shrinkage of resin-based sealers may affect the bonding quality to root canal dentin and different core materials [24]. While Sure-Seal bioceramic sealer is a premixed ready-to use, injectable calcium aluminosilicate paste which sets in the presence of moisture. Bioceramic sealers are composed mainly of calcium silicates, calcium phosphate monobasic, calcium hydroxide, zirconium oxide and thickening agents [25]. They have high dimensional stability with the least amount of shrinkage because of their ability to penetrate the dentinal tubules and to interact with dentine moisture [26]. The hydration reaction of calcium silicates usually results in the formation of calcium silicate hydrogel and calcium hydroxide which tend to react with phosphate ions forming a hydroxyapatite layer [27].

17% EDTA solution has been long used as a chelating agent where it can effectively remove the smear layer from the canal walls in all root thirds. However, EDTA has a limited antibacterial activity. In addition, prolonged exposure to EDTA negatively affects dentin microhardness and may result in erosion of peritubular and intertubular dentin [4]. On the other hand, using 0.2% chitosan solution (5 ml) as a final irrigant for 3 min removed the smear layer effectively with minimal dentin erosion [28]. It also reinforces collagen structure which improve dentinal surface resistance to degradation by collagenase [8].

Chitosan was used in combination with EDTA (1:1) to obtain the ultimate chelating effect as proposed in a previous study. Chitosan-EDTA (1:1) was effective in removal of the smear layer and caused less erosion of dentin in the coronal and middle portions with significant antimicrobial activity against Enterococcus faecalis. It was proven to be a potential endodontic irrigant with dual function; root canal disinfection and smear layer removal [29].

Nanoparticles have advanced physical and chemical properties in comparison to their bulk materials in terms of ultrasmall structure, large surface area/mass ratio and increased chemical reactivity [30]. Studies showed that chitosan nanoparticles (CNPs) can be used as a final irrigant in root canal treatment and serve as an alternative to EDTA, since it has the ability to remove the smear layer effectively and inhibit bacterial recolonization on root dentin [12,13].

The study was conducted on freshly extracted single-rooted mandibular premolars with approximately similar apical diameters (size #20) and similar root length of (16 + 1 mm) to ensure maximum standardization of the experimental groups.

A little amount of sticky wax was placed on the apex of each root in order to retain the irrigation solution within the canals, stimulate the *in vivo* apical counter pressure and prevent seepage of the irrigation solution during canal preparation [31].

Mechanical preparation was done using ProTaper Next rotary system. Rotary Ni-Ti files are preferred over manual stainless steel files because of their high flexibility and having a greater taper as compared to 2% taper of manual files. The ProTaper Next rotary files are standardized files with a matching sized gutta-percha. In addition, they provide better shaping advantages over ProTaper Universal system through the convergence of a variable tapered design on a single file, innovative M-Wire technology, and a unique offset mass of rotation [32].

Throughout preparation, canals were irrigated with 3 ml of 2.5% NaOCl between each file size. Sodium hypochlorite is the simplest available and most commonly used irrigating solution because of its antimicrobial properties and tissue dissolving action. It also has the ability of removing the organic components of the smear layer [33]. The root canals were finally flushed with sterile saline solution, as it was found that using NaOCl as a final rinse de-

creased the bond strength of resin-based sealers because it inhibits the polymerization process of resins [34].

All canals were obturated with single-cone technique using Pro-Taper Next matching gutta-percha cones. Single-cone obturation technique gives the same sealing effect of the lateral condensation technique as long as a resin root canal sealer was used [35]. The highest push-out bond strength values were obtained with the single-cone technique using matching 0.06-tapered master gutta-percha cones, as it provides a more uniform mass of gutta-percha and decreases the sealer amount which in turn minimizes the possibility of gap formation due to sealer shrinkage or dissolution [36].

This study compared the adhesion of epoxy resin and bioceramic-based sealers to root dentin by measuring their push-out bond strength using the universal testing machine. The results showed that AH Plus root canal sealer had a significantly higher bond strength values compared to Sure-Seal bioceramic sealer following the use of different chelating agents. The relatively high bond strength of AH Plus sealer may be explained by the fact that it bonds chemically to dentin, as the terminal reactive epoxide rings form a covalent bond with the exposed amino groups in the collagen network [23]. In addition, some previous studies reported that the high bond strength of AH Plus could be due to its low polymerization shrinkage and long-term dimensional stability [16,37]. While the bond strength of calcium silicate-based sealers depends mainly on the mechanical interlocking caused by the tag-like structures formed at the sealer-dentin interface, and the chemical interaction between the sealer and radicular dentine forming the "mineral infiltration zone" [38]; which seems to establish a weaker link to dentin compared to epoxy resin-based sealers [39]. These findings are supported by the fact that retreatability of calcium silicate-based sealers is more efficient than that of epoxy resin-based sealers [40].

Additionally, studies showed that removal of the smear layer increased the bond strength of AH Plus to root dentin [37,41], as it facilitates exposure of the collagen network and improves sealer penetration into the dentinal tubules [42]. On the other hand, it was found that removing the smear layer may adversely affect the bond strength of calcium silicate-based sealers [43]. The bioceramic sealer is a hydrophilic material that uses the moisture in the smear layer to form a hydroxyapatite-like precipitate during setting and form a chemical bond with dentin [44]. The moisture

present in the dentinal tubules following root canal dryness before obturation may not be sufficient for the material to set resulting in a lower bond strength [45]. Furthermore, the effect of the chelating agents is not restricted to removal of the smear layer which is rich in calcium and phosphate, it also changes the proportion of Ca:P in the tooth structure [46]. These changes may affect the adherence of calcium silicate-based sealers that depends mainly on the calcium ions present in dentine for the biomineralization process [43].

The push-out bond strength values varied from one study to another depending on the preparation taper, root canal sealer, core filling material, obturation technique, tooth portion, slice thickness, storage time, plunger diameter and load velocity [39]. The findings of the present study are in accordance with some recent studies that reported high bond strength values for AH Plus compared to calcium silicate-based sealers [39,43]. In these studies, the chelating agents were used for 3 to 5 minutes. Other studies showed no difference or superior bond strength values of calcium silicate-based sealers compared to AH Plus [1,47]. However, these studies are not comparable because different irrigation protocols were used in which less amount of the chelating agents were used or applied for a less period of time (1 min). In addition, some of these studies evaluated the bond strength only in the middle third of the root.

In the current study, results revealed that there is no significant difference in the bond strength of AH Plus sealer among the three groups (CNPs, CNPs:EDTA, EDTA), as studies showed that EDTA and CNPs have the same smear layer removal efficacy [12,13]. On the contrary, Sure-Seal bioceramic sealer displayed a significantly higher bond strength values in the CNPs group compared to the EDTA group, because chitosan results in less dentin erosion as well as less alterations in surface structure and Ca/P ratio [9].

In the coronal third, AH Plus showed a significantly higher bond strength values compared to Sure- Seal bioceramic sealer within the three experimental groups. While there is no statistically significant difference between the two sealers in the middle third. In the apical third, Sure-Seal bioceramic sealer recorded significantly higher bond strength values compared to AH Plus except in the EDTA group where there is no difference between the two sealers. This can be explained by the higher tubular density in the coronal third compared to the middle and apical thirds which increases the number of the resin tags, enhancing AH Plus bond strength [48]. In

addition, the smear layer removal efficacy of the chelating agents decrease from coronal to apical direction [13]. Therefore, Sure-Seal bioceramic sealer had a higher bond strength in the apical third compared to AH Plus except in the EDTA group, as it causes more erosion in dentin affecting the bond strength of calcium silicate-based sealers [43]. Moreover, the higher bond strength of Sure-Seal bioceramic sealer in the apical region may be due to its higher flowability and smaller particle size compared to AH Plus, which enhances sealer penetration into the dentinal tubules and allows the sealer to fill radicular dentin irregularities and minor spaces of difficult access [49].

At different root levels, the highest push-out bond strength values for AH Plus were recorded in the coronal third with a statistically significant difference from the middle and apical thirds, as the number of the dentinal tubules and the tubular diameter decreases from coronal to apical direction [48]. No significant difference was found between the middle and apical thirds because of the circular cross section of the root canal at the apical third, which provides a high resistance to dislodgment during the push-out test [50]. For Sure-Seal bioceramic sealer, the apical third showed significantly higher push-out bond strength values followed by the middle and coronal thirds. This may be attributed to the canal circular cross section in the apical areas and limited accessibility of the chelating agents to these areas [13,50], which decrease the smear layer removal efficacy and minimize changes in the Ca:P proportion of root dentin.

Regarding the failure mode, the present results obtained for AH Plus and Sure-Seal bioceramic sealer are in accordance with previously reported findings [39,47]. AH Plus and Sure-Seal bioceramic sealer predominantly showed cohesive failure modes. This is likely due to the stronger link formed between these sealers and radicular dentin compared to their link with gutta-percha [39].

Conclusion

Within the limitations of this study, it could be concluded that:

- The push-out bond strength of resin-based and bioceramicbased root canal sealers was influenced by the use of different chelating agents.
- Removing the smear layer improves the bond strength of AH Plus to root dentin, but negatively affects that of Sure-Seal bioceramic sealer.

- CNPs had less adverse effects on the bond strength and adherence of Sure-Seal bioceramic sealer compared to EDTA.
- The combined use of CNPs/EDTA (1:1) showed the same effect on the bond strength of both sealers compared to each individual irrigant.

Conflict of Interest

There is no conflict of interest.

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