



Impact of Green Tea Extract on Intraradicular Dentin Wettability and Bond Strength of Resin-Based Sealer Following Two Chelating Agents

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ABSTRACT

Purpose: To evaluate the effect of 3% green tea extract (GTE) on wettability, depth of penetration, and push out bond strength (PBS) of AH Plus sealer to intraradicular dentin following the use of 0.2% Nano chitosan or 17% EDTA. **Materials and methods:** Twenty human radicular dentin segments were used for wettability assessment. Following immersion in 2.6% NaOCl solution, samples were divided into 2 groups (n=10) according to the chelating agent used: group I: 0.2% Nano chitosan, and group II: 17% EDTA, each used for 3 min, then the contact angle (CA) was measured. The samples were further immersed in 3% GTE solution for 5 min followed by CA measurement. Twenty human mandibular premolars were prepared up to # X4 ProTaper Next files, then divided into two groups as mentioned previously. Each group was subdivided into two subgroups based on the final flush used (n=5); subgroups A and B; no treatment, and 3% GTE, respectively. Samples were obturated by cold lateral compaction using AH Plus sealer and gutta-percha. PBS was measured using a universal testing machine and sealer penetration was assessed using confocal laser scanning microscopy. **Results:** Using 3% GTE following the use of 0.2% Nano chitosan significantly increased the wettability and PBS of AH Plus sealer to intraradicular dentin, while its use following 17% EDTA significantly decreased the wettability and PBS of AH Plus sealer. Using GTE increased the maximum depth of AH Plus sealer penetration into the dentinal tubules. **Conclusion:** GTE improved the wettability, depth of penetration, and push out bond strength of AH Plus sealer to intraradicular dentin treated with Nano-chitosan. Nevertheless, it adversely affected those properties of AH Plus sealer to EDTA-treated dentin.

KEYWORDS

Green tea extract,
Chitosan nanoparticles,
Wettability, AH Plus.

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INTRODUCTION

Adhesion is one of the most essential clinical properties of a root canal (RC) sealer. This property describes the sealer's ability to adhere to RC walls as well as to the gutta-percha cones⁽¹⁾, thus reducing microleakage and improving the sealer's clinical performance⁽²⁾.

Different RC sealers are available with different compositions and properties. Epoxy resin-based sealers, including AH Plus, are generally characterized by their dimensional stability, minimal polymerization shrinkage, low solubility and high mechanical properties⁽³⁾. Many authors reported higher bond strength of AH Plus compared to methacrylates sealers⁽⁴⁻⁶⁾.

The wettability of the intraradicular dentin surface has a significant impact on sealer's adhesion⁽⁷⁾. However, studies showed that irrigation protocols can greatly affect the sealer's wettability and adhesion to RC walls⁽⁸⁻¹¹⁾. The main role of irrigant is to eliminate microorganisms, necrotic tissue and smear layer formed during endodontic treatment, which improves the seal to RC dentin⁽¹²⁾.

Sodium hypochlorite (NaOCl) is the most commonly used irrigant for canal disinfection and dissolution of pulp tissue remnants. The application of NaOCl alone has negligible effect on smear layer removal (SLR)⁽¹³⁾. Therefore, it is recommended to be used in conjunction with chelating agent, such as ethylenediaminetetraacetic acid (EDTA), to ensure efficient SLR⁽¹⁴⁾. However, a previous study reported that EDTA can cause extensive demineralization of the RC walls, which decreases the Ca/P ratio⁽¹⁵⁾. Moreover, significant decrease in dentin microhardness was reported^(16, 17). Therefore, the chemico-mechanical properties of dentin is greatly affected by the chelating agent's concentration and duration of application^(14, 18).

Researchers are in quest of an irrigant that is more biocompatible than EDTA, aiming to minimizing its harmful effects on dentin. Recently, natural products have attracted the attention in the dental field as

alternatives to synthetic ones. Chitosan is a natural biopolymer that is acquired from shrimp or crabs shells, and through a deacetylation process of chitin, it can be easily prepared. It has unique properties, such as antibacterial activity, biocompatibility, mucoadhesivity, high chelating ability for metal ions, easy availability, and cost effectiveness⁽¹⁹⁾.

The unique properties of chitosan encouraged its use in many dental applications, including drug carriers, dentin bonding agents and restorative materials modifications, and enamel repair⁽²⁰⁾. Furthermore, chitosan has been used in endodontics due to its chelating and antibacterial properties⁽²¹⁻²⁵⁾. Interestingly, chitosan nanoparticles (CSNPs) were demonstrated to be effective in eliminating the smear layer when utilized as a chelating agent⁽²⁵⁾. However, studies concerning the effect of CSNPs on the adhesion of endodontic sealers to RC walls are limited⁽²⁶⁾.

Green tea, extracted from the *Camellia sinensis* plant, is one of the most widely consumed health beverages. Green tea extract (GTE) includes polyphenols, including epigallocatechin-3-gallate (EGCG), which gives GTE its antioxidant, anticarcinogenic, anti-inflammatory, and antibacterial effects⁽²⁷⁾. Interestingly, GTE has collagen-stabilizing ability⁽²⁸⁾. Moreover, it has an inhibitory activity against matrix metalloproteinases (MMPs), which is one of the causes of dentin collagen breakdown⁽²⁹⁾. Therefore, the use of GTE as a final flush could improve the resin-dentin adhesion.

This research aimed to evaluate the effect of 3% GTE on wettability, depth of penetration, and push out bond strength of AH Plus sealer to intraradicular dentin treated with either 0.2% Nano chitosan or 17% EDTA. The null hypothesis tested was that the use of 3% GTE had no influence on wettability, depth of penetration, and PBS of AH Plus sealer to intraradicular dentin following the use of two chelating agents (0.2% Nano chitosan versus 17% EDTA).

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee, Faculty of Dental Medicine for Girls, Al-Azhar University, Egypt (# REC-PD-21-13).

Samples' size calculation:

The sample size was determined based on previous studies^(30,31) using the G*power software 3.1.9.2, where large effect sizes of 2.97 and 1.37 were detected, respectively. Using a two-sided hypothesis test, the significance level (α -error) was set at 0.05 and the power ($1 - \beta$ error) was set at 0.8. The estimated sample size was 8 for each group (contact angle measurement) + 2 samples per group were added to gain extra power and 10 for each group (push out bond strength measurement), summing up a total sample size of 40.

Preparation of irrigating solutions:

- ***Preparation of Nano chitosan solution:***

CSNPs preparation was achieved through two steps; firstly, 0.2g of chitosan powder (85% degree of deacetylation, Sigma Co., Egypt) was dissolved in 100ml of 1% acetic acid (pH=4, Alpha Chemika, India), and stirring the mixture for 6 hours with a magnetic stirrer. Then, the ionotropic gelation process⁽³²⁾ was followed, where 150 mL of tripolyphosphate (TPP) (0.2 percent w/v) was added drop-wisely into the chitosan solution under magnetic stirring for 20 minutes to precipitate CSNPs. Centrifugation (10,000 rpm, 10 min) was used to separate CSNPs and ensure homogenous dispersion in the solution.

- ***Preparation of GTE solution:***

In a Waring blender, fifteen green tea capsules (Mepaco Green Tea, Egypt), each containing 300 mg of green tea dry extract, were crushed and then mixed with 150 mL of distilled water at room temperature to achieve 150 mL with a concentration of 30 mg/mL of GTE⁽³³⁾. After filtration with a nylon filter, the supernatant was discarded, and the filtrate was kept in a screw-capped vial and refrigerated.

Characterization of the prepared solutions:

The structural analysis of CSNPs and GTE was estimated by Fourier-Transform Infrared Spectroscopy (FTIR, Vetex70 RAM II, Germany) using KBr discs. The morphology and particle size of CSNPs were determined using a transmission electron microscope (TEM) (JEOL100 CX, Japan).

Wettability assessment:

- ***Samples' preparation:***

Ten single-rooted human mandibular premolars were examined using dental operating microscope (Zeiss, Germany) for the presence of any defects as cracks or resorption. Teeth were cleaned and stored in distilled water until use.

Twenty longitudinal radicular dentin segments (5x5x2 mm, 2 segments/tooth) were prepared using a low-speed diamond disc (Diatech, Goltène AG, Altstätten, Switzerland) under water coolant. The intraradicular dentin surface of each segment was flattened using sandpaper (100 grit) to obtain smooth flat dentin surface for contact angle measurement. Ultrasonic vibrations were applied for 5 minutes to clean the dentin segments from any debris in distilled water. The samples were immersed in 10 mL of freshly prepared 2.6% NaOCl (Alex. Detergents and Chemical Co., Egypt) solution for 5 min.

- ***Samples' grouping and irrigating protocols:***

The samples were randomly assigned into two groups ($n=10$), using a Microsoft Excel random generator software, according to the chelating agent used as follows: group I, 0.2% Nano chitosan; and group II, 17% EDTA (DHRAMA Calix-E, USA). In each group, the samples were immersed in 5mL of each chelating agent for 3 min^(34,35), as described by Hu et al. (2010)⁽³⁶⁾. The samples were dried using paper points (Meta Dental Co., Ltd., Korea), followed by CA measurement. Then, the same samples were further immersed in 10 mL of 3% GTE solution for 5 min⁽³³⁾ followed by CA measurement. Accordingly, each sample serves as its own control.

Water contact angle (WCA) measurement:

The WCA of the treated dentin surfaces was measured by the sessile drop technique ⁽³⁷⁾, using contact angle goniometer (VCA Video System, Germany). With a micro-syringe, one drop (2 μ L) of deionized water was deposited on each dentin surface at room temperature (Fig. 1). The contact angle values were determined by analyzing the captured images with the drop using Image J software (version 1.4, Image J, NIH).

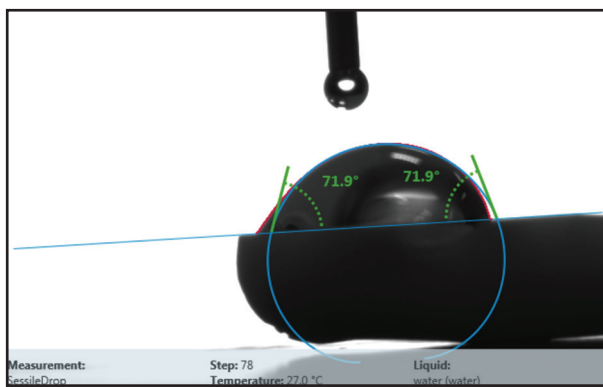


Figure (1) A photograph showing water contact angle measurement by the sessile drop method.

Push out bond strength (PBS) evaluation:

• Samples' preparation:

Twenty single-rooted human mandibular premolars with single RC were selected based on radiographic evaluation. The root length was standardized to 15 mm by decapitating each tooth at the cemento-enamel junction (CEJ) with a diamond disc under water coolant. Teeth were cleaned and stored in distilled water until use.

• Root canal preparation:

#10 K-file (MANI Inc., Japan) was used to maintain the RC patency, and the working length (WL) was calculated by reducing 1mm from length when the file's tip became visible at the apical foramen. The ProTaper Next system (Dentsply, Switzerland) was used with a handpiece operated by a torque-controlled endomotor (EndoEst motor mini, Geosoft Dent, Russia) at a speed of 300 rpm and 2 N.cm torque. After creating a gliding path

with #15 and 20 K-files, a set of four instruments (X1, X2, X3 and X4) were used to the WL.

Irrigation with 2 ml of freshly prepared 2.6 % NaOCl solution for 1 min was performed after each instrument use, using a 28 gauge side vent irrigating needle (Endo-Eze tip, Ultradent product, Inc, USA). The needle was placed into the RC as deeply as possible without binding. The samples were irrigated with a total volume of 10ml of 2.6 % NaOCl solution for 5 min. After RC preparation, the samples were assigned into two groups ($n=10$) on the basis of chelating agent used as mentioned previously. Then, each group was further subdivided into two subgroups (A and B) based on the final flush used ($n=5$); subgroup A: without GTE (no treatment), and subgroup B: 3% GTE solution. The samples of subgroup B were irrigated with 10mL of 3% GTE solution for 5 min. After irrigation, the samples were dried using paper points.

• Root canal obturation:

The samples were obturated using ProTaper gutta-percha (GP) cones (#X4) (Dentsply, Switzerland) and AH Plus sealer (Dentsply, DeTrey, Germany) that was mixed as instructed by the manufacturer. The sealer was applied to the RC walls using a master cone that moved vertically up and down inside the canal to ensure that the sealer covered the whole canal wall. Cold lateral compaction technique with auxiliary GP cones (#25, 0.02 taper) was used to complete the RC filling. With the use of a heated instrument, the excess GP was removed. The samples were sealed with a temporary filling, kept in gauze moistened with saline, and then sealed in a tube for 7 days at 37°C to allow the sealer to completely set.

• Samples' preparation for PBS evaluation:

Each sample was sectioned perpendicular to the long axis of the root into three segments (2 mm-thick each) from the coronal (C), middle (M), and apical (A) thirds. A compressive load was applied to each root segment using a universal testing machine (Instron, England). For the C, M, and A thirds, the root filling (RF) was loaded using 0.9, 0.7, and 0.5 mm-diameter cylindrical plungers, respectively. The

plunger tip was positioned to cover the RF almost entirely without contacting the RC wall. The push-out load was delivered in an apico-coronal path at a cross-head speed of 1 mm/min until debonding occurred. Thereafter, the maximum load applied for debonding was measured in Newton (N).

The bond strength in megapascals (MPa) was obtained by dividing the recorded value in Newton (N) by the adhesion (bonding) surface area of the RC filling, using the following formula ⁽³⁸⁾: $\text{Area} = \pi h (r_1 + r_2)$, where $\pi=3.14$, r_1 ; apical radius, r_2 ; coronal radius, and h ; root segment's thickness in mm.

Sealer penetration depth measurement:

One sample from each group/subgroup, summing up four samples, was selected for sealer penetration depth measurement. Samples' obturation was performed by cold lateral compaction technique using GP and AH Plus sealer as mentioned previously. AH Plus sealer was manipulated according to manufacture instructions and mixed with 0.1% by weight of Rhodamine B dye (Sigma-Aldrich, GmbH). To enable the complete sealer's setting, the samples were enclosed in a tube for 7 days at 37°C. Each root was horizontally sectioned under water cooling into 2 mm-thick slices at the apical and middle RC levels using IsoMet 4000 micro-saw. At 10x magnification, samples were scanned with Leica confocal laser scanning microscopy (CLSM) using excitation wavelengths of 488-552 nm and emission wavelengths of 555-695 nm. The sealer's maximum penetration depth into the dentinal tubules was calculated and recorded ⁽³⁹⁾.

Statistical analysis:

The normality of the numerical data distribution was tested. The data were presented as mean and standard deviation or median and range, for parametric and non-parametric data respectively, and statistically analyzed using Version 20 of SPSS (SPSS Chicago, USA). A paired t-test was performed for the CA measurements within each group. An independent-t-test was used to compare between unpaired groups. Regarding PBS evaluation, the Mann-Whitney U test was performed for comparisons between groups and subgroups. Friedman's test was used to compare among root levels. The significance level was set at $P < 0.05$.

RESULTS

Characterization of the prepared solutions:

Results of the FTIR showed similarity between both prepared solutions, the CSNPs and GTE, due to resemblance of functional groups (Fig. 2a,b). For the CSNPs, the characteristic peak at 3360 cm^{-1} indicates stretching vibration mode of N-H that was also overlapped with O-H stretching mode. Another detected peaks at 2870 cm^{-1} was assigned to C-H stretching vibration mode, at 1530 cm^{-1} was assigned to N-O stretching vibration, and at 1630 cm^{-1} for NH bending vibration. These peaks indicated the cross-linking between the ammonium groups of chitosan and the tripoly-phosphate, forming CNPs. Concerning the particle size and morphology analysis, the transmission electron micrograph showed a spherical shape of CSNPs with an average particle size of 20nm (Fig. 3).

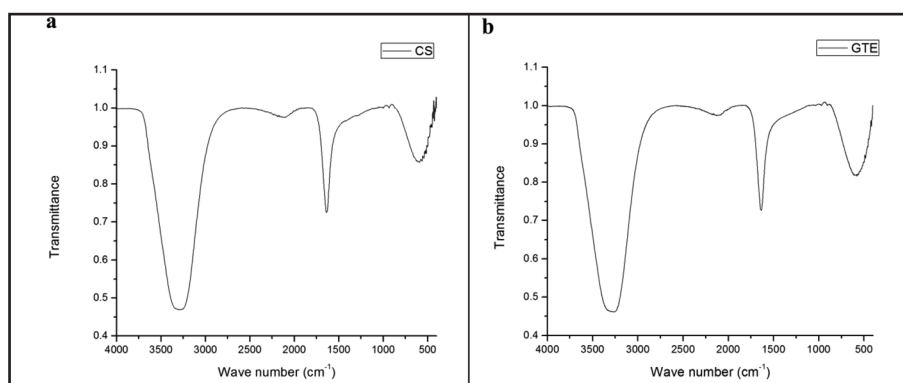


Figure (2)
FTIR spectra of (a) chitosan nanoparticles (CSNPs), and (b) green tea extract (GTE) solutions.

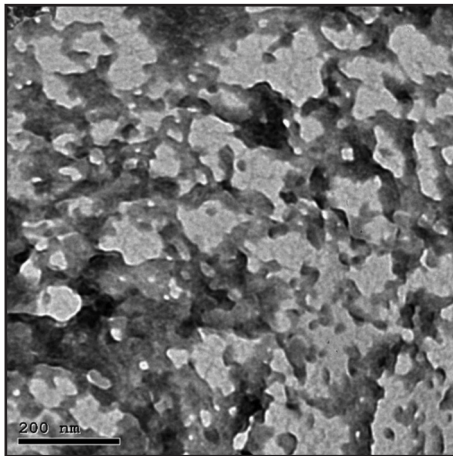


Figure (3) Transmission electron micrograph of chitosan nanoparticles (CSNPs).

Contact angle measurement:

All data were found to be parametric using normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests). The mean values and standard deviation of WCA of intraradicular dentin treated with different irrigating solutions were presented in Table (1) and (Fig. 4,5).

Evaluation of the contact angle (CA) of treated dentin surfaces revealed that, the use of 3% GTE as final flush following the use of 0.2% Nano chitosan significantly decreased the CA compared to that of Nano chitosan without GTE ($P=0.004$), indicating a significant increase in intraradicular dentin wettability. While, the use of 3% GTE as final flush following the use of 17% EDTA significantly increased the CA compared to that of EDTA without GTE ($P < 0.001$), indicating a significant decrease in intraradicular dentin wettability.

Regarding the effect of the chelating agent on CA, when 17% EDTA was used, the CA of treated dentin surfaces was significantly lower than those treated with 0.2% Nano chitosan ($P = 0.006$), denoting better wettability. Whereas when Nano chitosan + GTE were used, the CA of treated dentin surfaces was significantly lower than those treated with EDTA+ GTE ($P= 0.003$), denoting better wettability.

Table 1: Mean values and Standard deviation (SD) of contact angle ($^{\circ}$) of dentin surfaces treated with different irrigating solutions.

	Chelating agent	Group I (0.2% Nano chitosan)		Group II (17% EDTA)		P-value
		Mean	SD	Mean	SD	
	Final flush					
Contact angle (degrees)	Without GTE (no treatment)	66.3	13.7	35	3.9	0.006*
	3% GTE solution	34	13	68.1	3.7	0.003*
	P-value	0.004*		< 0.001*		

*: Significant at $P < 0.05$

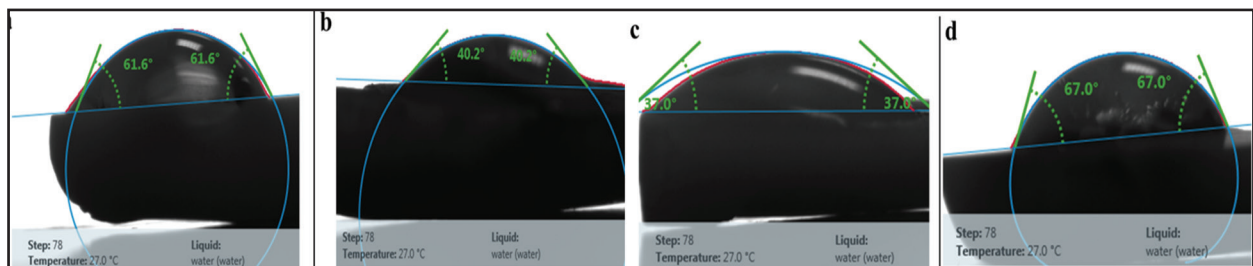


Figure (4) A photograph showing water contact angle of intraradicular dentin treated with, (a) Nano chitosan, (b) Nano chitosan + GTE, (c) EDTA, and (d) EDTA+GTE.

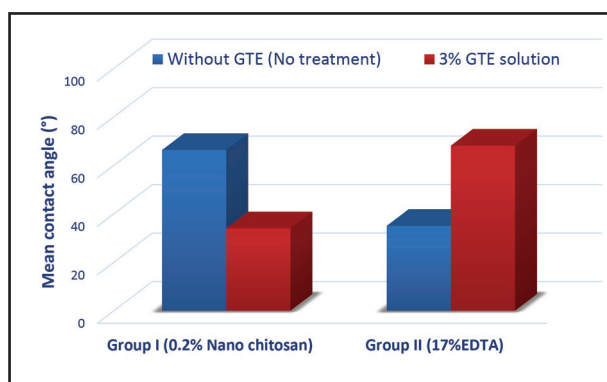


Figure (5) A bar chart comparing the mean contact angle with and without the use of 3% GTE within each group.

Push out bond strength results:

All data were non-parametric according to normality tests. The median and range of PBS of AH Plus sealer recorded for groups and subgroups were presented in Tables (2,3) and (Fig. 6,7).

The overall comparison between subgroups revealed that using 3% GTE as final flush following the use of 0.2% Nano chitosan significantly increased the PBS of AH Plus sealer to intraradicular dentin compared to the use of Nano chitosan without GTE ($P = 0.016$). While the use of 3% GTE following the

use of 17% EDTA significantly decreased the PBS of AH Plus sealer compared to the use of EDTA without GTE ($P = 0.037$) (Table 2) (Fig. 6).

Regarding the effect of the chelating agent on PBS, the results revealed that samples treated with 17% EDTA recorded statistically significant higher PBS values compared to those treated with 0.2% Nano chitosan at middle and apical levels ($P = 0.008$), while no statistically significant difference was recorded between 0.2% Nano chitosan and 17% EDTA at different RC levels ($P > 0.05$), when 3% GTE was used as final flush (Table 3).

Regarding the PBS results at different RC levels, within group I (0.2% Nano chitosan), the highest PBS value was found at the coronal and middle levels in subgroup A (no treatment) and subgroup B (3% GTE), respectively, while the apical level showed the lowest PBS value in both subgroups. There was no statistically significant difference in the median PBS values among the three RC levels ($P = 0.091$ and 0.247 for subgroup A and B, respectively). In group II (17% EDTA), the statistically highest PBS value was recorded at the middle level compared to the apical and coronal levels ($P = 0.015$) (Fig. 7).

Table 2: Median and range of push out bond strength (MPa) comparing the use of 3% GTE versus no treatment within each group at the coronal, middle and apical RC levels.

Chelating agent	Root level	Subgroup A (Without GTE) (no treatment)		Subgroup B (3% GTE solution)		P-value
		Median	Range	Median	Range	
Group I (0.2% Nano chitosan)	Coronal	7.11	3.36 - 8.30	7.15	5.88-7.58	1
	Middle	6.53	4.18 - 7.87	8.21	7.17 – 9.94	0.095
	Apical	3.64	2.37 – 4.49	6.75	4.90 – 8.77	0.008*
	Total	4.49	2.37 – 8.30	7.17	4.90 – 9.94	0.016*
Group II (17% EDTA)	Coronal	7.53	6.14 – 7.91	4.24	4.11 – 9.47	0.151
	Middle	9.41	8.36- 10.38	8.62	4.93 – 12.19	0.421
	Apical	7.91	7.58 – 8.34	5.01	3.33 – 6.46	0.008*
	Total	7.91	6.14 -10.38	5.02	3.33 – 12.19	0.037*

*: Significant at $P < 0.05$

Table 3: Median and range of push out bond strength (MPa) comparing Nano chitosan and EDTA within each subgroup at the coronal, middle and apical RC levels.

Final flush	Root level	Group I (0.2% Nano chitosan)		Group II (17% EDTA)		P-value
		Median	Range	Median	Range	
Subgroup A (Without GTE) (No treatment)	Coronal	7.11	3.36 - 8.30	7.53	6.14 – 7.91	1
	Middle	6.53	4.18 - 7.87	9.41	8.36- 10.38	0.008*
	Apical	3.64	2.37 – 4.49	7.91	7.58 – 8.34	0.008*
	Total	4.49	2.37 – 8.30	7.91	6.14 -10.38	0.000*
Subgroup B (3% GTE solution)	Coronal	7.15	5.88-7.58	4.24	4.11 – 9.47	0.151
	Middle	8.21	7.17 – 9.94	8.62	4.93 – 12.19	0.421
	Apical	6.75	4.90 – 8.77	5.01	3.33 – 6.46	0.056
	Total	7.17	4.90 – 9.94	5.02	3.33 – 12.19	0.126

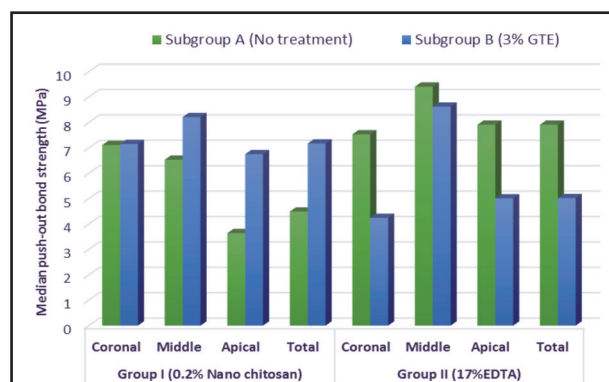
*: Significant at $P < 0.05$ 

Figure (6) A bar chart comparing the median push out bond strength of 3% GTE versus no treatment at the coronal, middle, and apical RC levels within each group.

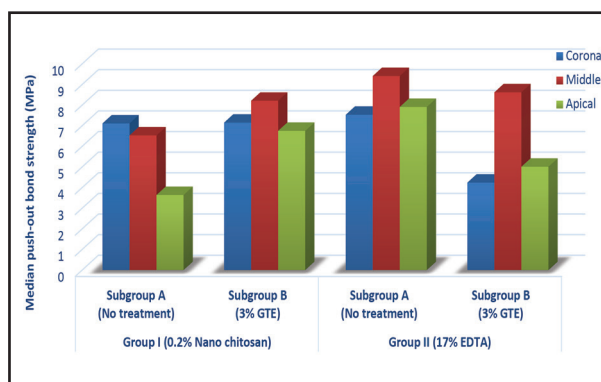


Figure (7) A bar chart comparing the median push out bond strength at the coronal, middle and apical RC levels.

Sealer penetration measurement:

CLSM analysis of AH Plus sealer penetration into the dentinal tubules at different RC levels revealed an increase in the maximum depth of sealer penetration in Nano chitosan-GTE treated samples

(1369.12 μ m) compared to those treated with Nano chitosan (1073.69 μ m). While a dramatic decrease in depth of sealer penetration was observed in samples treated with EDTA-GTE (206.35 μ m) compared to those treated with EDTA (1036.79 μ m) (Fig. 8).

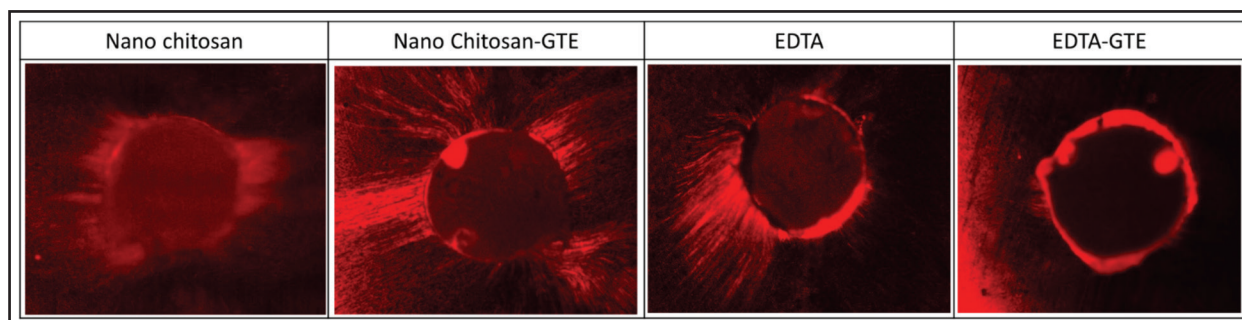


Figure (8) Representative images of CLSM at the middle level showing AH Plus sealer penetration depth into the dentinal tubules of radicular dentin treated with different irrigating solutions ($\times 10$).

DISCUSSION

Sealer's adequate flow, wetting and adhesion are crucial physicochemical properties during RC obturation^(9,10). However, intraradicular dentin treatment with various irrigants may affect the wettability and adhesion of a sealer to dentin surface⁽⁸⁻¹¹⁾. Unfortunately, none of the available irrigating solutions can act on both the organic and inorganic components of the smear layer concurrently. Therefore, a combination of NaOCl and EDTA has been recommended for effective SLR⁽⁴⁰⁾.

Despite the widespread use of EDTA, it has detrimental effects on chemico-mechanical properties of dentin⁽¹⁵⁻¹⁷⁾, it is considered an environmental pollutant, and furthermore, it is not easily biodegradable⁽⁴¹⁾. Chitosan is a natural biopolymer that has sparked interest in dental field due to its unique properties, abundance in nature, and low manufacturing cost⁽¹⁹⁾. Accordingly, chitosan has been used as a chelating agent⁽²¹⁻²³⁾.

Biodegradation of collagen in dentin has become the most significant concern with resin-based materials, resulting in interfacial bond failure⁽¹⁾. Cross-linking and reinforcing the collagen matrix, which can be accomplished by introducing a cross-linking agent such as GTE, has recently received more attention^(27,28). Therefore, this study aimed to evaluate the impact of 3% GTE solution, when applied after two chelating agents (0.2% Nano chitosan or 17% EDTA), on wettability, depth of penetration, and push out bond strength of AH Plus sealer to intraradicular dentin.

In the present study, the use of 5 ml of 0.2% Nano chitosan for 3 minutes was justified by a preliminary study found that applying 0.2% chitosan acetate for 3 minutes was the most effective chelating agent when compared to chitosan prepared with different concentrations and contact times⁽³⁴⁾. Moreover, CSNPs have been used to improve the effectiveness of chitosan, where nanomaterials have unique physicochemical properties such as large surface area and enhanced chemical reactivity, when compared to their bulk counterparts⁽⁴²⁾.

The samples were immersed in 5 ml of 17% EDTA for 3 minutes, to standardize the duration with the most effective irrigation regimen of 0.2% Nano chitosan solution. Furthermore, irrigation with 17% EDTA for 3 minutes contact time was found to be effective in eliminating the smear layer⁽³⁵⁾.

It has been suggested that applying a solution containing EGCG, the major polyphenol in green tea extract, enhances collagen stability and inhibits MMPs activity^(28,29). As a result, using GTE as a final flush may improve resin-dentin adhesion.

Wettability is among the most essential physicochemical properties of RC sealer. It is determined by the CA produced between a liquid drop and the surface of a solid⁽⁷⁾. It has been noted that surface roughness can affect wettability⁽⁸⁾, so intraradicular dentin surfaces were polished to standardize surface roughness.

In the current investigation, CA was measured using a controlled volume of water (2 μ L), as any

volumetric change could influence the contact angle value ⁽⁴³⁾. A captive bubble or a sessile drop technique can be used to determine the CA. By using the sessile drop method, it is possible to maintain the contact angles of a liquid drop on flat surfaces in a dry environment ⁽⁴⁴⁾.

For comparison of the wetting behavior of the dentin surface after different treatments, it is preferable to carry out the tests on the same surface conditions. Accordingly, each sample was treated with the chelating agent followed by CA measurement, and then GTE solution was applied followed by CA measurement. Therefore, each sample serves as its own control.

The cold lateral compaction technique was used for RC obturation, which is a widely known obturating technique that results in increased bond strength of the filling materials to RC dentin ⁽⁴⁵⁾. AH Plus is a common epoxy resin-based sealer that is considered a gold standard, due to its good physico-chemical qualities ⁽³⁾ and superior adhesion to root dentin when compared to other types of sealers ⁽⁴⁻⁶⁾. Dentin drying by air can cause collagen collapse, which increases the hydrophobicity of the dentin ⁽⁴⁶⁾. Therefore, the samples were dried with paper points to imitate the clinical scenario.

The bond strength of sealers to dentin has been evaluated using a variety of methods, such as push out, shear, and tensile tests. The push out test is a reliable and reproducible methodology that mimics clinical situations in which failure occurs parallel to the dentin/material interface ⁽⁴⁾. According to Pane et al. (2013) ⁽⁴⁷⁾, 2 mm thick slices were preferable to prevent premature debonding and sealer separation while slicing.

Increased sealer penetration into dentinal tubules is preferred because it creates a physical barrier, entombs residual bacteria, and improves the sealer-dentin interfacial integrity. Sealer penetration into dentinal tubules has been studied using scanning electron microscopy ⁽⁴⁸⁾, and CLSM ⁽³⁹⁾. CLSM has two key advantages: it does not require sample

processing and minimizes technical artifacts significantly ⁽³⁹⁾.

The results of the current study revealed that the use of 3% GTE solution had a significant impact on wettability and PBS of AH Plus sealer to intraradicular dentin treated with either 0.2% Nano chitosan or 17% EDTA. Moreover, the use of GTE improved AH Plus sealer penetration into dentinal tubules. As a result, the null hypothesis that evaluated was rejected.

Dentin treatment with 3% GTE as a final flush improved AH Plus sealer-dentin interaction by increasing surface wettability, sealer penetration into dentinal tubules, and PBS to Nanochitosan-treated dentin. This could be due to GTE's collagen stabilizing ability. GTE and collagen fibrils interact by forming complexes that are principally stabilized by hydrogen bonding between the hydroxyl groups of GTE and collagen's amide linkages. Additionally, GTE not only binds to collagen, but it may also block collagenase access to the active site and impede collagenase's collagenolytic action ⁽²⁸⁾.

Furthermore, due to the cationic nature of chitosan, the amino group can be protonated ⁽²¹⁾, allowing it to combine with other negatively charged molecules like GTE ⁽⁴⁹⁾. This could result in greater adsorption into the RC dentin and a significant decrease in the contact angle and surface tension, leading to better wettability and deeper penetration into the dentinal tubules.

On the contrary, the use of GTE significantly decreased wettability, sealer penetration into dentinal tubules, and PBS to EDTA-irrigated dentin. The negative effect of GTE following the use of EDTA, which represented a significant increase in the contact angle and a significant decrease in the PBS, could be related to the surface charge of both substances. As reported by Gomes et al. (2013) ⁽⁵⁰⁾, EDTA is anionic in nature owing to the presence of carboxylate groups. Therefore, the addition of another negatively charged substance like GTE leads to the creation of repulsive forces, which is reflected in increased surface tension, decreased wettability, and decreased PBS.

To our knowledge, there is no research on the effect of GTE on wettability and PBS of AH Plus sealer to intraradicular dentin treated with 0.2% Nano chitosan. Therefore, the current results cannot be directly compared to those of any previous study.

The results of the current study were in agreement with previous studies^(51,52), which reported that the EDTA-GT group produced lower PBS values compared to EDTA group. However, the results were not statistically significant. This could be due to differences in contact times of both EDTA and GT (1 min). On the other hand, the current results were inconsistent with previous research⁽³³⁾.

Regarding the effect of chelating agents, the CA of 17% EDTA-treated dentin surfaces was significantly lower than those treated with 0.2% Nano chitosan, denoting better wettability. Moreover, EDTA-treated dentin surfaces recorded statistically significantly higher PBS values compared to those treated with 0.2% Nano chitosan at middle and apical levels. Additionally, the use of EDTA resulted in an increase in AH Plus sealer penetration into the dentinal tubules compared to the use of Nano chitosan. This might be attributed to the addition of surfactant as cetrimide and sodium lauryl ether sulfate in the composition of EDTA that decreases the surface tension and improves the wettability of root dentin⁽⁵³⁾. This in turn improves the chelator's potential to penetrate the dentin walls, as revealed in the CLSM micrographs.

Chitosan is cationic in nature since it contains a large number of free hydroxyl and amino groups, enabling the ionic interaction with calcium dentin ions, thus providing its chelating action⁽²¹⁾. However, the 0.2% Nano chitosan group has a larger CA than the EDTA group due to its viscous nature.

The results of CA were in agreement with the findings of a previous study⁽⁸⁾, which found that using EDTA enhanced the wettability of AH Plus sealer. Conversely, the current results contradict the findings of earlier investigations^(9,54), which concluded that EDTA considerably decreased the

surface energy of dentin and reduced the wetting ability of the dentinal wall.

The current results were consistent with a recent study done by Choudhury et al. (2020)⁽⁵⁵⁾, who concluded that EDTA significantly produced higher PBS compared to 0.2% chitosan. Moreover, sealer penetration results were in agreement with Aydın et al (2019)⁽³⁹⁾, who concluded that EDTA enhanced sealer penetration that was superior to chitosan nanoparticle penetration. On the other hand, previous researches contradicted this study's findings, which reported that 0.2% chitosan provided the same bond strength as 17% EDTA^(26,31,56) and similar sealer penetration at the middle and apical thirds⁽³¹⁾.

Regarding the PBS results at different RC levels, 0.2% Nano chitosan group recorded the highest PBS value at the middle level in subgroup B (3% GTE). Moreover, EDTA group, either subgroup A or B, the highest PBS value was recorded at the middle level compared to the coronal level. This could be due to the fact that the diameter of dentinal tubules dropped dramatically from coronal to apical directions, resulting in smaller dentinal tubules diameter at the middle level compared to the coronal one⁽⁵⁷⁾, with more intertubular dentin containing collagen fibers to which AH Plus sealer bonds. This is in turn, increased the strength of the covalent bonds formed with the epoxide ring of AH Plus sealer.

The present study's main strength is that it is the first to provide a detailed assessment of the impact of GTE, when applied after 0.2% Nano chitosan, a naturally-based chelating agent, on wettability, depth of penetration, and push out bond strength of AH Plus sealer to intraradicular dentin.

CONCLUSION

Considering the limitation of the present study, GTE improved the wettability, depth of penetration, and push out bond strength of AH Plus sealer to intraradicular dentin treated with Nano chitosan. Nevertheless, it adversely affected those properties of AH Plus sealer to EDTA-treated dentin.

RECOMMENDATIONS

Further research is needed to determine the optimal GTE concentration and time of application, as well as to assess the collagen's biodegradation resistance over time that affects the interfacial bond durability.

Conflict of Interest:

The authors declares no conflict of interest.

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