

Comparative Evaluation of Different Irrigation Fluids on Smear Layer Removal, Root Canal Sealant Penetration Effect and Mechanical Properties of Human Dentin

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Research Article

Keywords: Root canal irrigation, Morinda citrifolia, Smear layer, Root canal sealer, Mechanical properties

Posted Date: August 19th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1949204/v1>

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Abstract

Objective: To compare the removal of smear layer from root canal wall with different irrigation solutions *in vitro* and evaluate the penetration of root canal sealers into dentinal tubules and their effects on the mechanical properties of dentin.

Methods: 69 human single-rooted premolars and 36 human molars were selected and randomly divided into 5 experimental groups and negative control group according to different irrigation regimens. 5.25%NaClO (A),6%MCJ (B),17%EDTA (C),5.25%NaClO + 17%EDTA (D),6%MCJ + 17%EDTA (E) and negative control group (Distilled water) (DW). Each group were selected for scanning electron microscopy, hot gutta-percha vertical compression filling and micro-hardness testing randomly (n=4). Each group were selected for flexural strength, compressive strength, and ultimate tensile strength testing (UTS), randomly (n=6).

Results: The removal effect of smear layer in groups D and E was better than that in groups A, B and the control group ($P < 0.05$). The maximum depth and penetration rate of root canal sealer penetration were significantly greater in groups D and E than the control group ($P < 0.05$). Group B and E significantly reduced the mechanical properties of dentine (micro-hardness, flexural strength, compressive strength and UTS) ($P > 0.05$).

Conclusion: The combination of MCJ and EDTA could better remove the smear layer and promote the penetration of root canal sealers. Furthermore, they had less detrimental effect on the mechanical properties of dentin than the combination of NaClO and EDTA.

Clinical Significance: Because traditional Chinese medicine root canal irrigation *Morinda citrifolia* has good biocompatibility, antibacterial properties and less adverse effects on the mechanical properties of dentin than NaClO, it is expected to replace NaClO as an ideal root canal irrigating solution in clinical practice.

1 Introduction

Root canal therapy is the first choice for the treatment of endodontic and periapical diseases, and root canal preparation is one of the key steps in root canal therapy, which includes mechanical and chemical preparation. During root canal preparation, a large amount of smear layer will be produced and tightly combined with the root canal wall. The presence of smear layer hinders the entry of root canal disinfectants and root canal sealers into the dentinal tubules. Therefore, root canal chemical preparation plays an important role in the disinfection of the root canal system and the removal of smear layer.

Sodium hypochlorite (NaClO) is the main irrigating solution widely used in the endodontic treatment due to its antimicrobial action and solvent capacity on organic tissues [1]. The tissue solubility of NaClO and the toxicity of periapical tissues are concentration-dependent. High concentrations of NaClO could dissolve infected or necrotic tissues in the root canal and has a better cleaning and bactericidal effect on

the root canal system, but its toxicity to periapical tissues is also increased [2]. Furthermore, NaClO decreases the mechanical properties of dentin [3, 4] and promotes structural changes in inorganic and organic dentin components, especially collagen [5]. As one of the most effective calcium chelating agents at present, Ethylenediaminetetraacetic acid (EDTA) has a lytic effect on dentin, which can not only remove the smear layer to a certain extent [3], but also lubricate and suspend dentin debris, and help expand narrow, fine, calcified or obstructed root canals [6, 7].

Morinda citrifolia has been demonstrated to have a wide range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, antihypertensive, and immunopotentiating effects [8]. There are relatively few relevant studies on the application of *Morinda citrifolia* in the field of dentistry at home and abroad, but some studies have also confirmed that *Morinda citrifolia* has the possibility of application in root canal therapy or oral mouthwash due to its good antibacterial effect in recent years. Our previous study has confirmed that compared with NaClO, *Morinda citrifolia* had less effect on cell proliferation, cell morphology, and cell migration of human stem cells from the apical papilla (hSCAPs) and human periodontal ligament fibroblasts (hPDLFs); and it had less effect on odontoblastic differentiation and mineralization of hSCAPs. Therefore, the present study will further evaluate the removal effect of *Morinda citrifolia* in the smear layer, the penetration effect of root canal sealer and its effect on the mechanical properties of dentin, in order to provide an experimental basis for the clinical application of *Morinda citrifolia* as a root canal irrigant.

2 Materials And Methods

2.1 Sample selection

This study was approved by the Medical Ethics Expert Committee of Harbin Medical University. 69 healthy and intact single-rooted human extracted teeth with no previous root canal treatment and 36 non-carious human third molars were collected and stored frozen. Visual and radiographic evaluations were employed to select teeth with a single canal, normal pulp chambers, no root curvatures, patent root canals, and entirely formed apices without any evidence of resorption.

2.2 The effect of irrigation fluid on smear layer removal and penetration of root canal sealer

48 single-rooted premolars were randomly selected and 12 ± 0.5 mm standard root length was obtained after crown removal using a low-speed diamond saw (SYJ-150, Shenyang Kejing Automated Equipment Co., Ltd.). For conventional pulp extraction, a 15# hand stainless steel K-file (Mani, Japan) was inserted into the root canal until the rasp was seen in the apical foramen, subtracted by 1 mm as the root canal working length, and the apex 3 mm was closed with resin (Japanese Pinus). Protaper hand nickel-titanium files (Dentsply, USA) were used to prepare to F3, during which root canals were irrigated with 2 ml of 5.25% NaClO solution for 1 min and dried for later use.

The prepared samples were randomly divided into 6 groups (n = 8) according to different flushing protocols (Table 1). In the flushing process, the needle of syringe shall be deeply inserted as far as possible, with no resistance, no impaction and smooth reflux. After the completion of irrigation in each group, the root canals of each sample were irrigated with 5 ml of distilled water for 1 min to remove the residual irrigation fluid and wiped dry. The above procedures were performed by the same physician.

Table 1
Root canal irrigation program

Groups	Basal Wash (Dose, Time)	End Wash (Dose, Time)
(Control) DW	5.25%NaClO (2ml/1min)	DW (5ml/2min)
(A) NACLO	5.25%NaClO (2ml/1min)	5.25%NaClO (5ml/2min)
(B) MCJ	5.25%NaClO (2ml/1min)	6%MCJ (5ml/2min)
(C) EDTA	5.25%NaClO (2ml/1min)	17%EDTA (5ml/2min)
(D)NACLO-EDTA	5.25%NaClO (2ml/1min)	5.25%NaClO-17%EDTA (2.5 ml/1 min each)
(E)MCJ-EDTA	5.25%NaClO (2ml/1min)	6%MCJ-17%EDTA (2.5 ml/1 min each)

2.2.1 Smear layer of root canal wall observed and scored by scanning electron microscopy

Four samples were randomly selected from each group for scanning electron microscopy to observe the smear layer on the root canal wall. The root halves were dehydrated in ascending ethanol and dried in an incubator at 37°C for 24 hours. Subsequently, they were mounted on stubs and coated with 20-nm thick gold-palladium for the SEM evaluation. One representative image of the apical (2–3 mm), middle (5–4 mm), and coronal (8–9 mm) thirds from each sample were obtained at a magnification of 500×,1,000× and 2,000× [9].

The images were classified using a 4-category scoring system adapted from Gambarini [10]and Laszkiewicz [11] as follows: score 1, open dentinal tubules, with no debris; score 2, open dentinal tubules, with debris covering less than 50% of the area; score 3, open dentinal tubules, with debris covering more than 50% of the area; and score 4, dentinal tubules covered by debris in 100% of the area examined.

2.2.2 The evaluation of the penetration of root canal sealer in dentinal tubules by confocal laser scanning microscopy

The remaining four samples in each group were root canal obturated using AH Plus root canal sealer (Dengshibai, USA) and hot gutta-percha vertical pressurization, and 0.1% rhodamine B dye was added to the sealer for observation and analysis under a confocal laser scanning microscope. Each sample was

axotomized using a diamond cutter with 1 mm thick cross-sectional sections at 2 mm (apical), 5 mm (middle), and 8 mm (crown) from the apex. Samples were placed on glass slides and viewed and analyzed under a Zeiss LSM880 laser confocal microscope at 10 × magnification, which has a wavelength of 543 nm.

Image J software were used to measure the maximum depth of root canal sealant penetration into dentinal tubules, the distance from the root canal wall to the maximum depth of root canal sealant penetration into dentinal tubules, as well as the root canal sealant infiltration rate and calculate with the following formula:

(1) For numbered lists

Displayed equation Percentage of tubular dentin sealer penetration = $\frac{\text{area filled by sealer} - \text{root canal area}}{\text{dentin area}} \times 100$

2.3 The detection of mechanical properties of dentin

2.3.1 Root dentin micro-hardness assessment

A total of 12 human single rooted premolar teeth were selected and the crowns were removed at the CEJ using a low-speed diamond saw (SYJ-150) under water-cooling. 12 roots were sectioned vertically with diamond disc to produce 24 root halves from each group, which were embedded in acrylic resin. The specimens were polished with abrasive papers to remove any surface scratches [12]. All samples were soaked in 2 ml of 5.25% NaClO, 6% MCJ, 17% EDTA, 5.25% NaClO + 17% EDTA, and 6% MCJ + 17% EDTA for 5 min. The negative control group was soaked for 5 min in distilled water. Then, the samples of the experimental group were thoroughly rinsed with distilled water for 2 min, respectively. The roots were divided into three regions of coronal, middle and apical (Fig. 2), and three points were taken 500 μm from the root canal wall in each region for Vickers hardness test, and the applied load parameter was tested to be 100 g, maintained for 15 s, and the values were recorded.

2.3.2 Flexural strength

Three-point flexural strength assessment was performed using molar crown dentin. Eighteen molars were randomly selected. Mid-coronal dentin was cut perpendicular to the longitudinal axis using a low-speed diamond saw (SYJ-150) under abundant water-cooling to obtain dentin disks. The disks were further sectioned using a slow-speed diamond saw (SYJ-150) to produce rectangular-shaped beams (1.79 mm ± 0.19 mm thickness × 1.98 mm ± 0.53 mm width × 9.30 mm ± 1.30 mm length) per tooth. Thirty-six dentin beams were randomly divided into six groups as the groups above (n = 6), and soaked for 5 min. Then, the samples were thoroughly rinsed with distilled water for 2 min to prevent continuous demineralization of the dentin surface.

Flexural testing was performed using a miniature three-point flexure device with a 6-mm support span. Each 9.30 mm ± 1.30 mm long beam was placed on top of the support span and loaded to fracture under water using a universal testing machine (Instron Corporation, USA) at a crosshead speed of 0.5 mm/min.

Flexural strength (in MPa) was calculated using the formula $3PL/2bd^2$, where P = load at fracture [N], L = length of support span [mm], b = beam width [mm] and d = beam thickness [mm] [13] (Fig. 3).

2.3.3 Compressive strength

Compressive strength assessment was performed using molar crown dentin. Eighteen molars were treated as the methods in 2.4.2. With the universal material testing machine, each sample was successively placed between the upper and lower platens of the test device, and the strain rate for the compression strength test was set at 5 mm/min (Fig. 4).

2.3.4 Ultimate tensile strength (UTS)

A parallel cut to the long axis of single-rooted extracted teeth was made by a slow-speed diamond saw (SYJ-150) to obtain two halves of the root canal. Each half was sectioned at the center perpendicular to the long axis and 4 specimens were obtained from each root. The specimens were manually trimmed to an hourglass shape by using a cylindrical diamond bur with a cross-sectional area of approximately 1.0 mm². Then, each specimen was tested in tensile load at 5 mm/min in a testing machine. The cross-sectional area at the fracture site was measured with a digital caliper and the UTS was calculated by the formula F/A , where F (N) is the force used and A (mm²) is the area in the fracture site [4], and expressed in MPa (Fig. 5).

2.4 Statistical analysis

All data were statistically processed using SPSS 25.0 software. Comparisons of smear layer scores between the groups were performed using the Kruskal-Wallis H-test. Quartile non-parametric tests were used to compare the root canal sealer penetration depth and permeability data between the groups. The data of each group of micro-hardness, Flexural strength, compression strength, and ultimate tensile strength were tested for normality and homogeneity of variance, respectively. If the variances were homogeneous, one-way analysis of variance was used to compare multiple groups of data, the LSD method was used for multiple comparisons, and if the variances were heterogeneous, the Welch test was used and the Games-Howell method was used for multiple comparisons. $P < 0.05$ was statistically significant. Data are expressed $\bar{x} \pm s$.

3 Results

3.1 Smear Layer Removal of Root Canal Wall by Different End Irrigation Fluid

The results of scanning electron microscopy showed that almost all dentinal tubules were covered with smear layer in groups A and B. In areas C, D, and E, a large number of dentin tubules were observed to be open in the crown, and only some dentin tubules were open in the middle, while the number of dentin tubules was the least in the apical.

In the crown, the removal effect of smear layer in groups D and E was better than that in group A ($P < 0.05$). There was no significant difference in smear layer between middle and apical groups ($P > 0.05$). The removal effect of smear layer in the crown of C, D and E was significantly better than that in the apical ($P < 0.05$), as shown in Table 2 and Fig. 6.

Table 2
Comparison of smear layer score among groups after different irrigant activation methods

Groups	Coronal	Middle	Apical	<i>P</i>
(Control) DW	4 (4;4) aA	4 (4;4) aA	4 (4;4) aA	1
(A) NACLO	4 (4;4) aA	4 (4;4) aA	4 (4;4) aA	1
(B) MCJ	4 (3;4) aA	3 (3;4) aA	3 (3;4) aA	0.533
(C) EDTA	1 (1;2) aB	2 (1.8;3) abB	3 (3;3.3) bB	0.009
(D) NACIO-EDTA	1 (1;2) aB	2 (2;2.3) abB	3 (3;3) bB	0.001
(E) MCJ-EDTA	1 (1;3) aB	2 (1.8;2) abB	3 (3;3) bB	0.001
<i>P</i>	<0.001	<0.001	<0.001	
Note: Different uppercase letters indicated significant differences among groups; different lowercase letters indicated significant differences among different parts in each group				

3.2 The effect of different terminal irrigation protocols on the penetration of root canal sealer

3.2.1 Maximum penetration depth of root canal sealer

In the crown, the maximum penetration depth of root canal sealers in groups D and E were significantly greater than that in the control group ($P < 0.05$).

In C, D, E and control groups, there was no significant difference between the crown and the middle, and there were significant differences compared with the apical ($P < 0.05$), as shown in Table 3 and Fig. 7.

3.2.2 The root canal sealant infiltration rate

In the crown, the penetration rate of root canal sealer in groups D and E was significantly greater than that in the control and group A ($P < 0.05$); in the middle, the penetration rate of root canal sealer in groups C, D, and E was significantly greater than that in the control and group A ($P < 0.05$); in the apical, the penetration rate of root canal sealer in group D was significantly greater than that in the other five groups ($P < 0.05$).

In group C, the root canal sealer permeability was similar between the crown and the middle, but significantly greater than that in the apical ($P < 0.05$), as shown in Table 4 and Fig. 7.

Table 3

Comparison of the maximum depth of sealer penetration among groups after different irrigating solution

Groups	Coronal	Middle	Apical	F	P
(Control)DW	1167.00 ± 78.31aA	1353.67 ± 147.77aA	721.33 ± 90.36bA	33.425	0.001
(A)NACLO	1920.00 ± 1223.50aAB	1389.33 ± 906.03aA	921.33 ± 986.31aA	0.683	0.541
(B)MCJ	1749.00 ± 166.90aAB	1528.67 ± 222.06aA	1695.67 ± 378.22aA	0.540	0.609
(C)EDTA	2468.00 ± 128.29aAB	2239.67 ± 372.04aA	1208.33 ± 323.46bA	15.622	0.004
(D)NACLO-EDTA	2776.67 ± 395.44aB	2497.00 ± 443.47aA	1902.00 ± 199.87bA	4.570	0.062
(E)MCJ-EDTA	2615.33 ± 346.07aB	2286.67 ± 219.08aA	1253.00 ± 692.70bA	7.024	0.027
F	3.718	3.718	2.072		
P	0.029	0.029	0.140		
Note: Different capital letters indicated significant differences between groups; Different lowercase letters indicated significant differences in the components within each group.					

Table 4

Comparison of the percentage (%) of sealer penetration among groups after different irrigant activation methods

Groups	Coronal	Middle	Apical	F	P
(Control) DW	3.96 ± 0.24aA	2.53 ± 0.14aA	2.54 ± 0.16aA	59.322	<0.001
(A) NACLO	5.03 ± 2.05aA	3.13 ± 2.38aA	1.99 ± 1.88aA	1.581	0.281
(B) MCJ	9.68 ± 1.83aAB	8.17 ± 3.95aAB	3.80 ± 1.22aA	4.108	0.075
(C) EDTA	13.42 ± 5.13aAB	15.43 ± 4.31aB	4.68 ± 2.74bA	5.609	0.042
(D) NACLO-EDTA	27.81 ± 11.23aC	17.49 ± 5.23aB	20.75 ± 11.88aB	0.851	0.473
(E) MCJ-EDTA	21.95 ± 5.42aBC	18.35 ± 7.73aB	7.38 ± 4.88aA	4.592	0.062
F	8.623	7.291	5.065		
P	0.001	0.002	0.01		
Note: Different capital letters indicated significant differences between groups; Different lowercase letters indicated significant differences in the components within each group.					

3.3 Effects of different irrigating fluids on mechanical properties of dentin

3.3.1 Results of dentin micro-hardness

In the crown, the dentin micro-hardness of groups B and E were not significantly different from that of the control group, while the dentin micro-hardness of groups B and E were significantly greater than that of groups A, C and D ($P < 0.05$); in the middle, the dentin micro-hardness of group B was not significantly different from that of the control group, while the dentin micro-hardness of group B was significantly greater than that of groups A, C, D, and E ($P < 0.05$); in the apical, the dentin micro-hardness of groups B and E were not significantly different from that of groups C and D, but were significantly greater than that of group A ($P < 0.05$) (Table 5).

3.3.2 Results of dentin flexural strength test

The dentinal flexural strength of groups A, B, C, D and E was significantly lower than that of the control group ($P < 0.05$). The dentin flexural strength of group B was significantly higher than that of group A, and the dentin bending strength of group E was significantly higher than that of group D ($P < 0.05$) (Table 6).

3.3.3 Experimental analysis of dentin compression strength

The dentin compression strength of groups A and D was significantly lower than that of the control group ($P < 0.05$), and the dentin compression strength of groups B and E was not significantly different from that of the control group. The dentin compression strength of groups B and E was significantly higher than that of groups A and D ($P < 0.05$) (Table 6).

3.3.4 Results of dentin ultimate tensile strength

The ultimate tensile strength of groups A, B, C, D and E were significantly lower than that of the control group, and the ultimate tensile strength of dentin of groups B, D and E were significantly higher than that of groups A and C ($P < 0.05$) (Table 6).

Table 5
Descriptive statistics of inter group mean difference comparison of micro-hardness test among different groups.

Groups	Coronal/HV	Middle/HV	Apical/HV	F	P
(Control) DW	66.85 ± 3.08ACDE	71.95 ± 4.38ACDE	61.95 ± 3.82AD	-	-
(A) NACLO	47.90 ± 8.48BD	55.33 ± 4.71BCE	51.65 ± 9.66BCE	1.326	0.295
(B) MCJ	61.38 ± 4.85ACD	70.13 ± 2.64ACDE	59.73 ± 3.87A	12.367	0.001
(C) EDTA	50.08 ± 4.13BE	61.95 ± 2.36ABD	58.71 ± 6.93A	9.589	0.002
(D) NACLO-EDTA	49.62 ± 4.70BE	54.93 ± 2.36BCE	54.65 ± 3.39	4.107	0.038
(E) MCJ-EDTA	56.83 ± 1.10ACD	62.08 ± 3.86ABD	59.23 ± 1.53A	6.746	0.008
F	14.214	24.778	2.792		
P	< 0.001	< 0.001	0.035		
*One way ANOVA test was used for coronal, middle and apical.					
Different capital letters represented significant differences among groups.					

Table 6
Mean and standard deviation (SD) of flexural strength, compressive strength and ultimate tensile strength and statistical categories.

Groups	Flexural strength /MPa	Compressive strength/MPa	Ultimate tensile strength/MPa
(Control) DW	199.39 ± 6.25ABCDE	75.06 ± 0.04AD	43.25 ± 10.90ABCDE
(A) NACLO	96.80 ± 24.55B	65.77 ± 7.73BCDE	8.22 ± 2.38BCDE
(B) MCJ	138.63 ± 23.77ACDE	74.00 ± 2.56AD	21.97 ± 3.15AC
(C) EDTA	99.43 ± 11.71BD	74.76 ± 0.67AD	14.12 ± 2.68ABDE
(D) NACLO- EDTA	79.48 ± 11.71BCE	58.32 ± 8.68BCE	20.00 ± 1.13AC
(E) MCJ-EDTA	114.16 ± 11.20BD	75.03 ± 0.04AD	24.58 ± 2.01AC
F	41.573	12.279	35.022
P	< 0.001	< 0.001	< 0.001
*One way ANOVA test was used for flexible strength, compressive strength and ultimate tensile strength.			
Different capital letters represented significant differences among groups.			

4 Discussion

Cleaning and tight three-dimensional filling of the root canal system is the key to successful root canal therapy. The root canal system is extremely complex, it consists of the main root canal and many lateral accessory root canals, and mechanical preparation alone can only remove part of the infectious material. Therefore, it is clinically necessary to supplement root canal irrigation to further clean the root canal system. NaClO is a highly effective and rapid broad-spectrum antibacterial agent that can effectively kill a variety of pathogenic microorganisms such as bacteria, phages, fungi, and spores. Its commonly used concentration is 0.5% ~ 5.25%. With the increase of concentration, its antibacterial effect is also enhanced [14]. However, it has the disadvantages of high toxicity, strong corrosion and lack of continuous antibacterial ability [15, 16]. EDTA is a potent chelating agent with the effects of lubricating the root canal wall and removing the smear layer, but it is corrosive to the root canal wall, and the longer the contact time, the more severe the degree of corrosion [17]. Studies have confirmed that EDTA can completely remove the smear layer in contact with the root canal wall for 1 min [18], so in this experiment, the combined irrigation time of the two irrigation solutions was set as 1 min for each irrigation solution and 2 min for a single irrigation solution.

The smear layer in the root canal, composed of organic and inorganic substances, including dentin debris, bacteria, and residual pulp, can occlude dentinal tubules and contribute to the adhesion and colonization of bacteria and endotoxin on the root canal walls [19]. Studies have shown that NaClO or MCJ alone cannot completely remove the smear layer [20], and the results of smear layer score in the present study were consistent with them, so it needed to be used in combination with chelating agents such as EDTA. The smear layer removal effect was similar between group D (NaClO-EDTA) and group E (MCJ-EDTA) in the crown, middle and apical (Fig. 2), which is consistent with the findings of Peter E. Murray et al [20]. In the crown and middle, the smear layer removal effect was significantly better in group D (NaClO-EDTA) than in the negative control group ($P < 0.05$), indicating that in the combined irrigation protocol, NaClO as the initial irrigation fluid could improve the ability of EDTA to remove the smear layer, which may be due to the fact that NaClO could remove the organic material from the smear layer and form a diffusion channel, thus allowing EDTA to penetrate into the dentinal tubules and peritubular dentin faster [21].

In general, the effect of terminal irrigation from the crown to the apical on smear layer removal ability and root canal sealer penetration ability is gradually reduced, which may be due to two reasons. Firstly, the smaller root canal diameter in the apical region resulted in little flow of irrigant reaching this region. Secondly, the presence of more sclerosing dentin in the apical region was not conducive to the delivery of irrigation fluid and the removal of smear layer, which directly affected the penetration effect of root canal sealers [22]. In the present study, group C, group D and group E had better smear layer removal effect in the crown, while the apical had the worst effect, which was consistent with the results of previous studies [23, 24].

Gutta-percha is the main material for root canal obturation, but it has poor sealing properties. Root canal sealer can fill the space between gutta-percha and gutta-percha, and between gutta-percha and root canal wall, and play a stabilizing role in irregular root canal structure [25]. In addition, its penetration in dentinal tubules can kill residual microorganisms and their toxins, thus preventing the occurrence of root canal therapy failure and reinfection [26]. The resin root canal sealer AH-Plus is used as the gold standard for root canal sealer research due to its good biocompatibility, physicochemical properties, as well as tissue tolerance [27]. Therefore, this experiment evaluated the effect of different terminal irrigation protocols on the maximum depth and permeability of resin root canal sealers to penetrate in dentinal tubules. In this experiment, the maximum penetration depth of group D and group E in the crown was significantly greater than that of the control group (Table 3). In the crown, only the root canal sealer permeability of group D and group E was significantly greater than that of the control group. In the middle, the root canal sealer permeability was significantly greater in groups C, D and E than in the control group. However, in the apical, root canal sealer penetration in group D was the greatest compared with other groups (Table 4). The apical area is the main area where the lateral branch root canal exists, and the traditional mechanochemical preparation method reaches it, which leads to the failure of root canal therapy due to residual microorganisms and their by-products. In the apical, root canal sealer penetration was greatest in group D, but it was not completely consistent with the results of smear layer scoring. It has been shown that the penetration effect of root canal sealers in dentinal tubules is affected by a variety of factors,

including the removal of smear layer, the permeability of dentin, and the filling technique [28–30]. The physical and chemical properties of root canal sealers can affect the penetration effect, such as the fluidity of sealers, the time and temperature of harmony, the number of lifts and insertions when introducing the root canal [31].

Dentin is a matrix with complex organic and inorganic structures, which is composed of 22% water and organic matter, most of which are composed of type I collagen and inorganic enhanced phase of carbonate apatite, which plays a great role in maintaining its mechanical properties [32]. Dentin should have good mechanical properties to function normally during mastication. Clinically, it is generally believed that teeth that have undergone root canal therapy are more fragile and more prone to fracture than normal teeth [33,34]. Carter JM et al. [35] suggested that the reduction of tooth structure and dehydration of dentinal tubules are the main reasons for the fragility and increased fragility of root-treated pulpless teeth. However, Reeh ES et al. [36] showed that tooth hardness was reduced by only 5% during root canal therapy. Therefore, the role of water in the biomechanical properties of the tooth has not been truly determined, and it is believed that there is no difference in mechanical properties between vital and pulpless teeth after root canal therapy [37]. The micro-hardness, flexural strength, compressive strength, and ultimate tensile strength of dentin [30] are common parameters to assess the effect of irrigation fluid on the hard tissue of the remaining healthy tooth. Marending M et al. [38] showed that NaClO solution had a significant concentration-dependent effect on the mechanical properties of dentin, so the highest concentration of 5.25% NaClO in the safe range was selected in this experiment. Moreira DM et al. [39] have shown that the use of NaClO as a root canal irrigating solution leads to disorganized organic structure and loss of structure of dentin in the wall. Since the destruction of the organic components of dentin by NaClO affects the mechanical properties of dentin, it is necessary to propose alternative cleaning agents that are antibacterial and do not impair mechanical properties of tissue. Since a single load rarely appears in the mouth and the geometry of the tooth is irregular, the tooth is frequently subjected to multiple uncertain loads [40]. The test indexes of dentin mechanical properties of different irrigation fluids are relatively single, so four indexes, micro-hardness, flexural strength, compression strength, and ultimate tensile strength, were selected in this experiment to comprehensively evaluate the effects of different irrigation fluids on dentin mechanical properties.

Hardness refers to the ability of the material itself to resist local deformation when an object is pressed into the material surface, and is an indicator for evaluating the softness and hardness of the material. Microhardness is used as a measure of the overall strength or fracture resistance of dental tissue [41], and its changes reveal changes in the organic and inorganic components of dentin [42]. The Vickers hardness test used in this experiment is not easily affected by surface conditions. Because diamond shaped indentations are easily observed during testing and are more sensitive to measurement error when using the same load, the microhardness measurement is more accurate [43]. Dentin is a natural heterogeneous material, in addition, dentin microhardness is related to the location of the dent, and its value decreases more significantly the closer to the inner wall of the root canal [44]. Therefore, this experimental microhardness test was performed as described by Cruz-Filho et al. [45] with indentation on a straight line parallel to the inner wall edge of the root canal. As shown in Table 5, in the crown, middle

and apical, the dentin micro-hardness of group A and C were significantly lower than that of the control group, while the dentin microhardness of group B was not significantly changed, which was consistent with the result that MCJ did not have a significant adverse effect on dentin microhardness obtained by AR et al. [46] ; And Barcellos DPDC et al. [47], similarly showed that 17% EDTA significantly reduced the micro-hardness of dentin, which in turn showed the potential to promote continuous demineralization of the dentin wall. This potential is due to the ability of EDTA to bind to calcified components of dentin via a chelating mechanism, leading to tissue demineralization and softening [46]. Therefore, after soaking the dentin blocks in 17% EDTA for 5 minutes, rinsing with distilled water must be used to prevent the continuous demineralization of the experimental samples. In the crown and middle, the dentin micro-hardness of group E was significantly higher than that of group D, while there was no significant difference in the apical between group E and group D. It showed that the adverse effect of combined irrigation with 6% MCJ and 17% EDTA on dentin micro-hardness was much smaller than that of combined irrigation with 5.25% NaClO and 17% EDTA, but there was no significant difference between the two in the apical, possibly due to the inability of dentin in the apical area to fully contact with the irrigation fluid. At the same time, it cannot be excluded that the inevitable experimental error is caused by the irregular diamond shape when the micro-hardness test is performed when the sample surface is uneven. Studies have shown that when 17% EDTA persists in the root canal for 10 minutes or for a period of time, the micro-hardness of dentin significantly reduced after root canal therapy [48], while 17% EDTA would be used in combination with 6% MCJ due to the short time of 17% EDTA remaining in the root canal during combined irrigation [49], which had little effect on the micro-hardness of dentin. In the crown, dentin micro-hardness was significantly higher in group E than in group C ($P < 0.05$), which also indicated that the combination of 6%MCJ and 17% EDTA alleviated the adverse effect of 17% EDTA alone on dentin micro-hardness.

In the process of dentin mechanical properties testing, different scholars use different test methods, which can be broadly divided into mechanical test method, ultrasonic test method and ultrasonic resonance test method. In this experiment, the universal testing machine is used, and the displacement method in the mechanical test method is used, that is, the dimensional strain change of the specimen is measured by the displacement sensor, and the magnitude of the force is measured by the force sensor, so as to complete the measurement of the specimen. Strength refers to the maximum stress that the material under load can endure when it fails to reach fracture. For the flexural test, it is expressed by the flexural strength [50]. Flexural strength plays a very important role in maintaining dentin properties, avoiding increased dentin fragility and reducing the risk of vertical fracture of teeth. Therefore, the adverse effects of irrigation fluid on the bending strength of dentin should be minimized. As shown in Table 6, the dentin flexural strength of groups A, B, C, D and E were significantly lower than that of the control group, the dentin flexural strength of group B was significantly higher than that of group A, and the dentin flexural strength of group E was significantly higher than that of group D ($P < 0.05$), which is consistent with the findings that Doglas Cecchin et al. [51] concluded that the use of 6% NaClO significantly reduced the mechanical properties (flexural strength, ultimate tensile strength) of dentin. These results showed that regardless of the irrigation fluid, the experimental group had an adverse effect

on the flexural strength of dentin, but the adverse effect of combined irrigation with 6% MCJ and 17% EDTA on the flexural strength of dentin was much less than that of combined irrigation with 5.25% NaClO and 17% EDTA. The reason why 5.25% NaClO could significantly reduce the flexural strength of dentin was because NaClO can cause disintegration of collagen by breaking down bonds between carbon atoms and oxidizing the protein primary frame [52]. Therefore, the adverse effects of NaClO on the organic matrix of dentin resulted in increased stress concentration and reduced the fracture strength of the tooth [53]. For the compression test, it is expressed as compression strength, which refers to the maximum stress that dentin can withstand when it fails to reach fracture under load. The dentin compression strengths of groups A and D were significantly lower than those of the control group, and the dentin compression strengths of groups B and E were not significantly different from those of the control group ($P < 0.05$). The dentin compressive strength of groups B and E were significantly higher than that of groups A and D ($P < 0.05$). These results indicated that 6% MCJ had less detrimental effect on dentin compressive strength, and the combined irrigation with 6% MCJ and 17% EDTA had less detrimental effect on dentin compressive strength than combined irrigation with 5.25% NaClO and 17% EDTA. The ultimate tensile strength of groups A, B, C, D and E were significantly different from that of the control group ($P < 0.05$), and the ultimate tensile strength of dentin in groups B, D and E were significantly higher than that of groups A and C ($P < 0.05$), which were consistent with the conclusion that 2.5% NaClO and 17% EDTA significantly reduced the ultimate tensile strength of dentin by Farina A.P. et al. [4], indicating that 6% MCJ produced less adverse effects on the ultimate tensile strength of dentin. And when 17% EDTA was used in combination with 5.25% NaClO and 6% MCJ, respectively, it could significantly reduce the detrimental effect on the ultimate tensile strength of dentin when 17% EDTA was used alone as an irrigant. According to the mechanism of drug action, both EDTA and NaClO can significantly change the dentin chemical composition by causing dentin demineralization [53,54]. Changes in the chemical composition of dentin can affect dentin properties and further affect the mechanical properties of teeth [55].

5 Conclusion

In the present study, the adverse effect of traditional Chinese medicine root canal irrigation *Morinda citrifolia* on dentin mechanical properties was significantly lower than that of NaClO. However, because the smear layer could not be completely removed, it needed to be used in combination with EDTA, whose smear layer removal effect and penetration effect of root canal sealer were similar to those of NaClO and EDTA, while the adverse effect on dentin mechanical properties was significantly lower than that of NaClO and EDTA. In view of the good biocompatibility and antibacterial properties of *Morinda citrifolia*, it is expected to replace NaClO as an ideal root canal irrigating solution in clinical practice.

Declarations

Funding

This research was supported by the National Natural Science Foundation of China (No. 81970924) and the National Natural Science Foundation of China (No. LH2021H045).

Conflicts of interest

The authors declare no conflict of interest.

Author Contributions Statement

Author 1 Lan Zhang (First Author): Conceptualization, Methodology, Software, Investigation, Formal Analysis, Writing - Original Draft; Author 2 Ting Zhao: Data Curation, Writing - Original Draft; Author 3 Lina He: Visualization, Investigation; Author 4 Shuang Pan: Resources, Supervision; Author 5 Ranxu Wang: Software, Validation; Author 6 Yanping Li: Visualization, Writing - Review & Editing; Author 7 Yumei Niu (Corresponding Author): Conceptualization, Funding Acquisition, Resources, Supervision, Writing - Review & Editing

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Figures

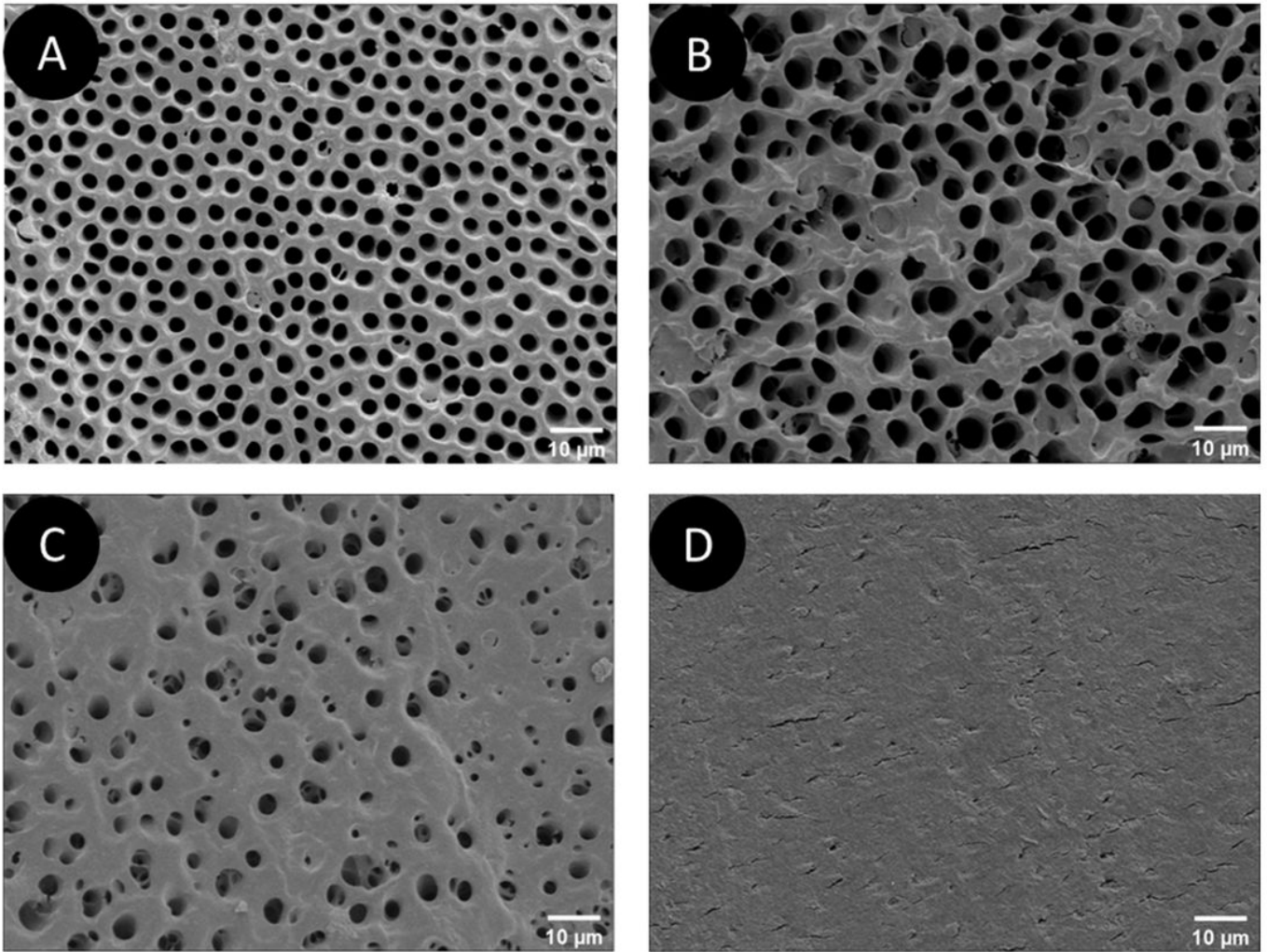


Figure 1

Repersentive images of scores (A) score 1,(B) score 2,(C) score 3,(D) score 4

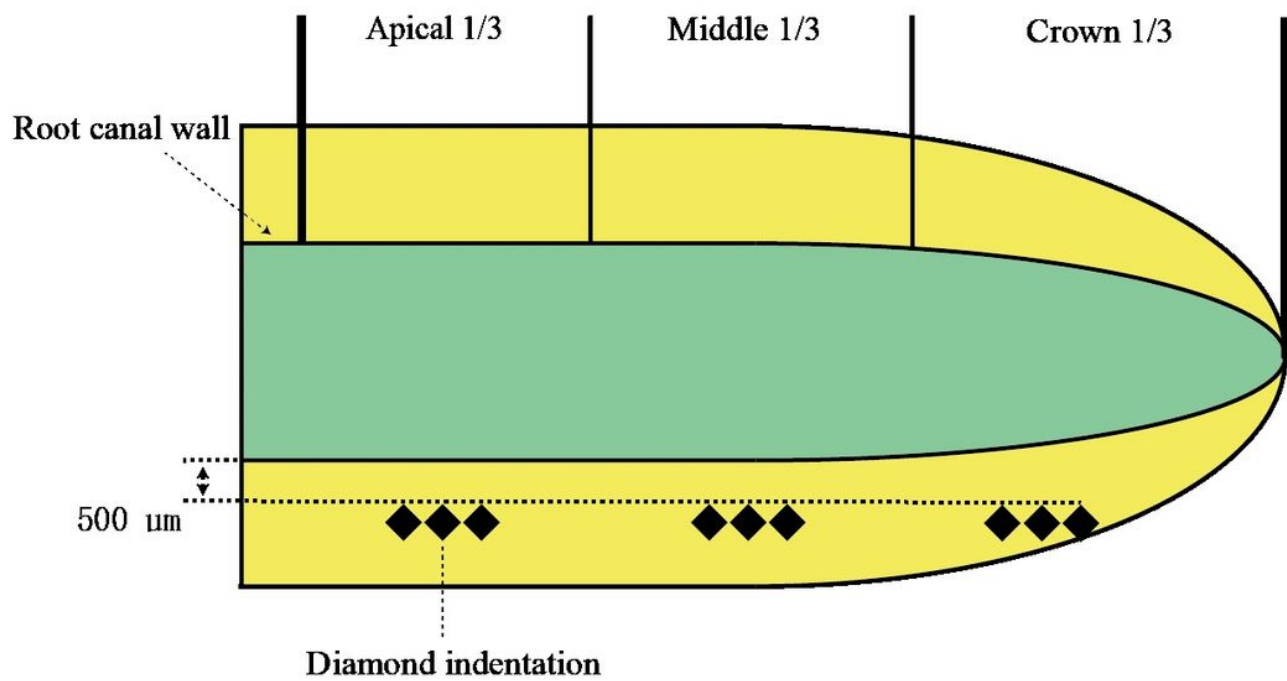


Figure 2

Diagram of diamond indentation in microhardness test

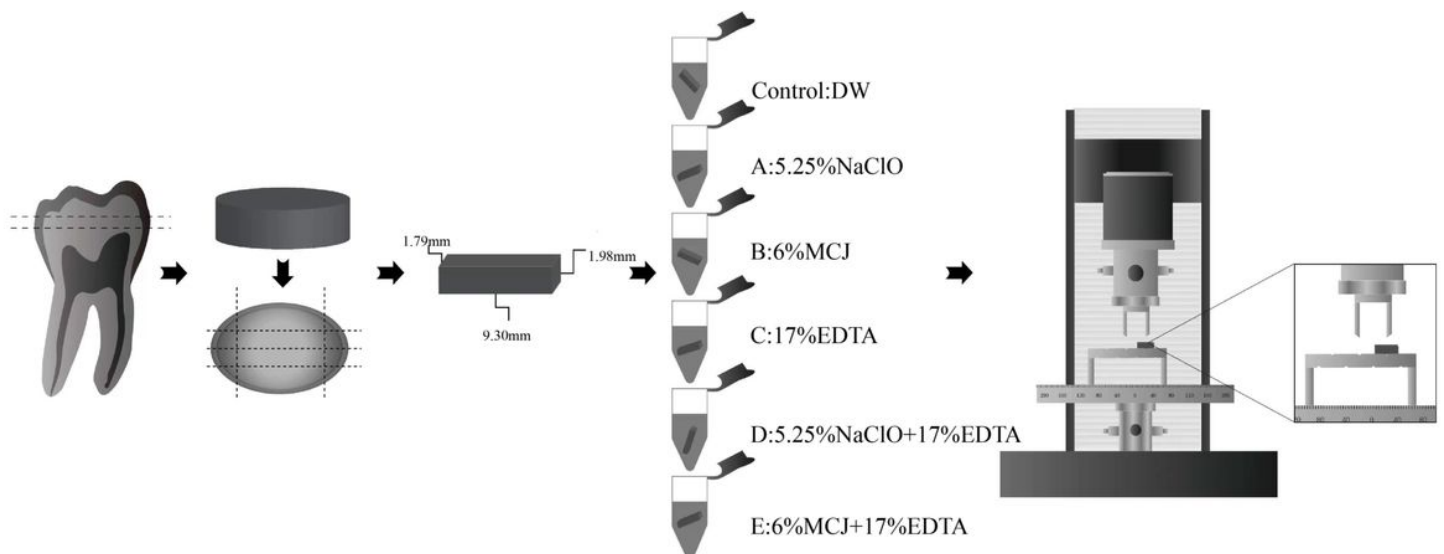


Figure 3

Flexural strength

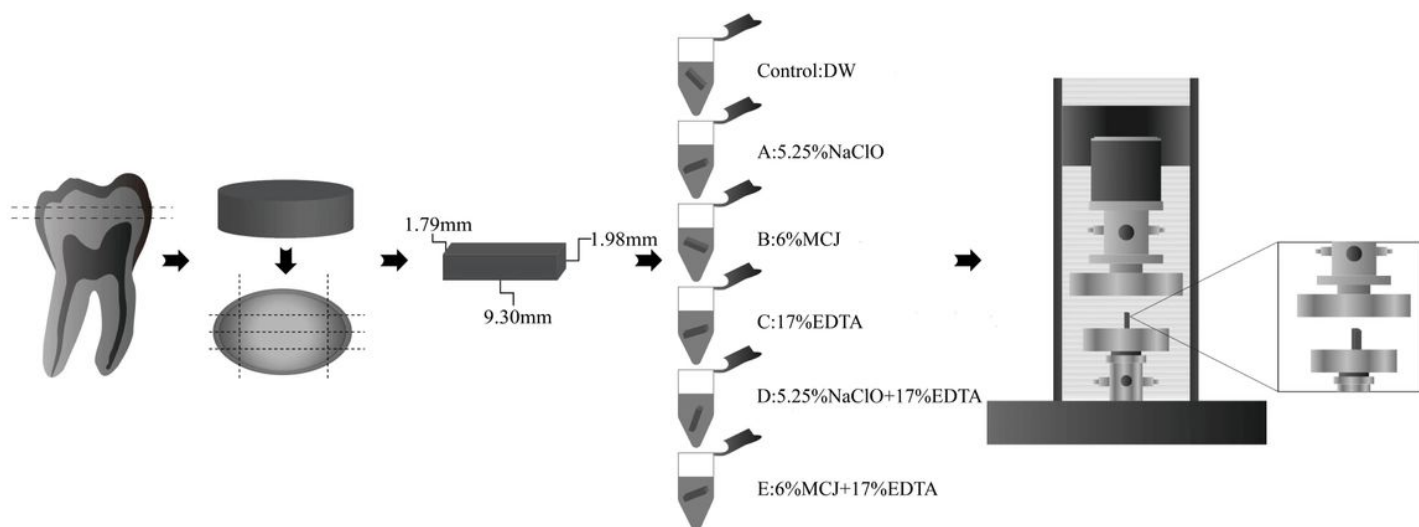


Figure 4

Compressive strength

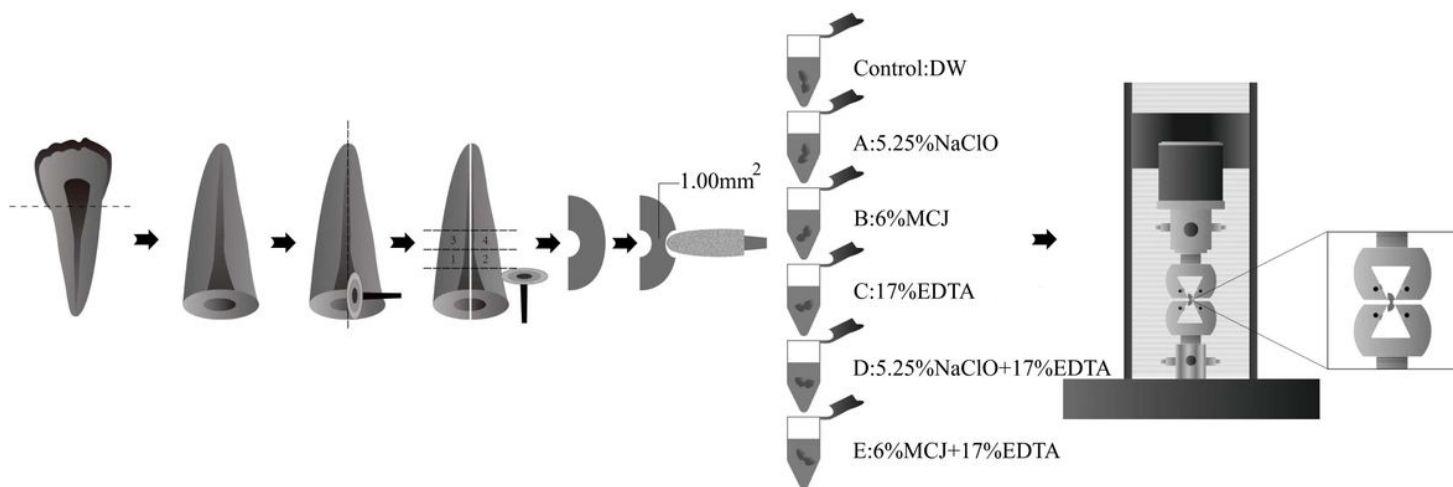


Figure 5

Ultimate tensile strength

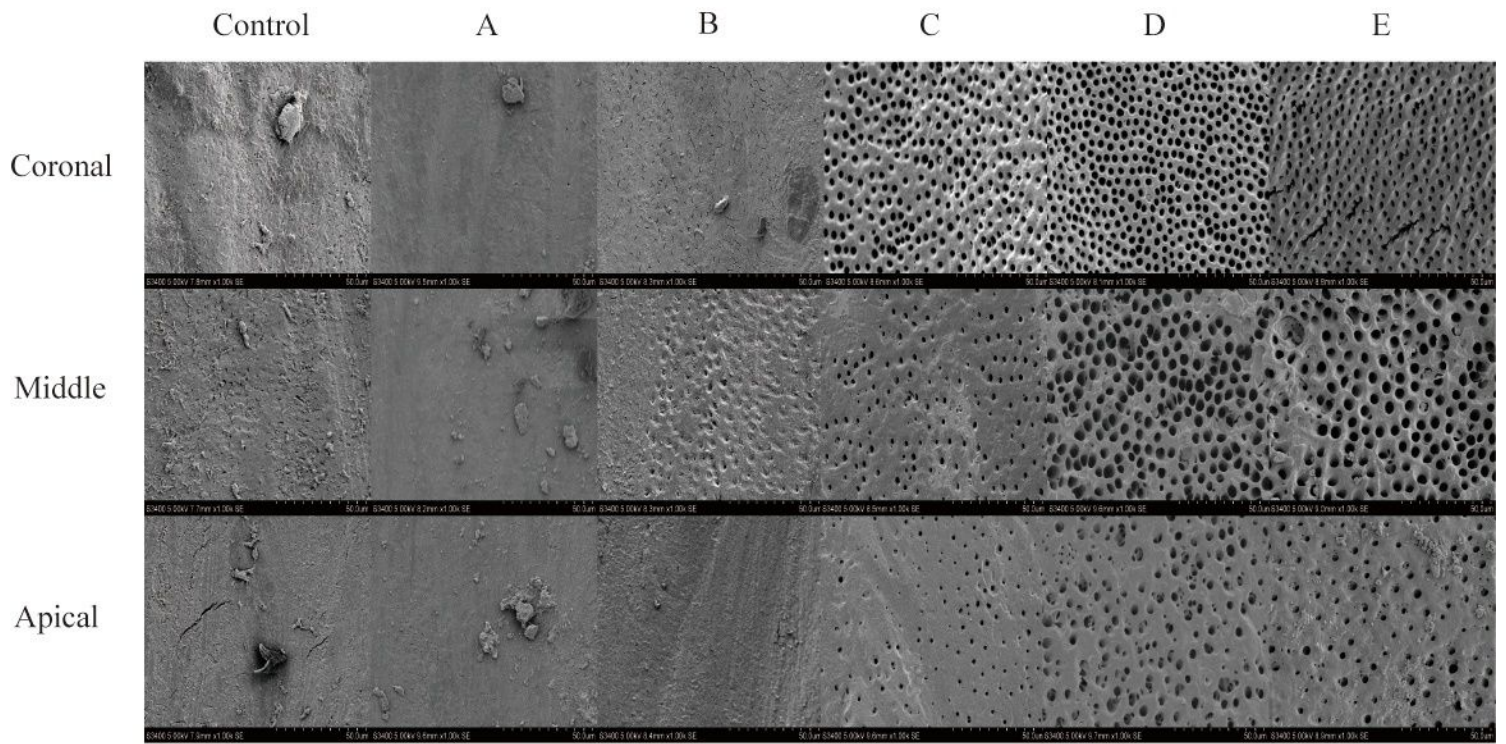


Figure 6

The smear layer removal effect of 6 groups of irrigation methods on various parts of the root canal wall was observed under scanning electron microscope ($\times 1000$)

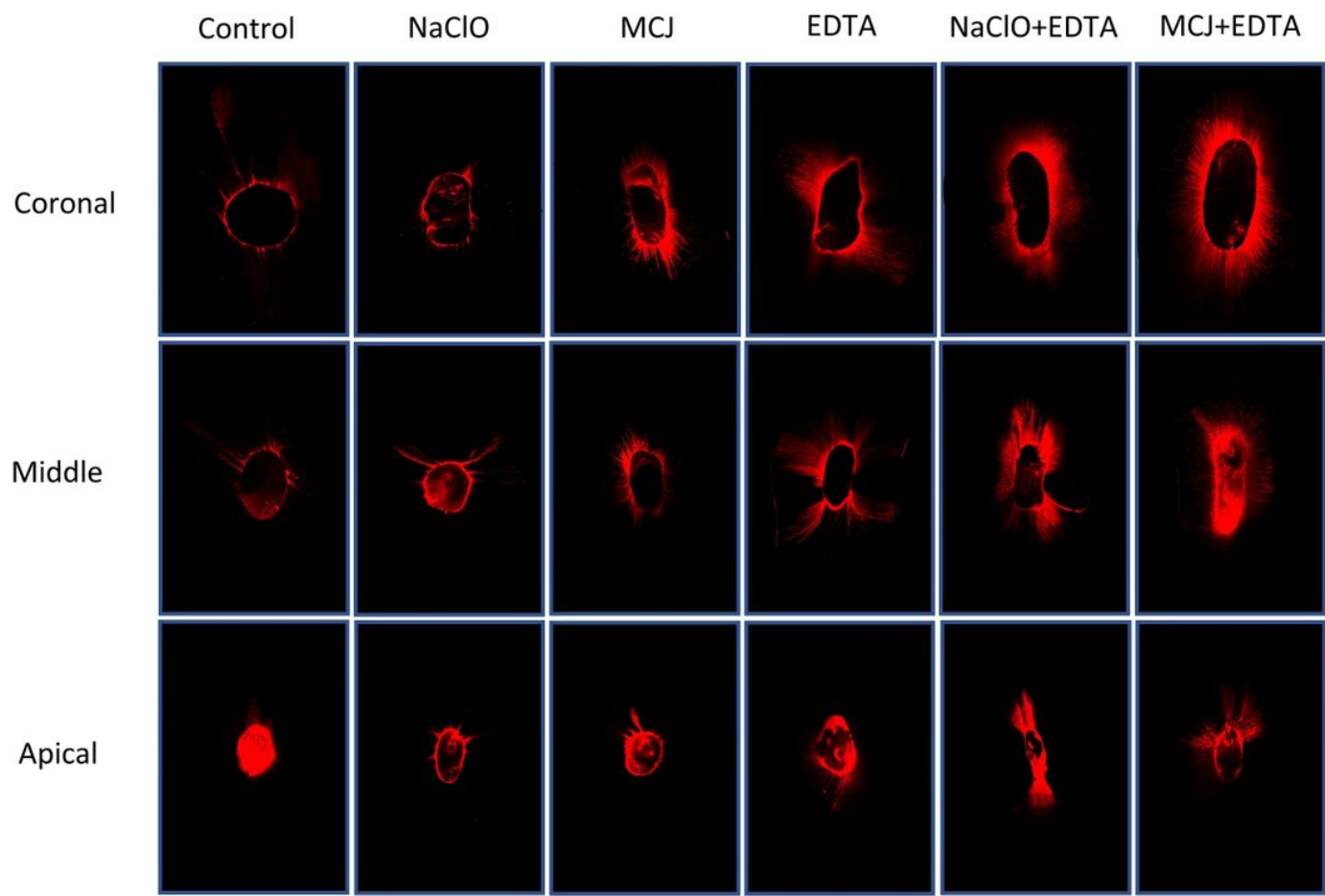


Figure 7

Representative CLSM images of sealer penetration around the root canal walls in each group and root third with a magnification at $\times 10$