JOURNAL OF CONSERVATIVE DENTISTRY

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J Conserv Dent. 2022 Jul-Aug; 25(4): 431–435. PMCID: PMC9520656
Published online 2022 Aug 2. doi: 10.4103/jcd.jcd_178_22: 10.4103/jcd.jcd_178_22 PMID: 36187868

The effect of chitosan nanoparticle, citric acid, and ethylenediaminetetraacetic acid on dentin smear layer using two different irrigation needles: A scanning electron microscope study

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Received 2022 Mar 31; Revised 2022 May 8; Accepted 2022 May 17.

Abstract

Objectives:

The objective of this study is to compare the efficacy of chitosan (CS) nanoparticles (CNPs), citric acid (CA), and ethylenediaminetetraacetic acid (EDTA) in removing the smear layer using two different irrigation needles.

Materials and Methods:

Palatal roots of 70 maxillary first molars were decoronated, instrumented, and divided into four experimental groups (n = 20) and one control group (n = 10). The groups received a final rinse of 0.5% CNPs, 10% CA, 17% EDTA, and distilled water for 3 min. Every group was subdivided into two subsections: IrriFlex® endodontic or ProRinse® irrigation needles. Specimens were divided lengthwise and viewed under a scanning electron microscope for evaluation.

Statistical Analysis Used:

Nonparametric Kruskal–Wallis and Mann–Whitney U-tests were used to compare the results (P < 0.05).

Results:

CNPs were as efficient as CA and EDTA as a chelating agent. However, significantly more efficient apically. At all three levels, there was no significant difference between A1 and A2. At the coronal and middle levels, there was a significant difference between B1 and B2, as well as apically between C1 and C2.

Conclusions:

CNPs remove the smear layer with the same efficiency as other irrigants utilized in this study at coronal and middle levels and more efficiently at the apical levels. IrriFlex® was more effective than ProRinse® in removing the smear layer when used with EDTA and CA, while there was no difference when used with CNPs.

Keywords: Chitosan nanoparticles, citric acid, ethylenediaminetetraacetic acid, IrriFlex®, ProRinse®, smear layer

INTRODUCTION

Root canal treatment comprises three-dimensional cleaning, shaping, and obturation of the root canal system.[1]

During mechanical preparation of the root canal, mineralized debris formed called the smear layer.

The smear layer is a physical barrier that prevents root canal medications and irrigating solutions from diffusing into the dentin matrix. Moreover, the smear layer can trap the bacteria remaining inside the dentin tubules, which occasionally escapes from the chemicals utilized.[2]

Because the smear layer contains both inorganic and organic debris, no currently available root canal irrigants, including sodium hypochlorite (NaOCl), can remove it alone. It is necessary to utilize NaOCl followed by a chelating agent or an acid that dissolves inorganic tissue.[3]

Ethylenediaminetetraacetic acid (EDTA) dissolves the inorganic component of the smear layer, softens dentin, and aids in the breakdown of calcifications that obliterate the root canal. The typical concentration is 17%; removing the smear layer occurs after 1-min contact.[4]

Nonetheless, this substance has a substantial demineralizing effect, widening the dentinal tubules, softening the dentin, and denaturing the collagen fibers. These factors make it harder for the obturator material to penetrate the walls of the root canals. An additional drawback is EDTA which is deemed a pollutant as this matter is not found in nature. Researchers seek alternatives to EDTA by searching for biocompatible solutions that can minimize their harmful influence on the periapical tissues. Citric acid (CA), which is organic and weak, is able to react rapidly with calcium ions, besides having comparatively low cytotoxicity.[5]

In previous studies, this acidic solution was utilized with an array of concentrations ranging from 1% to 50%.[6]

Chitosan (CS) is a natural polysaccharide that is widely utilized in dental research due to its lack of toxicity, biodegradability, biocompatibility, and bioadhesion. It is attained by deacetylation of chitin and discovered in shrimp shells and crab.[7]

There is limited documentation on the chelating properties of CS on dentin.

According to research, the final treatment with 0.2% CS nanoparticles (CNPs) had a similar influence on eliminating the smear layer as 17% EDTA; nevertheless, 0.2% CNP produced more microhardness and minor surface roughness over dentin than 17% EDTA.[8]

Various irrigation methods and devices are being utilized to enhance disinfection of the canals. Conventional endodontic irrigation syringes and needles are the most often utilized because they are simple to handle and allow for precise management of the needle depth as well as irrigant volume supplied.[9]

ProRinse®side-vented needle (Dentsply Tulsa Dental, Johnson City, TN, USA) is an endodontic irrigation needle can successfully remove microbes and pulpal debris from the root canal. They have a round tip and side-window port dispersal that blocks debris and solution from being expressed through the apical foramen.

Another endodontic needle is IrriFlex® (Produits Dentaires SA (PD), Vevey, Switzerland) which has two side vents at the end, located on a single plane. Its manufacturer claims that this unique feature enables balanced irrigant expulsion using two accurate jets aimed precisely at the dentinal walls. As the fluid spreads to the coronal third, the flow thickness of the irrigant is steady, and it maximizes shear force and eliminates debris, smear layer, and biofilm.

MATERIALS AND METHODS

Seventy extracted human maxillary first molars with intact palatal roots and an initial file # 20 (Dentsply Maillefer, Ballaigues, Switzerland) were used in this study. Dental crowns were removed using a high rotation drill, leaving a 13 mm root length. Working length was calculated by deducting 1 mm of root length. Rotary files HyFlex EDM (Coltene/Whaledent, Altstätten, Switzerland) were used to prepare the root. All root canals had been instrumented to Master Apical File corresponding to size 40.

During cleaning and shaping, the canals were irrigated with 5.25% NaOCl (Golden Falcon, Dubai, UAE) and 1–2 mm of working length.[10] All canals were then cleaned with 5 mL distilled water and dried with an absorbent paper point before receiving final irrigation for smear layer removal.

Final irrigation procedures

After biomechanical preparation, all teeth were divided randomly into four groups (n = 20), with the exception of the control group (n = 10) for examination. Group-A: 0.5% CNP (Chitosan Nanoparticles), Group-B: CA (Citric acid) 10%, Group-C: 17% EDTA, and Group-D: Distilled water. After that, every group was subdivided into two subgroups of 10 samples, except for the control group (five specimens). Each subgroup was irrigated with a ProRinse® endodontic nee-

dle and the second with an IrriFlex® endodontic needle. The total volume for final irrigation is 5 ml for 3 min. Last, the root canals were rinsed with 5 mL of distilled water and dried with paper point #40 [Figure 1].

After the final irrigations, the root was separated lengthwise into two halves. The selected half had been examined at three levels: 2.5 mm, 6 mm, and 10 mm from the root apex using a scanning electron microscope (FESEM, TESCAN, Mira 3). All photos taken at ×2000 and ×3000 are reviewed by two calibrated and blinded examiners. The Kappa test (Kappa 0.75) was used to measure the level of agreement between the two examiners.

To measure the quantity of smear layer that has been removed, a scoring system with a range of 1–4 established on the scores defined by Hulsmann was used.[11]

- Score 1: dentinal tubules are fully opened
- Score 2: more than 50% of dentinal tubules are opened
- Score 3: <50% of the dentinal tubules are opened
- Score 4: almost all dentinal tubules are coated with a smear layer.

Data obtained were studied utilizing Kruskal–Wallis, followed by Mann–Whitney *U*-test, with statistical significance ($P < 0.05$).

Preparation of 0.5% Chitosan-Tripoly Phosphate (CS-TPP) nanoparticles: 0.1g Chitosan provided by HIMEDIA (Mumbai, India) was dispersed in 20 ml of 1% (v/v) acetic acid then stirred for 8 h. They were then subjected to a 40-min sonication. Separately, 0.01 g TPP was added to 10 ml distilled water and then stirred for 8 h. After that, for 40 min, it had been sonicated. The TPP solution had been added dropwise to the CS solution utilizing a 1 ml syringe at a rate of 15 drops/min until the CS: TPP ratio reached 2:1. The solution was then agitated for a further 8 h and then sonicated for 45 min.[12]

Evaluation of particles size

Dynamic light scattering was used to determine the size of CS–TPP nanoparticles using a NanoBrook 90Plus Particle Size Analyzer (Brookhaven Instruments, USA).

Evaluation of suspension sedimentation

A centrifuge was used to test the suspension of CS–TPP nanoparticles (Hitachi CF16RXII). The specimens were placed in centrifuge tubes and centrifuged for 2 h at 6,000 revolutions per minute. Following that, the suspension was monitored for sedimentation. If no sedimentation occurs, the suspension has particles with a size range of nanometers.

RESULTS

Kappa tests revealed high agreement between two examiners, with values of 0.8 or more for all of the various categories.

A particle size analyzer was used to determine the particle size. The effective diameter of CS suspension was 230.9 nm. The outcome of the CS–TPP nanoparticles suspension sedimentation test shows that the particles remained homogeneously distributed and no precipitation occurred.

The result at three levels shows that all first three irrigants remove the smear layer more significantly predominant than the control group [Table 1].

CNPs were as efficient as CA and EDTA as a chelating agent at coronal and middle levels, while it was significantly different and more efficient than CA and EDTA apically [Figure 2].

This study shows that there was no significant difference between A1 and A2 at all three different levels, while there was a significant difference between B1 and B2 at coronal and middle levels, and C1 and C2 apically. In which IrriFlex® endodontic needle removes smear layer more efficiently than ProRinse® irrigation needles at those mentioned levels [Table 2].

DISCUSSION

The ability of an irrigating solution to remove smear layers from the coronal, middle, and apical thirds of a canal wall depends on the aggressiveness of the irrigant and the manner in which the irrigant is delivered.[13] In this research, the effectiveness of three different irrigations has been studied with two types of newly developed irrigation needles. According to the findings of this study, 0.5% of CNPs had the same effect on removing the smear layer as CA 10% and 17% EDTA at coronal and middle levels as shown in Figure 2. This finding is supported by a prior study done by Silva *et al.*, which concluded that all three irrigant solutions had a similar capability of removing the smear layer with a significant difference ($P < 0.05$) from the control group.[7] Final irrigation with various solutions appears to dissolve the smear layer, particularly the inorganic material, although in a variety of ways.

Two hypotheses were used to investigate CS's chelating mechanism. First, the bridge model postulates that CS has two or more amino groups that interact with the same metal ion. Second, the pendant model implies that the binding involves just one amino group and that the metal ion is suspended from the amino group same as a pendant.

Either of these two mechanisms might be responsible for calcium ion chelation in dentin, resulting in the degradation of inorganic materials in the smear layer.[14]

In contrast, CNPs remove the smear layer better than CA and EDTA 17% at apical thirds. This result was similar to another study done by Hassan and Negm, which indicates that the capability of smear layer elimination by CNPs is better than EDTA at 17% and CA at the apical area.[15]

It is more challenging to get rid of the smear layer in the apical part of the root canal as the diameter is narrower.[16] The nanoparticle size of CS can increase irrigation fluid flow into dentinal tubules, hence increasing smear layer clearance.[17] Besides, CS polymer is hydrophilic. Thus, it supports close contact with root canal dentin that enables it to be easily adsorbed by the walls of the root canal and supplied deeper to the dentinal tubules.[18]

Irrigation efficiency varies according to the irrigant delivery system and irrigating solution. According to the present study, there was no significant difference between ProRinse® and IrriFlex® endodontic needles when used with 0.5% CNP at all three different levels. However, when used with 10% CA, IrriFlex ® significantly more efficiently removes the smear layer than ProRinse® at coronal and middle levels and with EDTA 17% apically.

IrriFlex® is a unique plastic irrigation needle with a soft polypropylene body and back-to-back 2-side vent design which delivers solutions closer to the apex. Therefore, IrriFlex® provides excellent reachability and flexibility for improving irrigation treatments.[19]

CONCLUSIONS

Based on the results, our study concluded that all three tested irrigants removed the smear layer from the coronal, middle, and apical third. However, among these irrigants, CNPs were most efficient in the apical third.

In addition, the two types of irrigation needles had a minimum effect in removing the smear layer when used with CNPs, while IrriFlex® showed better cleaning efficiency than ProRinse® when used with CA and EDTA at different levels.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Acknowledgment

The authors would like to thank Mustansiriyah University (www. uomustasiryah.edu.iq), Baghdad, Iraq, for its support in the present work.

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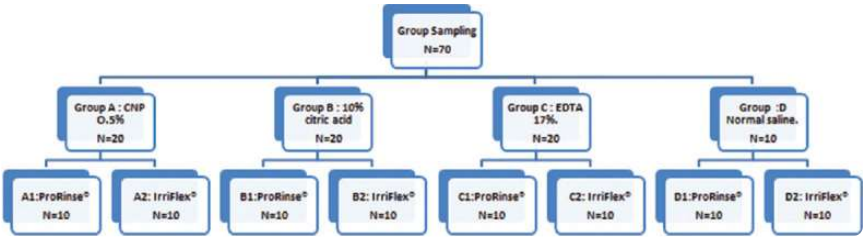
Figures and Tables

Figure 1



Group sampling according to final irrigation protocol and type of needle used

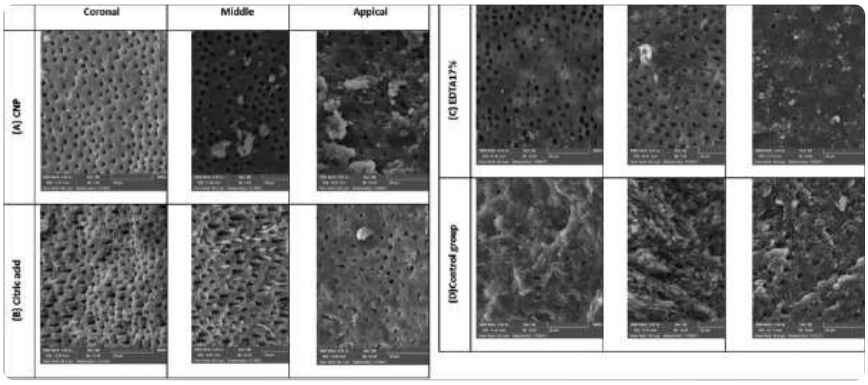
Table 1

Kruskal-Wallis and Mann-Whitney *U*-tests of the three levels of root canal after using different irrigating solutions

Ranks										
	Groups	***n***	**Mean rank**	***P*** *	**Pair-wise comparison****					
					A-B	**A-C**	**A-D**	**B-C**	**B-D**	**C-D**
Coronal	A	20	25.60	0.001	0.007	0.506	0.001	0.012	0.001	0.001
	B	20	40.35							
	C	20	28.15							
	D	10	60.30							
Middle	A	20	28.75	0.001	0.202	0.760	0.001	0.423	0.01	0.001
	B	20	34.85							
	C	20	30.95							
	D	10	59.40							
Apical	A	20	20.25	0.001	0.001	0.022	0.001	0.014	0.013	0.001
	B	20	44.05							
	C	20	30.95							
	D	10	58.00							

*P value,** Mann-Whitny U-test. A=CNP 0.5%, B=10% CA, C=EDTA 17%, and D=Controlled group. CNP: Chitosan nanoparticle, CA: Citric acid, EDTA: Ethylenediaminetetraacetic acid

Figure 2



Scanning electron microscope photographs at ×3000 showing; root samples treated with (a) CNP (b) citric acid (c) EDTA (d) and distilled water at different levels of root canal coronal, middle, and apical. CN *P*: Chitosan nanoparticle, EDTA: Ethylenediaminetetraacetic acid

Table 2

Comparisons among the mean ranks of smear layer removal of three levels between each two subgroups

	Subgroup	*n*	Median (Q1-Q3)	Mean rank	*P*
A					
Coronal	A1	10	2 (2-3)	12.40	0.109
	A2	10	2 (1-2)	8.60	
Middle	A1	10	3 (2-3)	11.50	0.383
	A2	10	2 (2-3)	9.50	
Apical	A1	10	3 (2-3)	11.50	0.342
	A2	10	3 (2-3)	9.50	
B					
Coronal	B1	10	3 (3-3)	13.50	0.004
	B2	10	2 (2-3)	7.50	
Middle	B1	10	3 (3-3)	13.50	0.04
	B2	10	2 (2-3)	7.50	
Apical	B1	10	3 (3-4)	10.00	0.661
	B2	10	4 (3-4)	11.00	
C					
Coronal	C1	10	2 (2-3)	11.5	0.342
	C2	10	2 (2-2)	9.5	
Middle	C1	10	3 (2-3)	11.9	0.240
	C2	10	2 (2-3)	9.1	
Apical	C1	10	3 (3-4)	13.1	0.015
	C2	10	3 (2.75-3)	7.9	
D					
Coronal	D1	5	4 (3-4)	6.5	0.221
	D2	5	3 (3-4)	4.5	
Middle	D1	5	4 (3-4)	6	0.513
	D2	5	4 (3-4)	5	
Apical	D1	5	4 (4-4)	5.5	1
	D2	5	4 (4-4)	5.5	