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EFFECTS OF DIFFERENT NATURAL IRRIGANT SOLUTIONS ON SURFACE ROUGHNESS OF ROOT CANAL DENTIN USING QUANTITATIVE TOPOGRAPHICAL 3D SURFACE TEXTURE ANALYSIS

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ABSTRACT

Objective: was to investigate the effect of 0.2% chitosan, 0.2% nano chitosan, and 5% apple vinegar compared to 17% EDTA on intraradicular dentin surface roughness. **Materials and Methods:** Twenty single-rooted human teeth were used in this study, after decoronation, working length determination, and mechanical preparation using ProTaper Next rotary files with irrigation after each file using 5ml of 2.6% NaOCl. The specimens were randomly divided into four groups according to the final irrigating solution. **Group I** used 17%EDTA, **group II** with 0.2% chitosan, **group III** with 0.2% nano-chitosan, and **group IV** with 5% apple vinegar. Specimens were sectioned longitudinally and evaluated for surface roughness changes using Environmental Scanning Electron Microscope (ESEM) and a photomicrograph was analyzed using a 3D software system. **Results:** The highest mean value of surface roughness was recorded in **group IV** treated with 5% apple vinegar with a statistically significant difference from the other groups (P value< 0.05). The lowest mean value was observed in **group III** used 0.2% nano-chitosan followed by **group II** irrigated with 0.2% chitosan which was lower than the control **group I** used 17% EDTA with statistically significant difference between the three groups (P value< 0.05). **Conclusion:** 0.2% nano-chitosan as the final irrigating solution had minimal drawbacks on dentin micro-structures by producing a slight change of surface roughness**.**

KEYWORDS: Surface roughness, nano-chitosan, apple vinegar, EDTA, ESEM.

INTRODUCTION

Biomechanical preparation is a fundamental phase in endodontic treatment, in which irrigation is complementary to mechanical instrumentation functioning to eliminate the microorganisms, cleaning the debris, remove the smear layer including both organic and inorganic components [(1)]. Alternative use of Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA)

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solutions is the most common irrigation protocol to get rid of organic and inorganic structures of the smear layer[2]. EDTA is the gold standard final irrigating solution masterly eliminate the inorganic part of smear layer through its chelating characteristic, however; the extended exposure of EDTA can resulted in abstraction of peritubular and intertubular dentin causing alteration in dentin ultrastructure, mechanical property and surface roughness[3,4]. Since synthetic chemical irrigants currently used in the root canal treatment lack the ideal irrigating material characteristics with prospect side effects, naturally sourced substitute has begun to be scrutinized.

Apple vinegar is extremely biocompatible solution with low cost and easy accessibility attracted attentiveness and has been investigated for use as auxiliary solution for biomechanical cleaning of the root canal system [5]. It is known to have bactericidal effect towards microorganisms correlating with endodontic infections as *Enterococcus faecalis* and *Staphylococcus aureus* [6]. Apple vinegar was reported to be eligible for smear layer cleanliness that covers the root canal wall disserve the dentinal tubule promoted better cleaning with interaction mechanism comparable to that of EDTA on mineral content of intraradicular dentin [7].

Chitosan, another natural product that become included the dental research studies, it is a natural polysaccharide that consists of a long biopolymer chain of N-acetylglucosamine. It has the advantages of being biocompatible and has no genotoxic or toxicological effect [8]. In the field of endodontics chitosan tested to replace EDTA as final irrigating solution, it has chelation ability comparable to that of EDTA which perfectly removes the smear layer through dissolving its inorganic components. Moreover, chitosan has an advantage over EDTA of possessing antimicrobial impact against broad range of microorganisms and that are isolated from root canal infection as Enterococcus *faecalis* and *Candida albicans* had been demonstrated [9,10].

Nanodentistry reveals implementation of nanomaterials in dental treatment aiming to improve comprehensive oral health, in the endodontic field this new technology applied in different aspects like solutions for irrigation [11]. Nano materials provide unique physicochemical characteristics of ultra-small particle sizes, increased surface area related to mass ration, and improved chemical reactivity compared to their bulk material form [12]. Chitosan nanoparticles have been introduced to mend the root canal disinfection since it showed better antibiofilm capacity and bacterial degradation, and has chelating effect capable to remove the smear layer with the ability to penetrate deeper into the root canal irregularities and dentinal tubules criteria that make it suitable to be used as finishing rinse during root canal irrigation [13].

So, the null hypothesis of this research was that there will be no difference in surface roughness change on the root canal dentin between 17% EDTA, 0.2% chitosan, 0.2% nano-chitosan, and 5% apple vinegar when used as final irrigating solutions.

MATERIALS AND METHODS

The current experimental study was designed as randomized controlled trial, and conducted in Endodontic Department, Faculty of Dental Medicine for Girls, Al-Azhar University. Ethical approval for the use of extracted human teeth was obtained in accordance with guidelines from Research Ethic Committee (REC) with approval code (REC-PD-22-07), Faculty of Dental Medicine for Girls, Al-Azhar University. The experiment was directed to evaluate dentin surface roughness of the root canal dentin using 20 extracted teeth.

Question in this study was addressed in terms of PICO question which involves 4 elements: problem (P), intervention (I), comparison(C) and outcome (O)] as following:

P. Surface roughness change (problem).

I. Using 17% EDTA final irrigating solution (intervention).

C. Using natural irrigating solutions (0.2% chitosan, 0.2% nano chitosan, and 5% apple vinegar) (comparison).

O. Minimal change in surface roughness (Outcome).

Sample size estimation and statistical power:

The sample size was calculated on OpenEpi program and according to a previous study done by Ratih et al. (2020) who stated that the Mean and standard deviation of the contact time effect of final irrigation solutions on the surface roughness of root canal dentin at 3 minutes in 17% EDTA group was 2.41 ± 0.09 and in Chitosan nanoparticle group was found 0.93 ± 0.26 and adjusting the confidence level to 95% and power of the test to 80%; the required sample size per group was found 4 [14].

Teeth selection:

Twenty fully formed permanent human single rooted teeth were selected which recently extracted for periodontal or orthodontic causes, after confirmation of being free from root caries, root canal configuration Vertucci's type I, or previous endodontic treatment. All teeth were thoroughly cleaned off soft tissue and hard debris stored in 0.1% thymol solution until use.

Specimens' preparation and grouping:

All selected specimens were decoronated at the cemento-enamel junction to establish a standardized tooth length of 16 ±1 mm using low speed double-faced diamond disc (Diatech, Coltene, Switzerland) under copious water coolant to get uniform specimens. After working lengths confirmation through subtracting 1 mm of size 10 k-file (MANI Inc., Japan) length after being observed from apical foramen then root apices were sealed with sticky wax. Root canal preparation were performed using performed using ProTaper Next rotary files (Dentsply, Maillfer, Switzerland) in a crown-down manner up to #X3, speed and torque for each file were adjusted according to manufacturers' recommendations. In between each file Irrigation was performed with 5ml of 2.6% NaOCl solution (Alex. Detergents and Chemical Co., Egypt) for 1 minute, then rinsed with 5ml distilled water.

All specimens were then randomly divided into four groups (n= 5) according to final irrigating solution used. Each specimen was placed in uncoded, sealed, opaque envelop and dental practitioner who was not percipient about the experiment asked to distribute the specimens equally to the four groups. Specimens were finally irrigated as the following; **Group I** 7% EDTA solution (Prevest Denpro limited, Digiana, Jammu, India) 5ml/3min, **Group II**: 0.2% chitosan 5ml/3min, **Group III**: 0.2% Chitosan nanoparticle (CNP) 5ml/3min, and **Group IV** 5% apple vinegar (Industrial Zone, Badr City, Cairo,Egypt) 5ml/3min, finally all the root canals were flushed with 5mL of distilled water. The irrigating solutions were dispensed using 31-gauge irrigating needle (Navi-Tip, Ultradent product, South Jourdan, UT) which inserted in the root canal 2mm off working length. Root canals were then dried using sterile paper points # 35.

Chitosan acetate 0.2% solution preparation was performed via dissolving amount of 0.2g of chitosan powder (90% degree of deacetylation) (Sigma Co., Egypt) in 100 ml of 1% acetic acid and the mixture was stirred for 2 hrs using a magnetic stirrer until a homogenous clear solution was obtained, then preserved in the refrigerator and used within one week.

Nano-chitosan CNP (Al-Azhar Technology Incubator (ATI)); Chitosan was milled in a multidimensional swipe nano-ball-milling machine in a process based on inotropic gelation of CS. Then 0.2 grams were diluted in 100 ml of 1% acetic acid which was then stirred for 2 hours using a magnetic stirring machine till a crystalline homogenous solution was produces. Then, sodium tripolyphosphate solution of 0.8% was a prepared by dissolving 80mg

of TPP in 10ml of deionized water. Nano-chitosan CNP preparation was mixed with a Polytron homogenizer at 5,000 rpm with drop-wise addition of the tripolyphosphate solution (TPP) under magnetic stirring at room temperature in the ratio 2.5: 1(v/v) (chitosan: TPP). Blank nanoparticles were obtained upon the addition of Tripolyphosphate (TPP) aqueous solution to Chitosan solution [15].

All roots were sectioned longitudinally by making groove at nearly midline of both buccal and lingual aspects of the root surface without penetrating deep to the root canal space using a low-speed diamond disk. Each root specimen was split into two halves using a sharp chisel, cleaned from dentin chips by using soft brush and distilled water, and inspected to select the best one represent the total canal anatomy with evident area of the apex and coded for evaluation of intraradicular dentin surface roughness.

Scanning electron microscopy (SEM) analysis:

The coded specimens were measured using a caliper to determine the length from cementoenamel junction to the root apex to mark coronal, middle, and apical thirds. Specimens were then mounted on metal stubs using a conductive adhesive, examined under SEM10 (JEOL JSM-5510, Tokyo, Japan) at X2000 magnification and digital photomicrographs were taken at the center of each third to visualize the dentin surface after application of final irrigants. Analysis of the dentinal surface of the root canal topography was conducted using the 3D Roughness Reconstruction program (XT document roughness software, x-ray tungsten filament for microanalysis measurements), specimens were placed at the analyzing chamber and center of coronal, middle, and apical levels was specified to be captured and analyzed by roughness software (XT document) to convert the image from 2D to 3D metrology monitoring surface texture and data was tabulated for statistical analysis (fig.1).

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges. Also, qualitative variables were presented as number and percentages. The comparison between more than two groups regarding quantitative data and parametric distribution was done by using One Way ANOVA test followed by post hoc analysis using LSD test. The comparison between more than two paired groups was done by using Repeated Measures ANOVA test followed by post hoc analysis using Bonferroni test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the *P*-value was considered significant < 0.05.

RESULTS

The means and standard deviations of the root dentin roughness values for the experimental groups and control group are represented in Table 1, figure 1.

Comparison between the experimental groups revealed that the highest roughness mean values and standard deviations were recorded in the specimens received 5% apple vinegar as final resin with statistically significant difference at coronal and apical thirds with the remaining tested irrigants (*P*-value< 0.05), followed by that of group I treated with 17% EDTA (control group) which represent statistical significant difference at the coronal and apical levels with group III used 0.2% chitosan nano-particles (*P*-value< 0.05). However; group II treated with 0.2% chitosan bulk material showed lower mean values and standard deviations in comparison with group I 17% EDTA (the control) with statistically significant difference at coronal and apical levels, while showed mean value and standard deviations of surface roughness higher than that of group III (nano-chitosan) with statistically significant difference (*P*-value< 0.05).

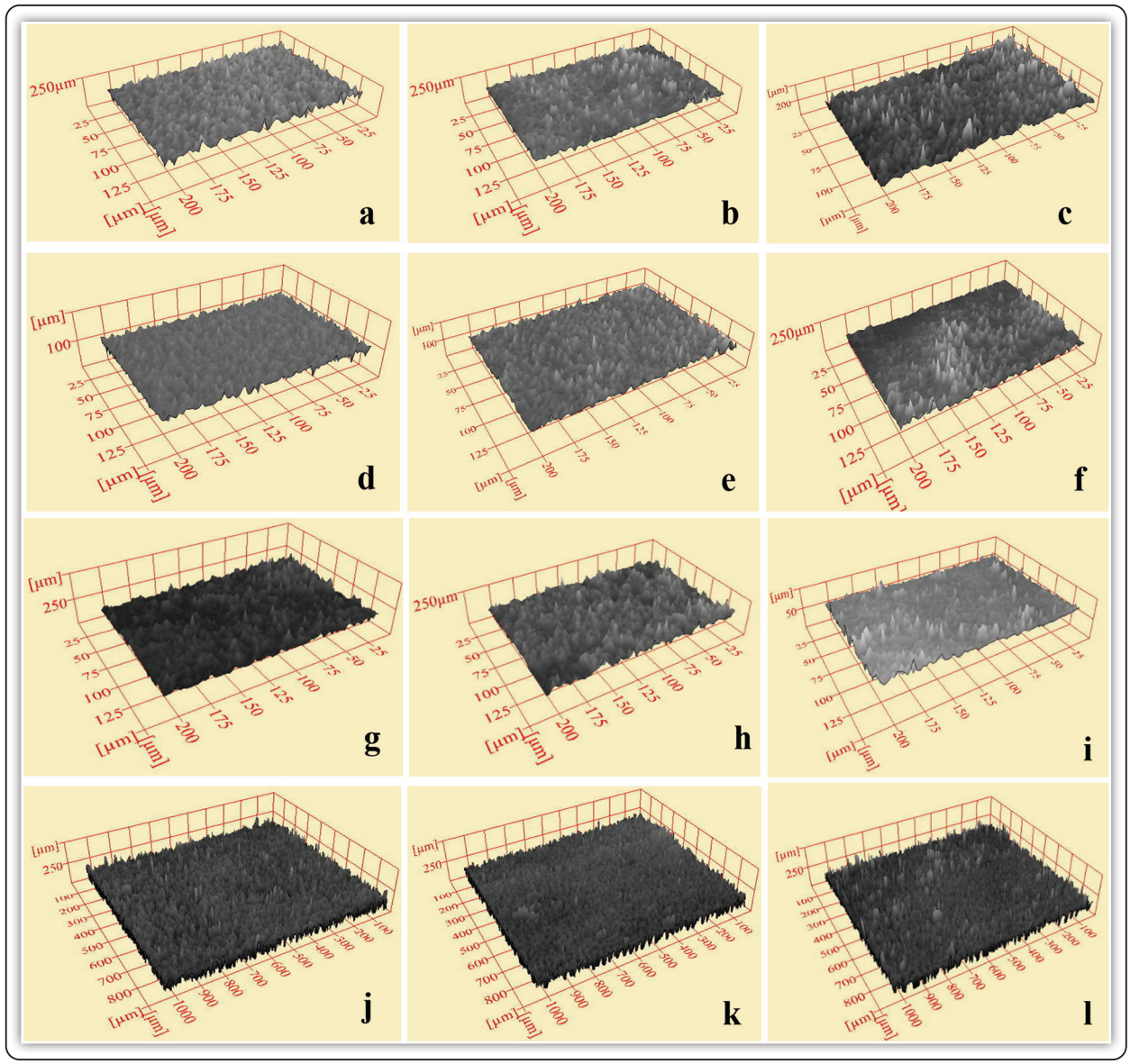

FIG (1) Topographical 3D images of Group I (EDTA); (a) Coronal third, (b) Middle third, (c) Apical third. Group II (Chitosan); (d) Coronal third, (e) Middle third, (f) Apical third. Group III (Chitosan Nanoparticles); (g) Coronal third, (h) Middle third, (i) Apical third. Group IV (Apple vinegar); (j) Coronal third, (k) Middle third, (l) Apical third.

With regarding to radicular region comparison, it was observed that apical third recorded the lowest mean value and standard deviations regarding to the middle and coronal thirds, with statistical significant difference with the coronal third in group I (17%EDTA) (*P*-value< 0.05), and high statistical significant difference with the coronal and middle thirds in group III (0.2% nano chitosan) (*P*-value< 0.01). However, the middle third showed lower roughness mean values and standard deviations when compared with the coronal among the tested group except that of group II (0.2% chitosan) but with statistically non-significant difference (*P*-value> 0.05).

TABLE (1) The means and standard deviations of the root dentin roughness values for the experimental groups and control group

Ra (μm)		Group I (EDTA)	Group II (Chitosan)	Group III (Nano-chitosan)	Group IV (Apple Vinegar)	Test value	P-value	Sig.
		No. = 5	No. = 5	No. = 5	No. = 5			
Coronal	Mean ± SD	0.183 ± 0.031[a]	0.130 ± 0.039[a,b]	0.091 ± 0.010	0.256 ± 0.002[a,b,c]	39.461•	0.000	HS
	Range	0.137 – 0.205	0.101 – 0.195	0.082 – 0.104	0.253 – 0.259			
Middle	Mean ± SD	0.142 ± 0.044	0.186 ± 0.189	0.105 ± 0.013	0.258 ± 0.003	2.274•	0.119	NS
	Range	0.099 – 0.204	0.086 – 0.523	0.084 – 0.118	0.254 – 0.262			
P-value Vs coronal		0.162	–	0.448				
Apical	Mean ± SD	0.093 ± 0.036[a]	0.061 ± 0.007[a,b]	0.031 ± 0.005	0.256 ± 0.003[a,b,c]	144.810•	0.000	HS
	Range	0.048 – 0.132	0.052 – 0.072	0.026 – 0.038	0.253 – 0.258			
P-value Vs coronal		**0.025**	–	**0.000**	–			
P-value Vs middle		0.057	–	**0.000**	–			
Repeated Measures ANOVA test	**F**	7.788	1.831	97.210	1.256			
	P-value	**0.017**	0.247	**0.000**	0.335			

P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant

•: One Way ANOVA test

Post hoc analysis between groups: [a]: Significant from Nano-Chitosan group; [b]: Significant from EDTA group; [c]: Significant from Chitosan group

DISCUSSION

Clinical endodontic treatment setting including application of synthetic chemical irrigating solutions in a sequence as adjuncts to facilitate preparation, improve disinfection, and clean the smear layer [16]. While performing those functions, root canal dentin that representing the bulk of the tooth with unique composite material of mineralized connective tissue is sensitive to thematic modulation that resulted in structural and surface changes with subsequent weaken the root architecture, hence, representing worthy clinical implications [17]. Surface roughness is indicator of surface texture quantified by measurement of irregularities that are estimated by the deviation of typical surface from normal smooth one [18]. Using proteolytic and chelating agents in order to get rid of organic and inorganic components of smear resulted in irreversible erosion of the dentin microstructure and surface roughness [19].

Endodontics research is directed to develop strategies for replacing the synthetic chemical irrigating solutions with biomaterials that meet the cleaning and therapeutic irrigation objectives and minimize the physically and chemically induced damage [20]. Natural irrigation products such as chitosan, chitosan nano-particles, and apple vinegar were proved proper microbial disinfection, removing tissue residues, and completely clean the root canal wall off the smear layer [6,7,10,11]. Thus, this study was directed to evaluate those newly proposed irrigants in relation to the gold stander and more contemporary chelating agent EDTA when used as final irrigating solutions.

The surface texture analysis was conducted under 2000X magnification using environmental scanning electron microscope (ESEM) to evaluate changes in dentin surface roughness. ESEM which widely used in surface studies including surface erosion on root canal dentin evaluation, has benefits of providing technology for imaging biological samples with minimal manipulation, no need for conductive coatings, images had more clearly defined, and reduced the possibility of introducing artifacts [21]. Surface roughness photomicrograph was analyzed using 3D software system (Dektak XT document roughness software) which able to map 2D surfaces into 3D profiling. Although 2D average roughness is the sole parameter specific for monitoring surface texture, it provides a quick gauge of general roughness which may not a complete picture of the sample surface [22]. However; 3D metrology had advantages of providing clear picture that allow reliable characterization of dentin surfaces at high resolution quantitatively and qualitatively, moreover vision software includes a multi-region analysis that lets a user define and compare multiple features within a dataset, furthermore surface metrology using this method allows standardization of measurements and comparison of future studies which is of great importance [18, 23].

The result of this study demonstrated that 5% apple vinegar as final irrigation solution showed the highest mean value effect on the intraradicular dentin roughness among all tested irrigating solutions even 17% EDTA (the control). These results can be interpretated on the base that apple vinegar containing maleic acid as main ingredient with its demineralization capacity that related to strong acidic pH that produce higher dentin surface roughness than that of EDTA [24]. This results were in accordance with the study results reported that apple vinegar as fruit extract produced high surface roughness, and another reported that maleic acid recorded highest roughness compared to citric acid and EDTA [24-27]. However; it was in contrast with results of study that reported that 17% EDTA formed higher value of surface roughness compared to 5% apple vinegar, but the other study applied the irrigating solutions for 1 minute while in this study the were applied for 3 minutes which expected to permit extra chelation effect and greater surface roughness [25, 28].

Regarding to the surface roughness recorded by 0.2% nano chitosan, it showed the lowest mean roughness followed by 0.2% chitosan bulk material compared to the roughness created by 17% EDTA had higher mean value than both solutions. Thus, the effect of 0.2% chitosan nanoparticles was minimal on dentin surface with smaller demineralization changes with statistically significant difference with (the control) 17%EDTA, while; 0.2%chitosan bulk martial showed roughness that was lower than that of the control group and greater than nano chitosan treated group with statistically significant difference with both groups. EDTA solution had neutral pH act on intraradicular dentin through replacement of calcium in the hydroxyapatite by the hydrogen ions which resulted in increased dentin surface roughness due to chelation property that capable not only demineralize the smear layer but also affect peritubular and intertubular dentin lead to erosion and increased roughness [29]. In contrast, chitosan is a weak chelating agent, its impact confined to smear layer cleanliness and demineralizes less dentin surface, and makes contact with intraradicular dentin surface induce remineralization of demineralized dentin through covalent interaction of chitosan to dentin collagen which may be attributed to phosphate groups that can attract calcium ions that represent satisfactory surface for nucleation of crystals forming calcium-phosphate layer. This result was in accordance to the other studies reported that EDTA produced surface roughness greater than that of chitosan and its nano counterpart [28, 30-32].

This study results demonstrated that surface roughness produced by 0.2% chitosan nano-particles was lower than that produced by 0.2% chitosan bulk material, this can be attributed to the advanced chemical and physical properties of nano-particles

compared to the parent materials, of its ultra-small size, and larger available surface area to volume ratio compared with micro/macrostructures counterpart. However; the nanoparticles are less stable and exhibit weaker bonding and interaction with other molecules however; bulk material promotes intimate contact and adsorption which expected to prolong the chelating effect on dentin surface with subsequent increased surface roughness [11, 31].

With considering the different root canal levels, the apical third showed the lowest mean value of surface rough among the all-tested groups, this may relate to anatomical character of this area which is narrow which doesn't allow inadequate penetration of the irrigating solution into this portion of the canal which subsequently exposed to minimal effect on its surface structure [33]. This can be due to reduced surface tension of the solution as a result of alcohol content with other ingredient as acetic, citric, formic, lactic, succinate and tartaric acids which improve its flow through the apical part of the canal [34]. While, in group II and group III treated with chitosan and nano-chitosan the highest mean roughness value was observed at the middle level which may be related to its viscosity that interferes with flow capability that cause stagnation of the solution at middle part of the root canal for longer time before reaching the apical part which exposed the area to longer contact time and more chelating effect [35].

Thus, the null hypothesis of this study was rejected since there was statistical difference between the tested groups.

CONCLUSIONS

Within the limitations of this study, it could be concluded that; 0.2% nano-chitosan as final irrigating solution had minimal drawbacks on dentin micro-structures by producing slight change of surface roughnessm and 5 % apple cider vinegars may have softening effects on the root canal dentin that increase the dentin roughness.

AUTHORS' CONTRIBTIONS

Abdelkafy H, performed the practical part and interpreted the results. Elsheikh HM., major contributor in writing the manuscript. Marzouk RM, Seham i. Hallool and Enas M M, analyzed the results.

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