Journal of Oral Biology and Craniofacial Research 14 (2024) 578–584

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Effectiveness of chitosan nanoparticles, and novel chemical irrigants with surfactant on smear layer removal and microhardness alteration

Monika Khangwal [a,*], Ravinder Solanki [b], Hena Rahman [c], vinay [d], Neha Saini [b], Nilesh Bagde [b]

[a] Conservative Dentistry and Endodontics, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India
[b] Dept of Oral and Maxillofacial Surgery, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India
[c] Conservative Dentistry and Endodontics, GMC JALAUN, India
[d] Conservative Dentistry and Endodontics, BPS Government Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India

ARTICLE INFO

Keywords:
Irrigants
Microhardness
MTAD
Scanning electron microscopy
Smear layer

ABSTRACT

Objective: This current study was designed to compare and correlate between smear layer eradication and reduction in microhardness by natural 0.2%chitosan nanoparticles and novel chemical irrigants with surfactant at the apical root third.

Materials and method: One hundred and twenty straight single-rooted extracted lower premolars were decoronated and working length obtained with #10 K-file. Pro-taper rotary files were used till apical size F3. The canals were simultaneously flushed with assigned irrigant containing surfactant [(ChX-Ultra, NaOCl-Extra, Pro-EDTA, 0.2%chitosan nanoparticles, Biopure MTAD]. The samples were randomly divided into two equal groups (n = 60). InGroup S (n = 60), the residual smear layer was examined by scanning electron microscope and in Group M(n = 60) microhardness was determined by Vickers Microhardness Tester. Further both groups were divided into six equal groups (n = 10 each) according to assigned irrigating solutions.

For smear layer removal; Kruskal-Wallis tests followed by pair wise comparison using Mann Whitney *U* test was done. For change in microhardness ANOVA and post hoc Tukey tests was done.

Results: Maximum smear layer removal was recorded in Biopure MTAD (1.8 ± 0.63), followed by pro-EDTA (2.2 0 ± 0.63) then 0.2%chitosan (2.6 ± 0.51), then NaOCL Extra (3.5 ± 0.53)and least in CHX-Ultra (4.4 ± 0.52) and saline (5.0 ± 0.00). Pro-EDTA group (12.8 ± 2.47) revealed significant highest reduction in microhardness followed by Biopure MTAD (8.01 ± 3.06), 0.2%chitosan nanoparticles (5.48 ± 2.87), NaOCl-Extra (5.44 ± 1.62) and least recorded in CHX-Ultra (4.94 ± 1.43) and saline (3.04 ± 0.63).

Conclusion: The elimination of the smear layer is always accompanied by a reduction in microhardness. Moreover, irrigant with surfactant and chelators enhanced smear layer removal, with best perceived in Biopure MTAD.

1. Introduction

Endodontic treatment emphasizes on elimination and dissolution of necrotic tissues and microbial-laden smear layers. This objective can be achieved by biomechanical instrumentation and irrigation of complex root canals.[1] Existence of residual smear layer on canal surfaces hinders the penetration of sealers/irrigants, discourages adaptation of obturating material, and causes apical or coronal microleakage. Hence, the removal of the entire smear layer is necessary for the successful root canal treatment. Recently surface modifiers have integrated with irrigants to augment their functional activity. Surface modifiers can act as emulsifiers, foaming agents, detergents, wetting agents or dispersants, so it is called surfactants or surface active agents.[2] Incorporation of surfactant with irrigating solution decreases surface tension, thus, permitting its rapid penetration into multifarious anatomical structures and enhancing its antimicrobial effectiveness and clinical performance.[3] However, introduction of irrigating solution with chelators and surfactant may affect inorganic content and dentinal ultrastructure while eradication of smear layer.[2] Alteration in mineral content may decrease the microhardness of the root dentin.[4] Microhardness is an indirect valuable substantiation of inorganic content alteration in radicular dentin. These inorganic mineral changes influence adhesive properties

* Corresponding author.
E-mail addresses: dr.monikasolanki@gmail.com (M. Khangwal), ravindersolanki@hotmail.com (R. Solanki), henavns@gmail.com (H. Rahman), vinaysingh7493@gmail.com (vinay), Nehasaini.1360@gmail.com (N. Saini), Nileshbagde30@gmail.com (N. Bagde).

https://doi.org/10.1016/j.jobcr.2024.06.005
Received 3 February 2024; Received in revised form 11 June 2024; Accepted 27 June 2024

and the success of endodontically treated teeth.[5]

During instrumentation, the selection of an irrigant is of great significance because of the disparity in their function to act as lubricants, antimicrobials, and cleanser for the smear layer. Sodium hypochlorite (NaOCl) is the most commonly workable irrigating solution but it failed to dissolve inorganic content of smear layers.[6] So ethylenediaminetetraacetic acid (EDTA) is used concurrently due to its chelating action on the mineral content of smear layers.[6]

However, revelation with EDTA may change the substructural configuration of dentin, ensuing in compromised mechanical integrity and inducing surface irregularities.[2] Due to these shortcomings, Sodium hypochlorite Extra (NaOCl-Extra)and Pro-EDTA with surface modifiers were introduced and assessed for their efficiency in eradication of the smear layer. Chlorhexidine Ultra (ChX-Ultra)is another long-lasting commonly used irrigant with surface active agent. It is twice as fast in disintegrating multispecies biofilm and ten times faster against planktonic bacteria when compared with standard 2 % chlorhexidine.[7]Its surface modifiers enable it to penetrate inaccessible areas such as lateral canals and isthmuses.[7] A novel irrigant, BiopureMTAD comprise of a tetracycline isomer; an acid and a detergent. It can effectively clear the smear layer and eliminate or eradicate resistant microbes like enterococcus faecalis.[8] Preceding investigations demonstrated a correlation between smear layer eradication and alteration in elemental and morphological dentinal substructure, consequentially decreasing dentin micro-hardness and producing surface irregularities.[3,9] Recently chitosan has been broadly taken into clinical consideration known for its biocompatibility, biodegradability and bioadhesive polysaccharide, which is acquired from shells of shrimps/crabs.[10] At low pH, its chelating affinity for various metal ions (calcium, copper) is enhanced. It is abundantly available biological inexpensive cellulose which fascinatingly rationalized its utilization in the field of dentistry.[11] Del et al. in their research demonstrated chitosan as a chelating and antimicrobial agent.[12] For enhanced bonding of endodontic sealers, the multiplicity of relevant factors such as the presence of smear layers, microbial growth, maintenance and preservation of dentinal substructure are of the highest implication. Since these multifactorial reasons are determinants for the authenticity of the standard outcome of successful root canal therapy, thus to conclude with coherent superior final irrigants in the elimination of the smear layer without or with the least alteration in dentinal integrity is of paramount significance. Hence this in-vitro study was designed to assess and evaluate important parameters i.e. comparison and correlation of natural 0.2%chitosan nanoparticles and chemical irrigants (ChX-Ultra, NaOCl-Extra, Pro-EDTA, Biopure MTAD) with surfactant and also a correlation between smear layer eradication and alteration in dentin microhardness. T he null hypothesis was that there was no difference in smear layer removal and alteration in dentin microhardness with experimental irrigants.

2. Materials and method

This research project with all the prospective procedures was permitted by the ethical committee of the state government institute under reference no. 2499 dated 2-11-2020. This study was conducted per the Declaration of Helsinki. One hundred and twenty freshly extracted lower premolars of orthodontic rationale with relatively similar aspects and morphology were collected with the patient's consent. Confirmation of a single canal was verified with intraoral periapical radiographs at mesiodistal and buccolingual angulations. Selected teeth specimens were decontaminated as per the centre for disease control and prevention (CDC) by immersion in 5.25 % sodium hypochlorite solution for 5 min s and were stored at room temperature in saline solution. This research was designed into two appraisals i.e. smear layer removal and micro-hardness alteration assessment. The selected samples were decoronated at the cemento-enamel junction to obtain a 15 mm ± 0.5 mm root length using a diamond disk (3 M ESPE, St. Paul, MN, USA) under water coolant. After the endodontic access opening, the working length of all specimens was obtained with #15 K-file. Two longitudinal grooves of 0.5 mm depth were placed on the buccal and lingual surface to facilitate tooth splitting. The roots were then painted with nail polish and embedded in putty silicon to replicate a closed environment. ProTaperUniversal™ rotary system (Dentsply/Maillefer, Ballaigues, Switzerland) was used till apical size F3 to full working length for biomechanical preparation. The samples were randomly divided of into two groups G-S and G-M of sixty samples each. In Group-S (n = 60), smear layer removal was examined under SEM and in the second group Group-M (n = 60), surface microhardness of radicular dentin was determined. These groups were further subdivided into six equal groups (n = 10 for each group) according to the different endodontic irrigants used in the present study- ChX-Ultra (Coltene Endo), NaOCl-Extra (Coltene Endo), Pro-EDTA (Coltene Endo), 0.2 % chitosan nanoparticle, Biopure MTAD (Dentsply), Control group-normal saline.0.2 g m of chitosan powder (Aura Biotechnologies Private Limited, Chennai, Tamil Nadu) was dissolved and stimulated with a magnetic stirrer for 2 h in 1 % acetic acid of 100mlvolume to attain 0.2 % homogenous chitosan nanoparticle solution.[13]

2.1. Smear layer evaluation

For smear layer removal evaluation the samples were randomly divided into six groups i.e. Group S-1: ChX-Ultra, Group S-2: NaOCl-Extra, Group S-3: Pro-EDTA, Group S-4: 0.2 % chitosan nanoparticles, Group S-5: Biopure MTAD, Group S-6: (Control group) normal saline. During instrumentation all the samples of five groups were irrigated with 5 ml of 2.25 % sodium hypochlorite while sixth group (control group) was irrigated with 5 ml of normal saline. After complete biomechanical preparation, each group was primarily irrigated with 4 ml of the tested solution with constant 2 mm vertical movement of the side-vented 27-G needle. The irrigating solutions were aspirated using an endovac system (Sybron Endo) and canals were dried with paper point. Irrigation protocol for Group –S was elaborated in Table- 1.

Finally prepared specimens were flushed with 10 ml of distilled water, dried and the canal orifice was sealed with a small cotton pellet to prevent contamination of the root canal during sectioning. All the 60 root specimens were sectioned into two halves longitudinally with mallet and a chisel giving one twenty root halves. The apical third root sections were coded and observed under SEM (EVO LS 10, Zeiss, Germany) at a magnification of 2000×.as shown in figure A- CHx-Ultra, figure B– NaOCl-Ultra, figure C-pro EDTA, figure D-0.2 % chitosan nanoparticles, figure E– Biopure MTAD, figure F- normal Saline. Two blinded and regulating surveyors evaluated the amount of residual smear layer according to the following scoring criteria.[14]:

Score 1: patent dentinal tubules with no remaining smear layer.

Score 2: most of the dentinal tubuli were noticeable with less than 25 % of thin smear layer coverage.

Score 3: almost 50 % of inconsistent smear layer coverage.

Score 4: the complete canal surfaces are covered with a thin homogenous smear layer.

Score 5: the entire canal surface is covered with a thick homogeneous smear layer.

2.2. Microhardness evaluation

Canals of all samples of Group-M were prepared till F3 size of ProTaper Universal™ rotary system (Dentsply/Maillefer, Ballaigues, Switzerland) and irrigated with distilled water for baseline microhardness evaluation. Longitudinal grooves were prepared on the external root surface (buccally and lingually). Root specimens were then split with a chisel into two segments giving one twenty halves. All segments were embedded in acrylic resin blocks horizontally. Each root half was labeled with certain private number. For evaluation of post-irrigation microhardness, the samples were randomly divided into six groups i.e. GroupM-1: ChX-Ultra, GroupM-2: NaOCl-Extra, GroupM-3: Pro-EDTA,

GroupM-4: 0.2 % chitosan nanoparticles, GroupM-5: Biopure MTAD, GroupM-6: (Control group) normal saline according to irrigating solutions and treatment. Irrigation protocol for group-M was elaborated in table-1. All the samples were immersed in experimental irrigants for 5 min in closed glass tubes. Later these specimens were flushed with saline and dried with paper points.

Microhardness was evaluated and measured for each specimen at baseline and after treatment with different irrigants consisting of surface modifiers. Vickers Microhardness Tester was used at the apical third region at a depth of 100 μm, each using a load of 200 g m and for 20 s dwell time to assess baseline microhardness in all the samples as elaborated in fig- G. 0.5 mm indentation was made on dentin surface from root canal space. The diagonal lengths of indentation were measured and converted into Vicker's number. The alteration in dentin microhardness was measured as the disparity between baseline values and post treatment values i.e. after flushing in assigned irrigating solutions.

Statistical Analysis: Data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS v 26.0, IBMNY, USA). A scoring system was followed to assess the SEM images. The data for the smear layer removal score was graded on a scale; hence non-parametric tests have been used for the comparisons. Intergroup comparison was done using the Kruskall-Wallis test followed by pairwise comparison using the Mann Whitney *U* test. Comparison of frequencies of categories of variables with groups was done using the chi-square test. Microhardness data was explored for normality using the Shapiro-Wilk test and values across the groups were compared using parametric one-way analysis of variance ANOVA test and post hoc Tukey test, for all comparisons; $p < 0.05$ was considered to be statistically significant, keeping α error (type-I error) at 5 % and β error (type-II error) at 20 %, thus giving power to the study as 80 %.

TABLE-1

Groups-	Irrigation protocol for Smear layer removal evaluation	Irrigation protocol for microhardness alteration evaluation
ChX-Ultra	Irrigation was done with 5 ml of 2.5 % NaOCl followed by 5 ml of normal saline solution. Then, irrigated with 4 ml ChX-Ultra for 4 min s and left for 1min.	10 samples were immersed in 10 ml of ChX-Ultra for 5 min s in closed glass tubes
NaOCl-Extra	Irrigation was done with 5 ml of 2.5 % NaOCl followed by 5 ml of normal saline solution. Then, irrigated with 4 ml of NaOCl-Extra for 4 min and left for 1min.	10 samples were immersed in 10 ml NaOCl-Extra for 5 min s in closed glass tubes
Pro-EDTA	Irrigation was done with 5 ml of 2.5 % NaOCl followed by 5 ml of normal saline solution. Then, irrigated with 4 ml of Pro-EDTA for 4 min and left for 1min.	10 samples were immersed in 10 ml Pro-EDTA for 5 min s in closed glass tubes
0.2 % chitosan nanoparticles	Irrigation was done with 5 ml of 2.5 % NaOCl followed by 5 ml of normal saline solution. Then, irrigated with 4 ml of 0.2 % chitosan nanoparticles for 4 min s and left for 1min	10 samples were immersed in 10 ml of 0.2 % chitosan nanoparticles for 5 min s in closed glass tubes
Biopure MTAD	Irrigation was done with 5 ml of 2.5 % NaOCl followed by 5 ml of normal saline solution. Then, irrigated with 4 ml of Biopure MTAD for 4 min s and left for 1min.	10 samples were immersed in 10 ml of Biopure MTAD for 5 min s in closed glass tubes
Normal saline. (Control group)	Irrigation was done with 5 ml of normal saline, and then the canal was dried with sterile paper points.	10 samples were immersed in 10 ml of normal saline for 5 min s in closed glass tubes

3. Result-

Table 2 inferred that there was a statistically highly significant difference seen for the frequencies between the groups ($p < 0.01$) with higher frequencies for smear layer score 5 with saline group while scores 3 and 4 with NaOCl Extra while score 2 with Pro- EDTA, score 4 with CHX Ultra, score 2 with Biopure MTAD while scores 2 and 3 with 0.2% chitosan nanoparticles. Table −3 revealed that maximum removal of smear layer at apical root third was achieved with lowest mean smear layer removal score in Biopure MTAD (1.8 ± 0.63), followed by pro-EDTA (2.2 ± 0.63) then 0.2%chitosan nanoparticles (2.6 ± 0.52), then NaOCl-Extra (3.5 ± 0.53)and least eradication was noted in CHX-Ultra (4.4 ± 0.52) and saline (5.0 ± 0.00). Table 4 revealed that there was a statistically highly significant difference seen for the values of smear layer removal score between all the pairs of groups ($p < 0.01$) except for group CHX Ultra versus NaOCl Extra and CHX Ultra versus Pro- EDTA where there was a statistically non-significant difference seen for the values ($p > 0.05$). Table-5 showed that there was a statistically highly significant difference seen for the change in microhardness value at apical root third between the groups ($p < 0.01$) with higher values in Pro-EDTAgroup (12.8 ± 2.47) followed by Biopure MTAD (8.01 ± 3.06), then 0.2%chitosan nanoparticle (5.48 ± 2.87), then NaOCl-Extra (5.44 ± 1.62) and least recorded in CHX-Ultra (4.94 ± 1.43) and saline (3.04 ± 0.64). Table 6 revealed a comparison for change in microhardness between each pair of groups using the Post hoc Tukey test, there was a statistically highly significant difference seen for the values between all the pairs of groups ($p < 0.01$) except for group [CHX Ultra versus (NaOCL Extra, 0.2%chitosan nanoparticles, Saline)]; [NaOCl-Extra versus (0.2%chitosan nanoparticles, Biopure MTAD, Saline)]; [0.2%chitosan nanoparticles versus (Biopure MTAD, Saline)]where there was a statistically non-significant difference seen for the values ($p > 0.05$) (see Table 3).

Order of maximum Smear layer removal - Biopure MTAD (maximum) > pro-EDTA>0.2%chitosan nanoparticle > NaOCl-Extra > CHX-Ultra > saline (least).

Order of maximum reduction in dentin microhardness- Pro-EDTA (maximum) > Biopure MTAD>0.2%chitosan nanoparticle > NaOCl-Extra > CHX-Ultra > saline (least).

The results of smear layer removal were: as shown in Figure A- CHx-Ultra, Figure B- NaOCl-Ultra, Figure C-pro EDTA, Figure D- 0.2 % chitosan nanoparticles, Figure E- Biopure MTAD, Figure F- normal Saline (see Fig. 7).

4. Discussion

This research project aimed to evaluate and correlate the influence of ChX-Ultra, NaOCl-Extra, Pro-EDTA, 0.2 % chitosan nanoparticles, Biopure MTAD and normal saline on smear layer elimination and dentinal microhardness alteration. Irrigants with chelator and surfactant have doubled facet action i.e. eradication of smear layer along with alteration in calcium/phosphate ratio, consequentially decreasing microhardness.

Table −2
Inter group comparison of frequencies of smear layer removal score using chi square test.

	smear layer removal score					Total	Chi square value	p value
	1	2	3	4	5			
CHX Ultra	0	0	0	6	4	10		
NaOCL Extra	0	0	5	5	0	10		
Pro- EDTA	1	6	3	0	0	10	99.387	0.000[a]
0.2%chitosan nanoparticles	0	4	6	0	0	10		
Biopure MTAD	3	6	1	0	0	10		
Saline	0	0	0	0	10	10		
Total	4	16	15	11	14	60		

[a] = statistically highly significant difference ($p < 0.01$).

Table −3
Inter group comparison of smear layer removal score (considering the scores to be on ordinal scale) (n = 10 per group) using Kruskal-Wallis Test.

Groups	Mean ± Std. Deviation	Median	Chi square value	p value of Kruskal-Wallis Test
CHX Ultra	4.4 ± 0.52	4		
NaOCL Extra	3.5 ± 0.53	3.5		
Pro- EDTA	2.2 ± 0.63	2	50.060	0.000[a]
0.2%chitosan nanoparticles	2.6 ± 0.52	3		
Biopure MTAD	1.8 ± 0.63	2		
Saline	5.0 ± 0.00	5		

[a] = statistically highly significant difference ($p < 0.01$).

Table −4
Intra group comparison of smear layer removal score between each pair of group using Mann-Whitney *U* Test.

Groups	comparision	Mann-Whitney U	Z value	p value
CHX Ultra vs	NaOCL Extra	33.000	−13.450	0.147[c]
	Pro- EDTA	34.000	−1.378	0.168[c]
	0.2%chitosan nanoparticles	7.500	−3.395	0.001[b]
	Biopure MTAD	0.000	−3.905	0.000[b]
	Saline	0.000	−4.108	0.000[b]
NaOCL Extra vs	Pro- EDTA	19.000	−2.571	0.010[a]
	0.2%chitosan nanoparticles	15.000	−2.936	0.003[b]
	Biopure MTAD	0.000	−3.914	0.000[b]
	Saline	0.000	−4.119	0.000[b]
Pro- EDTA vs	0.2%chitosan nanoparticles	2.5000	−3.725	0.000[b]
	Biopure MTAD	0.000	−3.905	0.000[b]
	Saline	0.000	−4.108	0.000[b]
0.2%chitosan nanoparticles vs	Biopure MTAD	15.000	−2.936	0.007[b]
	Saline	0.000	−4.110	0.000[b]
Biopure MTAD vs	Saline	20.000	−2.854	0.004[b]

[a] = statistically significant difference ($p < 0.05$).
[b] = statistically highly significant difference ($p < 0.01$).
[c] = non significant difference ($p > 0.05$).

Table −5
Inter group comparison of change in dentin microhardness expressed in Mpa (mega pascal) by different irrigants at apical root third. (n = 10 per groups) using One way Anova.

Groups	Mean ± Std. Deviation	Std. Error	F value	p value of One way ANOVA
Chx-Ultra	4.94 ± 1.43	0.45		
NaOCl-Extra	5.44 ± 1.62	0.51		
Pro-EDTA	12.8 ± 2.47	0.78	24.36	0.000[a]
0.2%Chitosan nanoparticles	5.48 ± 2.87	0.91		
Biopure MTAD	8.01 ± 3.06	0.97		
Saline	3.04 ± 0.64	0.20		

[a] = statistically highly significant difference ($p < 0.01$).

An imperative amendment in the current research was to incorporate a newer irrigating solution with surfactants and chelatorsfor efficient absolute eradication of the smear layer. Important parameters i.e. smear layer removal and dentinal microhardness alterations were evaluated considering both chemical and natural irrigants using a scanning electron microscope and Vickers Microhardness test that accounts for the strength of the study. In this study single-rooted mandibular premolars were used as they are the most extracted teeth for orthodontic corrections and are thus easily available. We also used 27-gauge side-vented needle tips for irrigation which aid in deeper penetration.

The smear layer and morphological details of the treated canals were assessed with a scanning electron microscope because of their high resolution and magnification. The appraisal of the microhardness of specimens was investigated with the Vickers microhardness test. Hardness is calculated as the resistance to the penetration of an indenter that is stiffer than the model to be investigated.[15] Microhardness of dentin shows considerable variation among teeth, so in the current research for ascertaining rational evaluations; measurement was recorded as a difference between baseline and post-treatment value. To measure Vickers

Table- 6
Intra group comparison for change in microhardness between each pair of groups using Post hoc Tukey test.

Groups	Comparison Groups	P value
Chx-Ultra vs	NaOCl-Extra	1.000[c]
	Pro-EDTA	0.000[b]
	0.2%chitosan nanoparticles	1.000[c]
	Biopure MTAD	0.042[a]
	Saline	0.865[c]
NaOCl-Extra vs	Pro-EDTA	0.000[b]
	0.2%chitosan nanoparticles	1.000[c]
	Biopure MTAD	0.169[c]
	Saline	0.264[c]
Pro-EDTA vs	0.2%chitosan nanoparticles	0.000[b]
	Biopure MTAD	0.000[b]
	Saline	0.000[b]
0.2%chitosan nanoparticles vs	Biopure MTAD	0.188[c]
	Saline	0.238[c]
Biopure MTAD vs	Saline	0.000[b]

[a] = statistically significant difference ($p < 0.05$).
[b] = statistically highly significant difference ($p < 0.01$).
[c] = non significant difference ($p > 0.05$).

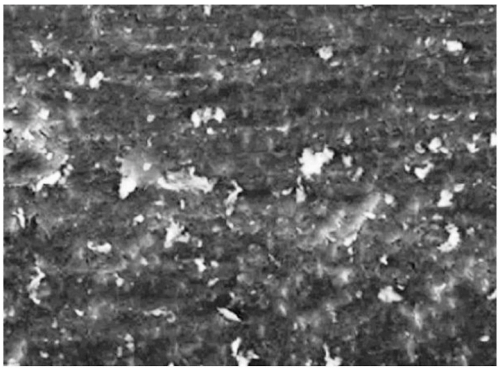

Fig. 1SEM image at 2000× magnification showing apical third root section irrigated with CHx-Ultra.

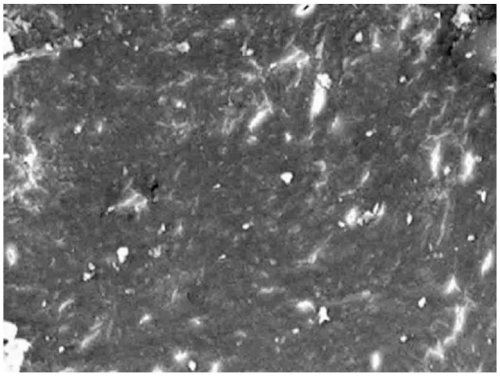

Fig. 2SEM image at 2000× magnification showing apical third root section irrigated with NaOCl-Ultra.

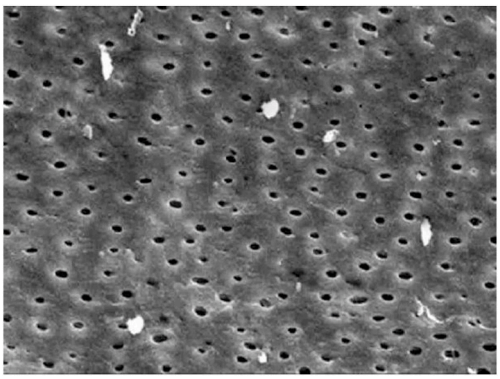

Fig. 3SEM image at 2000× magnification showing apical third root section irrigated with pro-EDTA.

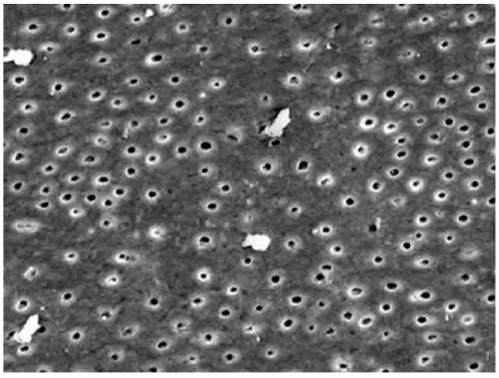

Fig. 4SEM image at 2000× magnification showing apical third root section irrigated with 0.2 % chitosan nanoparticles.

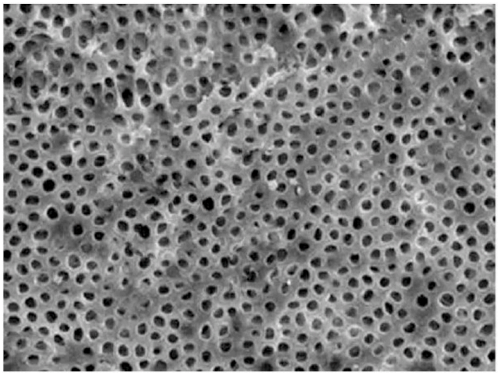

Fig. *5*SEM image at 2000× magnification showing apical third root section irrigated with Biopure MTAD.

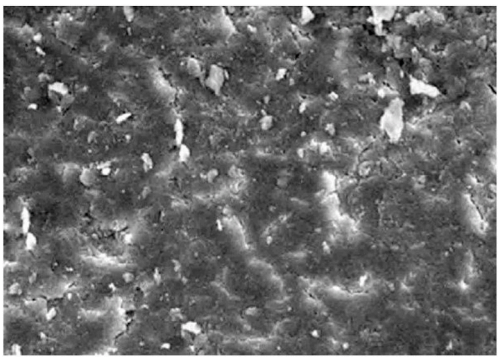

Fig. 6SEM image at 2000× magnification showing apical third root section irrigated with normal Saline.

Fig. 7image showing Vickers microhardness tester assessing baseline microhardness.

hardness values of dentin; an indentations test was done at the apical third region at a depth of 100 μm, each using a load of 200 g m and for 20 s dwell time.[16]

According to the investigation, observations recorded were in agreement with Torabinejad et al. conclusion that MTAD showed better results when compared with EDTA.[17] MTAD has both antibacterial as well as chelating properties. Its sustained antibacterial effect was due to doxycycline (bacteriostatic agent). Its chelating activity may be attributed to the synergistic action of citric acid, doxycycline, and detergent present in MTAD. Its low surface tension (34.5 mJ/m^2) permits deeper penetration and improves intimate contact of irrigant to dentin for the effective elimination of the smear layer.[18]It has a more acidic pH as compared to EDTA which might augment the removal of calcium ions from dentin.[18] MTAD, due to the presence of 4.25 % citric acid dissolves inorganic contents of the dentin that also counts for its decreased dentin microhardness.[19]

0.2 % chitosan is composed of Chitin dimer with N-acetyl-D glucosamine chain. In low pH atmospheres, protonation of amino groups results in negatively charged amines ($-NH_3^+$). These charged particles account for its adsorption and chelation to the metal resulting in the formation of a stable, water-soluble complex.[20,21] Two hypotheses challenged to explicate the chelating properties of chitosan. The first assumption is recognized as the "bridge model", which clarifies the attachment and adsorption of numerous amino acids of chitosan polysaccharides to comparable metallic ions. The subsequent conjecture is justified as a "free-arm model", in which barely one amino group of chitosan is concerned with the binding to the metal ions.[22] Recently, 0.2 % chitosan has been projected as the concluding irrigant and its

application for just 3 min was adequate for complete elimination of the smear layer.[22,23] 0.2%chitosan nanoparticleswere shown to havesmear layer removal abilitywith lesser dentinal micro-hardness reduction than pro-EDTA and Biopure MTAD in this study. Coherent justification to the statement is due to itshydrophilicnature and presence of free hydroxyl ions and amino groups resulting in ionic bonding between chelates and radicular dentinal calcium.[24] Additionally, chitosan has covalent bonding with dentinal collagen resulting in remineralization of demineralized peritubular and intertubular dentin by collagenase enzymatic action.[21] This observable fact arises due to the interaction of phosphate with calcium ions resulting in the nucleation of the calcium phosphate layer on the dentinal surface.[25]

Ulusoy and Gorgul, Torabinejad et al. and Moazayeni et al. inferred that 6 % NaOCl-Extra irrigant was unable to remove the inorganic content of the smear layer which is in agreement with the present study.[4,17,19] NaOCl being a deprotienizing agent dissolves organic content but is incapable in eradication of inorganic portion of the smear plug.[19] Naenni N. et al. concluded that CHX failed to dissipate the necrotic pulpal tissues and the smear layer.[26] Hence, the residual smear layer acts as an obstacle to limit contact with the dentin thus causing negligible alteration in microhardness.[5] Thepresent study noted that all irrigants, except saline, decrease dentin microhardness to some extent. Sayin et alalso stated that EDTA, EGTA, EDTAC and tetracycline HCl with and without consequent NaOCl treatment reduce dentin microhardness significantly.[27] These studies indicated that irrigation treatment had a potent direct effect on the dentin contents. Texeira et al. concluded that a neutral EDTA solution decreases mineral and non collageneous proteins (NCP's) of root dentin thus resulting in dentin microhardness reduction by elimination of bonded calcium ions.[28]

In this current study, normal saline was used as a control group. It acts in flushing and lubrication of the root canal. It is a biocompatible solution with no adverse effect even if extruded periapically, because its osmotic pressure is the same as that of the blood. However, it has no disinfectant or antimicrobial properties. Also does not remove the smear layer.

Novel irrigants such as ChX-Ultra, NaOCl-Extra, Pro-EDTA, and BioPure MTAD not only fascinatingly eradicate the smear layer but also cause dentinal microhardness reduction. These variations may influence the adhesive property of the endodontic sealer to the biomechanically prepared dentinal walls. Natural 0.2%chitosan nanoparticlesand chemical Biopure MTAD had a promising effect on removingthe smear layer with minimal alteration in microhardness.

One of the limitations of the current study was that it was an in vitro study and multiple aspects of clinically in vivo conditions could not be completely simulated. Therefore, further clinical trials of these irrigants are needed to appraise their clinical significance, efficiency and biocompatibility before they can be used clinically. However standardized conditions for all study groups allowed for comparable results.

5. Conclusion

Elimination of the smear layer is always accompanied bya reductioninmicrohardness (inverse correlation), the null hypothesis was rejected. Moreover, irrigants with surfactant and chelators enhanced smear layer removal which was best perceived in Biopure MTAD with less reduction in microhardnessthan pro-EDTA. Also, it can be accomplished that 0.2 % chitosan nanoparticles had a chelating effect with minimal alteration in microhardness than pro-EDTA and Biopure MTAD.

Ethical cleaarance

Ethical clearance taken and mentioned in manuscript.

Patient consent

Patient consent not required as this current study is in-vitro.

Source of funding

Nil.

Acknowledgements

done.

References

1. Hartmann MS, Barletta FB, Camargo Fontanella VR, Vanni JR. Canal transportation after root canal instrumentation: a comparative study with computed tomography. *J Endod*. 2007;33:962–965.
2. Schreier S, Malheiros SV, de Paula E. Surface active drugs: self-association and interaction with membranes and surfactants—physicochemical and biological aspects. *Biochim Biophys Acta*. 2000;1508:210–234.
3. Rossi-Fedele G, Prichard JW, Steier L, de Figueiredo JA. The effect of surface tension reduction on the clinical performance of sodium hypochlorite in endodontics. *Int Endod J*. 2013;46:492–498.
4. Ulusoy OIA, Gorgul G. Effects of different irrigation solutions on root dentine microhardness, smear layer removal and erosion. *Aust Endod J*. 2013;39:66–72.
5. Aslantas EE, Buzoglu HD, Altundasar E, Ahmet Serper A. Effect of EDTA, sodium hypochlorite, and chlorhexidine Gluconate with or without surface modifiers on dentin microhardness. *J Endod*. 2014;40:876–879.
6. Hargreaves KM, Berman LH, eds. *Cohen's Pathways of the Pulp*. eleventh ed. 2016.
7. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. *Int Endod J*. 2012;45:363–371.
8. Vemuri S, Kolanu SK, Varri S, Pabbati RK, Penumaka R, Bolla N. Effect of different final irrigating solutions on smear layer removal in apical third of the root canal: a scanning electron microscope study. *J Conserv Dent*. 2016;19:87–90.
9. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid in endodontics. *Eur J Dermatol*. 2013;7(Suppl.1):S135–S142.
10. Devadiga SB, Shetty D, Lakshmi G, Packiyam JE. Synthesis of chitosan silver nanoparticles from chitin of crustacean shell and its applications. *Int J Curr. Res Chem Pharmac Sci*. 2016;5:1–5.
11. Kurita K. Chemistry and application of chitin and chitosan. *Polym Degrad Stabil*. 1998;59:117–120.
12. Del C-PA, Kishen A, Shrestha A, Bramante CM. Antibacterial properties associated with chitosan nanoparticle treatment on root dentin and 2 types of endodontic sealers. *J Endod*. 2015;41:1353–1358.
13. Shafiei M, Jafarizadeh-Malmiri H, Rezaei M. Biological activities of chitosan and prepared chitosan-tripolyphosphate nanoparticles using ionic gelation method against various pathogenic bacteria and fungi strains. *Biologia*. 2019;7:1–8.
14. Schafer E, Lohmann D. Efficiency of rotary nickel-titanium FlexMaster instruments compared with stainless steel hand K Flexofile. Part 2. Cleaning effectiveness and instrumentation results in severely curved root canals of extracted teeth. *Int Endod J*. 2002;35:514e21.
15. De Deus G, Paciornik S, Mauricio MHP. Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine. *Int Endod J*. 2006;39:401–407.
16. Ballal NV, Mala K, Bhat KS. Evaluation of the effect of maliec acid and EDTA on the microhardness and surface roughness of human root canal dentin. *J Endod*. 2010;36:1385–1388.
17. Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. *J Endod*. 2003;29:170–175.
18. Single MG, Garg A, Gupta S. MTAD in endodontics: an update review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;112:70–76.
19. Mozayeni MA, Javaheri GH, Poorroosta P, Ashari MA, Javaheri HH. Effect of 17% EDTA and MTAD on intracanal smear layer removal: a scanning electron microscopic study. *Aust Endod J*. 2009;35:13–17.
20. Ratih DN, Enggardipta RA, Kartikaningtyas AT. The effect of chitosan nanoparticle as a final irrigation solution on the smear layer removal, micro-hardness and surface roughness of root canal dentin. *Open Dent J*. 2020;14:19–26.
21. Kamble AB, Abraham S, Kakde DD, , et alShashidhar C. Scanning electron microscopic evaluation of efficacy of 17% ethylenediaminetetraacetic acid and chitosan for smear layer removal with ultrasonics: an *in vitro* study. *Contemp Clin Dent*. 2017;8:621–626.
22. Silva PV, Guedes DFC, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. *Int Endod J*. 2013;46:332–338.
23. Bangun H, Tandiono S, Arianto A. Preparation and evaluation of chitosan-tripolyphosphate nanoparticles suspension as an antibacterial agent. *J Appl Pharmaceut Sci*. 2018;8:147–156.
24. Christian P, Von der Kammer F, Baalousha M, Hofmann T. Nanoparticles: structure, properties, preparation and behaviour in environmental media. *Ecotoxicology*. 2008;17:326–343.
25. Gusiyska A, Dyulgerova E, Vassileva R. The effectiveness of a chitosan citrate solution to remove the smear layer in root canal treatment - an *in vitro* study. *Indian J Sci Res*. 2016;5:1169–1174.

26. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. *J Endod*. 2004;30:785–787.
27. Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;104:418–424.
28. Teixeira CS, Felippe MCS, Felippe WT. The effect of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis. *Int Endod J*. 2005;38:285–290.