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Effect of Chitosan Nanoparticles on Root Dentin Roughness In Comparison With Sodium Hypochlorite And Ethylenediaminetetraacetic Acid: An In Vitro Study.

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Abstract:
Aim and Objectives: *The aim of this study is to evaluate the effect of chitosan nanoparticles on root dentin roughness in comparison with sodium hypochlorite and ethylenediaminetetraacetic acid.*
Materials and Methods: *Roots of 20 human caries free permanent maxillary anterior teeth were taken. They were sectioned longitudinally to obtain 40 dentin samples. Forty (40) dentin samples were divided into four groups (n=10). Group I – Distilled water (control group), Group II, III and IV are 5.25% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid and chitosan nanoparticle solution (1.5mg/ml) respectively. Roughness values of the dentin samples were evaluated by atomic force microscopy analysis on 4 groups (n=10), after each group was treated in one of the tested irrigant solution for 10 min.*
Statistical Analysis: *Values were statistically analysed by one-way ANOVA, followed by post hoc Tukey's test for pair wise comparison.*
Results: *17% EDTA demonstrated a significantly higher roughness value (Rq=53.56nm, Ra=42.40nm) compared to the other tested irrigants, while no significant differences were seen in 5.25%NaOCl (Rq=31.43nm, Ra=24.77nm) and chitosan nanoparticle solution (Rq=30.20nm, Ra=25.22) groups.*
Conclusion: *The newly introduced chitosan nanoparticles could increase dentin roughness similar to other commonly used irrigants and promotes adhesion of dentin to restorative materials.*
Key words: *Atomic force microscopy, Chitosan nanoparticles, Ethylenediaminetetraacetic acid, Root mean square, Surface roughness, Sodium hypochlorite.*

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I. Introduction

The main goal of antimicrobial irrigant solutions is to achieve bacterial elimination from the root canals.(1) There will be failure for complete removal of bacteria and dentinal debris if we use mechanical instrumentation only.(2) So, irrigating solutions should be used after instrumentation to eliminate microorganisms and dentinal debris.(1,3) 5.25% sodium hypochlorite (NaOCl) is most commonly used root canal irrigant due to its antimicrobial and high tissue dissolving property. The sequential use of 5.25% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraaceticacid (EDTA) as root canal irrigants had proved for its better antimicrobial activity and ability to remove smear layer.(3,4)

During the preparation of root canals with the use of irrigating solutions, removes the smear layer which in turn causes relative softening of dentinal walls.(5) It affects the adhesion and sealing ability of root canal sealers due to decrease in microhardness. The physicochemical properties of root dentin can be changed by irrigating solutions.(6,7)

The adhesion of restorative material to root canal walls depends mainly on the surface roughness of dentin.[8] According to wenzel equation increased dentin roughness resulted a decrease in contact angle.[7] Rough surfaces might be a clinical benefit as in case of micromechanical bonding of the adhesive materials that need the irregularities on the surface. Sodium hypochlorite and EDTA had shown increased dentin surface roughness in previous studies.[9] EDTA causes erosion of dentinal tubules due to hyper decalcification on dentin,[1] for that reason exploration for new irrigants is continuing. In this context, nanoparticles were being used due to their antimicrobial properties. Chitosan nanoparticles have come out as an effective antimicrobial agent due to its broad-spectrum effect.[11] However there is no report till now about these chitosan nanoparticles on root dentin roughness.

The aim of this in vitro study is to evaluate the effect of chitosan nanoparticles (1.5mg/ml) on root dentin roughness in comparison with 5.25% sodium hypochlorite and 17% ethylenediaminetetraacetic acid using Atomic force microscopy analysis.

II. Materials and Methods

The 20 human caries free permanent maxillary anterior teeth were collected (Fig 1a). The root canal irrigants included in this study are 5.25% NaOCl (Modi Surgico Healthcare Pvt Ltd, Maharashtra, India), 17% EDTA (PREVEST DenPro Pvt Ltd, Jammu, India), Chitosan nanoparticles solution (1.5mg/ml) (AURA Biotechnologies Pvt Ltd, Chennai, India). Distilled water (J. K. Laboratories, Maharashtra, India) was used as control. The 20 teeth were decoronated till the CEJ using diamond disk (Kwality diamond tools, Kalbadevi, Mumbai) under water coolant (Fig 1b). Then they are sectioned longitudinally using diamond disk under water coolant to obtain 40 dentin samples (Fig 1c & Fig 1d). The surfaces of these samples were polished with 400-, 600-, 800-, 1200-grit polishing papers (Bitec Paint & Refinishing suppliers) in order to remove surface irregularities (Fig 1e). Forty dentin samples were randomly divided into 4 groups (n=10) based on the irrigants into which the samples are immersed. Group I – Distilled water (control group), Group II, III and IV are 5.25% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid and chitosan nanoparticle solution (1.5mg/ml) respectively.

The samples of each group were treated with one of the tested irrigants (Distilled water, 5.25% NaOCl, 17% EDTA, Chitosan solution) for 10 minutes. Fresh liquid (5 ml) of Distilled water, 5.25% NaOCl, 17% EDTA, chitosan nanoparticles were used for each period of immersion (Fig 1f). Then samples were rinsed with deionized water and dried with filter paper (Fig 1g). The roughness of the samples was viewed under an AFM using contact mode imaging (Fig 1h). The portion of radicular dentin which is contacted with the AFM probe of each dentin sample was selected and viewed.

Figure 1a

Figure 1b

Figure 1c

Figure 1d

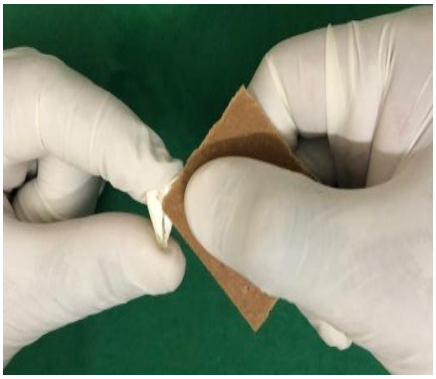

Figure 1e

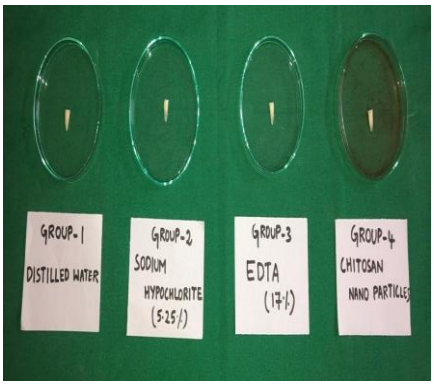

Figure 1f

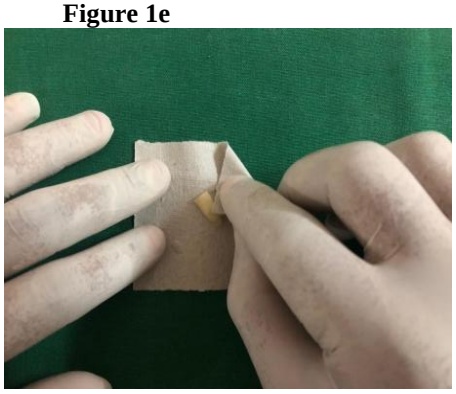

Figure 1g

Figure 1h

III. Statistical analysis

Roughness values were statistically analysed by one way ANOVA, followed by post hoc Tukey's test for pair wise comparison using statistical software IBM SPSS – 20.0 versions.

IV. Result

The atomic force microscopy images (Figure 2) had shown that 17% EDTA (Fig 2c) with maximum roughness followed by 5.25% NaOCl (Fig 2b), Chitosan (Fig 2d) and distilled water (Fig 2a) with least roughness. The one – way ANOVA shows that there is statistical significant difference ($P < 0.05$) for the root mean square (RMS) and surface roughness between the control (distilled water) and experimental groups (5.25% NaOCl, 17% EDTA, Chitosan Nanoparticles). The post – hoc test (Tukey HSD) shows that there is no statistical significant difference ($P > 0.05$) for both root mean square (RMS) and surface roughness between 5.25% NaOCl and chitosan nanoparticles (ChNPs) groups but there is significant difference from 17% EDTA and control groups (distilled water). 17% EDTA treated samples had shown highest surface roughness (Rq=53.56nm, Ra=42.40nm) and lowest was recorded in samples treated with distilled water (Rq=17.29nm, Ra=13.92nm), while it was similar statistically in samples treated with 5.25% NaOCl (Rq=31.43nm, Ra=24.77nm) and chitosan nanoparticles (Rq=30.20nm, Ra=25.22nm). (Table 1)

Table 1: Mean (SD) of roughness values of dentin samples for different irrigants (n=10). (Different letters in the column indicate a statistically significant difference)

Root canal irrigant	Root mean square (Rq)	Surface roughness (Ra)
Distilled water	17.29(0.84)[a]	13.92(0.72)[a]
5.25% NaOCl	31.43(0.96)[b]	24.77(1.10)[b]
17% EDTA	53.56(1.38)[c]	42.40(1.33)[c]
Chitosan Nanoparticles	30.20(1.48)[b]	25.22(1.53)[b]

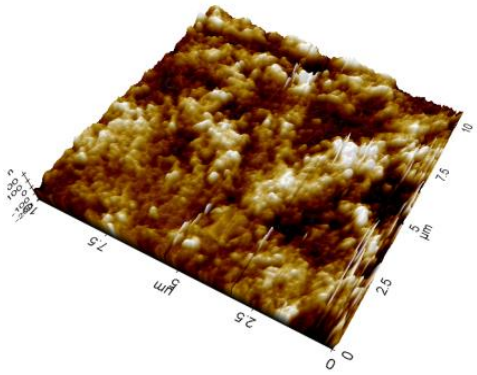

Figure 2a

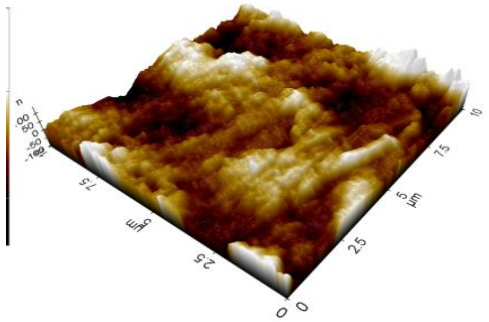

Figure 2b

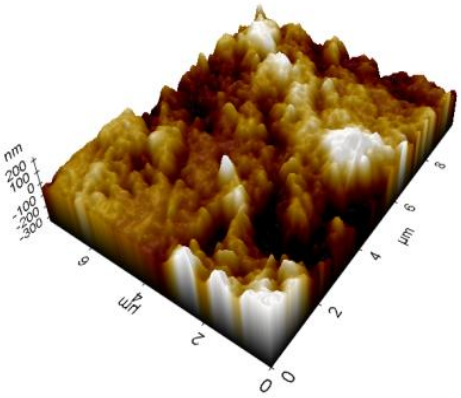

Figure 2c

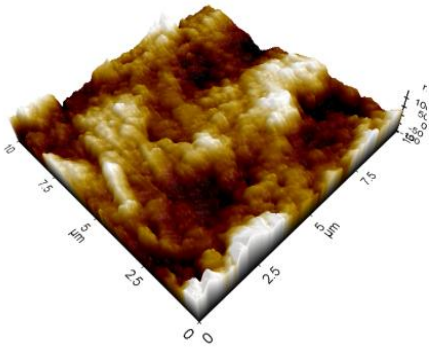

Figure 2d

Figure 2: AFM images of dentin surfaces after treatment with different root canal irrigants. Fig 2a - Distilled water, Fig 2b – 5.25% NaOCl, Fig 2c - 17% EDTA, Fig 2d – Chitosan Nanoparticles

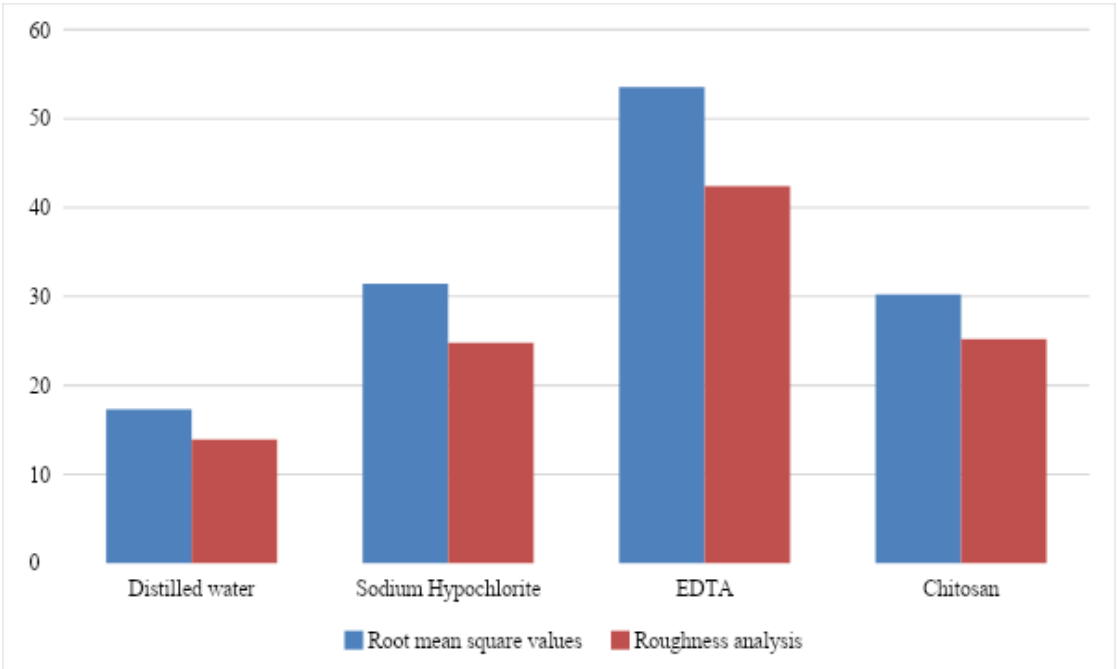

Figure 2: Graphical representation of both Root mean square (Rq) and Surface roughness (Ra) values of all dentin samples.

V. Discussion

The root canal irrigants are used along with mechanical instrumentation to achieve bacteria free environment in the root canals.[3] They cause softening of dentinal walls which ultimately affects adhesion and sealing ability of sealers.[5,6] However adhesion to root canals depends mainly on surface roughness as there will be decrease in contact angle.[7] Though previous studies had shown increased dentin surface roughness for sodium hypochlorite and EDTA[9] but irrigant like EDTA had shown erosion of dentinal tubules due to hyper decalcification on dentin.[1] So there is in need for introducing new irrigants to avoid detrimental effects on dentin. This study investigated the effect of chitosan nanoparticle irrigant solution on dentin roughness using AFM on contact mode comparing with common root canal irrigants (5.25% NaOCl, 17% EDTA). Various tools like stylus profilometer, computerized roughness tester and AFM etc., are there for measuring surface roughness. In spite of several methods to check dentin roughness, atomic force microscopy (AFM) is used in this study because surfaces are characterized at extremely high resolution.[10] Three dimensional images of surface topography also provided with this atomic force microscope.[13]

The dentin surface of all the samples are polished using polishing papers to remove surface irregularities which might have formed during longitudinal sectioning of samples using the diamond disks. Farshad M et al reported the dentin surface roughness using AFM after the immersion of samples in the irrigants for 10 minutes.[18] So, in this study all the samples were immersed in irrigant solutions for 10 minutes before evaluating surface roughness. The AFM probe will contact with the portion of dentin surface, the contacted portion will be viewed to evaluate the surface roughness. All the irrigants along with distilled water had shown surface roughness on all dentin samples. 17% EDTA treated samples had shown highest surface roughness (Rq=53.56nm, Ra=42.40nm) and lowest was recorded in samples treated with distilled water (Rq=17.29nm, Ra=13.92nm), while it was similar statistically in samples treated with 5.25% NaOCl (Rq=31.43nm, Ra=24.77nm) and chitosan nanoparticles (Rq=30.20nm, Ra=25.22nm).

To date there is no data regarding effect of chitosan nanoparticles on surface roughness of dentin. In the study 1.5 mg/ml ChNPs is taken, as it showed better antimicrobial activity. Surface roughness on dentin might be due to chelating action of chitosan which were explained by Bridge and Pendant model. These models explains that amino groups in chitosan bind to metal ions (Calcium) which results in depletion of inorganic matter from smear layer.[12]

The results which were obtained in this study regarding 5.25% NaOCl and 17% EDTA were similar to those which were previously reported by Hu et al, who used AFM in his study.[7] The results are also similar with findings by Ari et al, Patil CR et al who used computerized roughness tester and stylus profilometer in their study.[14]

EDTA at a neutral ph (7.3) acts through complex formation and protonation mechanism. The hydrogen ions in EDTA replace calcium in the hydroxyapatite. Thus the dentin surface roughness increases due to this chelation and smear layer removal property.[15] The NaOCl acts on the collagen (organic matrix) and make it dissolves thus causing the surface of dentin rough.[16,17] Further investigations should be carried out for this Chitosan Nanoparticles on physicochemical properties of dentin to prove its application clinically as a root canal irrigant.

VI. Conclusion

Within the limitations of this study, EDTA had shown maximum root dentin roughness. We also found that newly introduced chitosan nanoparticles could increase dentin roughness similar to other root canal irrigants and promotes adhesion of dentin to restorative materials.

References

[1]. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98.
[2]. Siqueira J, Machado A, Silveira R, Lopes H, Uzeda Md. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. Int Endod J. 1997;30(4):279-82.
[3]. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg OralMed Oral Pathol. 1983;55(3):307-12.
[4]. Johnson W, Noblett W. Cleaning and shaping in: endodontics: principles and practice. Saunders, Philadelphia, PA; 2009.
[5]. Cruz-Filho AMd, Paula EAd, Pecora JD, Sousa-Neto MDd. Effect of different EGTA concentrations on dentin microhardness. Braz Dent J. 2002;13(3):188-90.
[6]. Perdigao J, Eiriksson S, Rosa BT, Lopes M, Gomes G. Effect of calcium removal on dentin bond strengths. Quintessence Int. 2001;32(2).
[7]. Hu X, Ling J, Gao Y. Effects of irrigation solutions on dentin wettability and roughness. J Endod. 2010;36(6):1064-7.
[8]. Rosales J, Marshall G, Marshall S, Watanabe L, Toledano M, Cabrerizo M, Osorio R. Acid-etching and hydration influence on dentin roughness and wettability. J Dent Res. 1999;78(9):1554-9.
[9]. Saleh A, Ettman W. Effect of endodontic irrigation solutions on microhardness of root canal dentine. J Dent. 1999;27(1):43-6.
[10]. Farina M, Schemmel A, Weissmuller G, Cruz R, Kachar B, Bisch P. Atomic force microscopy study of tooth surfaces. J Struct Biol. 1999;125(1):39-49.

[11]. Alejandra Cardelle-Cobas, Patrica J.M.Reis, Eduardo Costa, Freni K.Tavaria, Manuela E.pintado. Chitosan impregnated gutta-percha points: antimicrobial in vitro evaluation and mechanical properties. International Journal of Polymeric Materials and Polymeric Biomaterials. 2018:68(9),481-488.
[12]. Del Carpio-Perochena A, Bramante CM, Duarte MA, de Moura MR, Aouada FA, Kishen A. Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restor Dent Endod. 2015;40(3):195–201.
[13]. Allison DP, Mortensen NP, Sullivan CJ, Doktycz MJ. Atomic force microscopy of biological samples. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010;2(6):618-34.
[14]. Ari H, Erdemir A, Belli S. Evaluation of the effect of endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. J Endod. 2004;30(11):792-5.
[15]. Eldeniz AU, Erdemir A, Belli S. Effect of EDTA and citric acid solutions on the microhardness and the roughness of human root canal dentin. J Endod. 2005;31(2):107-10.
[16]. Di Renzo M, Ellis T, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces:: II. Deproteination. Biomaterials. 2001;22(8):793-7.
[17]. Gordon TM, Damato D, Christner P. Solvent effect of various dilutions of sodium hypochlorite on vital and necrotic tissue. J Endod. 1981;7(10):466-9.
[18]. Farshad M, Abbaszadegan A, Ghahramani Y, Jamshidzadeh A. Effect of Imidazolium-Based Silver Nanoparticles on Root Dentin Roughness in Comparison with Three Common Root Canal Irrigants. Iran Endod J. 2017;12(1):83-6.

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