

## Research Article

### **Comparison of the Effect of Chitosan and Morinda citrifolia on Smear layer removal: An *in-vitro* study**

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**Abstract:** The aim of the study was to compare the effectiveness of Chitosan and Morinda citrifolia juice with EDTA on smear layer removal from the instrumented root canals. The methodology includes preparation of forty four extracted single rooted human permanent teeth by crown down preparation. During instrumentation, the canals were irrigated with 1ml of 3% Sodium Hypochlorite (NaOCl). Samples were divided into four groups based on the final irrigation. Group 1(n=11): 0.2% Chitosan (CH), Group 2(n=11): Morinda citrifolia juice (MCJ), Group 3(n=11): 17% Ethylene diaminetetraacetic acid (EDTA), Group 4(n=11): without any final irrigation (control group). Teeth were sectioned and observed for smear layer removal at apical, middle and coronal thirds under Scanning Electron Microscope (SEM). Results were analyzed statistically by Kruskal– Wallis test and Wilcoxon Signed Ranks test and it was shown that the three experimental groups were significantly more effective in smear layer removal than the control group. There was no significant difference between Chitosan and EDTA groups. Chitosan group was significantly better than MCJ group (p< 0.05). Smear layer removal was better at coronal third followed by middle third and apical third for all the groups. It was concluded that 0.2% chitosan and 17% EDTA effectively removed the smear layer when compared to Morinda citrifolia juice.

**Keywords:** Chitosan, EDTA, Irrigation, Morinda citrifolia, Root canal, Smear layer

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#### **INTRODUCTION**

Reduction of bacteria within the root canal system is achieved by mechanical shaping and cleaning procedures. Such procedures result in the formation of amorphous smear layer on the root canal walls, which not only promotes adhesion and colonization of microorganisms but also delays the effect of irrigants and medicaments[1]. It also interferes with the adaptation and penetration of the root canal sealers thus compromising the so called fluid tight seal[2]. Therefore removal of smear layer is deemed necessary, as it plays an important role in the success of the root canal therapy.

Since its introduction, EDTA has been the most widely used chelating agent. However, there are various concerns regarding the use of EDTA as it may cause damage to the periapical tissues and root canal dentin erosion depending on its concentration and application time. It is also considered a pollutant, as it is not found originally in nature[3].

Hence, the search for more biocompatible solutions continues. Chitosan, a natural polysaccharide

obtained from the deacetylation of chitin has been used in many applications like food, cosmetics, biomedical and pharmaceutical applications because of its properties like biocompatibility, biodegradability, bioadhesion and atoxicity to the human body[4,5]. It also shows remarkable chelating capacity for different metal ions, because of its acidic pH and hence used in various sectors of industry for recovery of metals[6]

Noni, with the botanical name *Morinda citrifolia* is a traditional folk medicinal plant that has been used for over 2000 years by Polynesians. It has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory, immune-enhancing effects[7,8].

Most of the studies on Morinda citrifolia juice (MCJ) have focused on antimicrobial activity[9] with very little literature on smear layer removal and there are no earlier studies comparing the efficacy of chitosan and MCJ. Hence, this study was conducted to compare the effect of 0.2% Chitosan and Morinda citrifolia juice

on the smear layer removal of endodontically treated teeth using Scanning Electron Microscope.

## MATERIALS AND METHODS

Forty four maxillary and mandibular, single-rooted, noncarious, extracted permanent human teeth with fully developed apices ranging in length from 21 to 25 mm with intact clinical crowns were included in this study and were stored in 0.1% thymol solution. Teeth with coronal restorations and root fillings were excluded. Teeth were randomly divided into three experimental groups and a control group of 11 each.

### Preparation of the Root Canal:

Conventional access preparation was made for each tooth and a 10 or 15 K-type file (Dentsply Maillefer, Switzerland) was introduced into the root canal until it could be seen at the apical foramen. It was then withdrawn to be within the apical foramen and the working length was established. Later the canals were prepared using crown down procedure using rotary protaper universal files (Dentsply Maillefer, Switzerland). After each file, the canal was irrigated with 1 ml of 3% NaOCl for 1 min. After shaping, the canals were finally irrigated with test solutions.

Samples were divided into 4 groups based on the final irrigation: Group 1(n=11): 1 ml of 0.2% Chitosan (CH) (Panvo Organics, Chennai, India) for 1 min, Group 2(n=11): 1 ml of Morinda citrifolia juice (MCJ) (Sri Vishnu Biotec Formulations, India) for 1 min, Group 3(n=11): 1 ml of 17% EDTA solution (EDTA) (Chen Chemicals, Chennai, India)for 1 min, Group 4(n=11): without any final irrigation (control group).

For preparation of the 0.2% chitosan solution, 0.2 g of chitosan (90% degree of deacetylation) was diluted in 100 ml of 1% acetic acid, and the mixture was stirred for 2 h using a magnetic stirrer.  
Scanning Electron Microscope Observation:

Longitudinal grooves were made on the buccolingual surfaces of the teeth using a silicon carbide disc without penetrating the canal and then the teeth were split into two halves with a chisel. The samples were then dehydrated and sputter-coated with gold and observed under Scanning Electron Microscope

(SEM) with a magnification of 1000x. Analysis of the SEM images was performed by three investigators who scored the presence of smear layer on the surface of the root canal at the coronal, middle and apical portion of each canal.

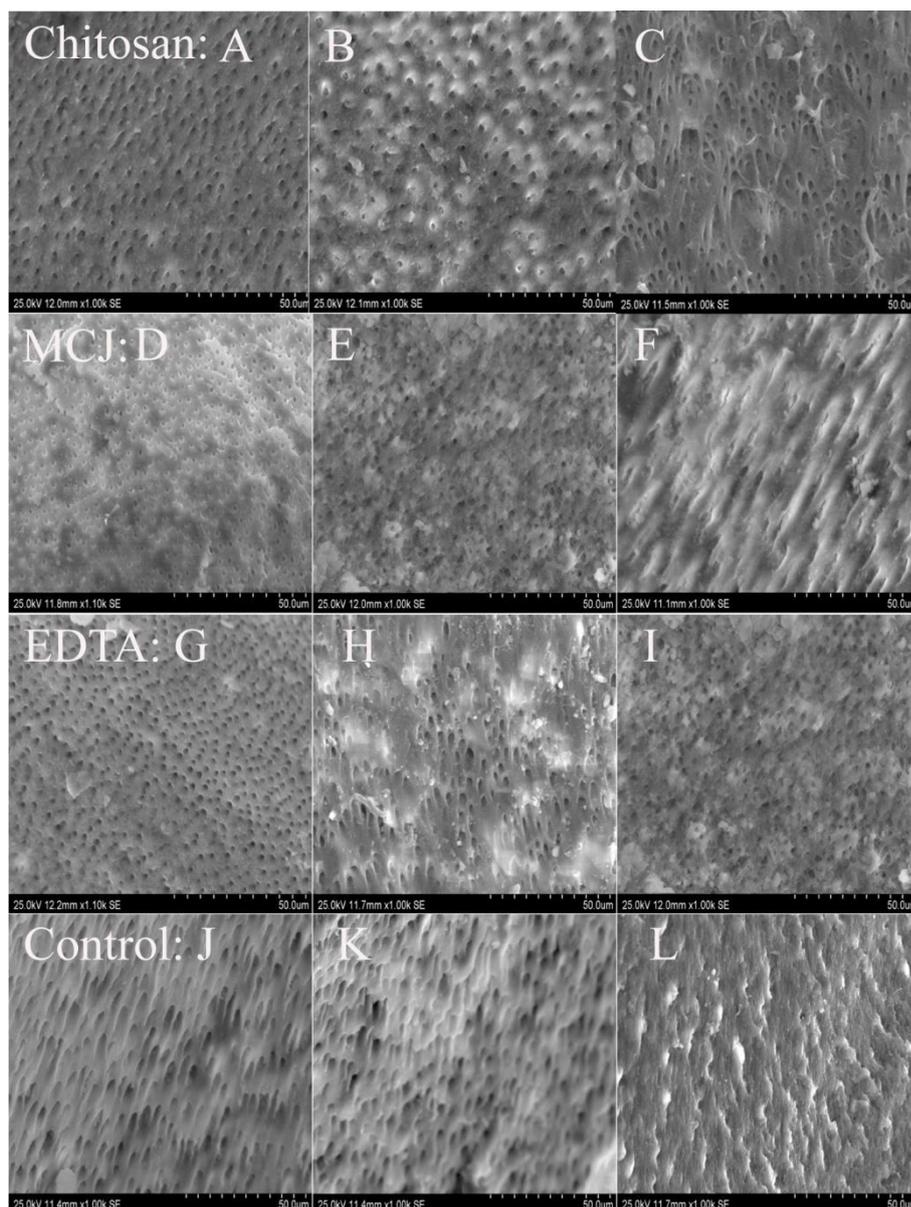
Scores were given based on the criteria described by Torabinejad et al:[10] Score 1- no smear layer, no smear layer on the surface of the root canals, and all tubules are clean and open, Score 2- moderate smear layer, no smear layer on the surface of the root canals, but tubules contain debris, Score 3- heavy smear layer, smear layer covers the root canal surface and the tubules

Data were further analyzed statistically by kruskal – Wallis test and Wilcoxon Signed Ranks test.

## RESULTS

Figure 1 represents the Scanning Electron Microscope images of Chitosan, Morinda citrifolia juice, EDTA and Control groups at coronal, middle and apical thirds. SEM images of Chitosan and EDTA groups shows no or only a small amount of smear layer in the coronal third, small to moderate amount in the middle third and moderate to heavy smear layer was present in the apical third; whereas for MCJ group, small to moderate amount was present in the coronal third, moderate to heavy in the middle and heavy smear layer in the apical third; and for control group, moderate to heavy amount in the coronal third and heavy smear layer in the middle and apical third.

Table 1 presents the mean values of the smear layer of the 4 groups. The three experimental groups effectively removed the smear layer and were statistically significant when compared to the control group. There was no statistical significance between Chitosan and EDTA groups. Chitosan group was significantly better than MCJ group ( $p < 0.05$ ). Thus to summarize, the Chitosan and EDTA were efficient in smear layer removal. The MCJ group was better than the control group but less efficient when compared to Chitosan and EDTA groups. When the different root canal levels of all the groups were compared, smear layer removal was better at the coronal third than the middle third. It was least at the apical third.



**Fig-1: Removal of smear layer of root canal walls in Chitosan, MCJ, EDTA and Control groups. A, B, C represents the smear layer removal at coronal, middle and apical thirds of Chitosan group respectively; D, E, F represents coronal, middle and apical thirds of MCJ group respectively; G, H, I represents coronal, middle and apical thirds of EDTA group respectively and J, K, L represents coronal, middle and apical thirds of Control group respectively.(1000x magnification)**

**Table 1: Mean value of the smear layer of the four groups**

	<b>CORONAL 3RD</b>	<b>MIDDLE 3RD</b>	<b>APICAL 3RD</b>
CHITOSAN	1.27 ± 0.47	1.82 ± 0.41	2.55 ± 0.52
MCJ	1.73 ± 0.47	2.18 ± 0.41	2.64 ± 0.51
EDTA	1.27 ± 0.47	1.64 ± 0.51	2.59 ± 0.51
CONTROL	2.00 ± 0.00	2.45 ± 0.52	3.00 ± 0.00

## DISCUSSION

Root canal instrumentation produces smear layer on the root canal walls which is composed of inorganic material like dentin chips and organic elements such as pulp tissue debris, bacteria and blood cells[11]. The presence of such layer increases the risk of microleakage and bacterial infection[12]. Hence, the

removal of smear layer is essential. Shahravan et al concluded that smear layer removal improves the fluid-tight seal of the root canal system whereas other factors such as the obturation technique or the sealer, did not produce significant effects[13].

NaOCl is routinely used for irrigating canals as it dissolves the necrotic tissue. Wu et al concluded that 3% NaOCl when irrigated for 1 min had removed the smear layer[14]. In the current study, smear layer removal was appreciated at the coronal third of the control group where 3% NaOCl was used as a basic irrigant between instrumentation without any other final irrigation.

EDTA is the most common decalcifying agent. Crumpton et al reported that the removal of smear layer was effective by using 1 ml of 17% EDTA for 1 min[15]. But the application of EDTA for more than 1min or in volumes greater than 1 ml led to the erosion of the root canal wall[10,16,17]. So, in the current study, 1ml of 17% EDTA was used for 1 min. To make valid comparisons among the three final irrigants, all the three experimental groups were irrigated with 1 ml of the final irrigant for 1 min respectively.

EDTA favours the smear layer removal by acting on its inorganic portion but it has various disadvantages. Sayin et al, concluded that the usage of EDTA either alone or in combination with NaOCl reduces the microhardness of root dentin significantly[18]. Thus to overcome the adverse effects of EDTA, search for biocompatible substances continues and Chitosan is one such biocompatible material. Chitosan at low concentration has removed smear layer similar to EDTA.

Chitosan acts on the inorganic portion of the smear layer favouring its removal. The formation of complexes between chitosan and metal ions most probably is due to the mechanisms of adsorption, ion exchange and chelation. The type of interaction that occurs depends on the involved ions, the chemical structure of chitosan and the pH of the solution[19]. Currently, there are two theories that explain the chelation process of chitosan. The first, known as the model of the bridge, which states that two or more amino groups of one chitosan chain will bind to the same metallic ion[20]. The second theory supports that only one amino group of the structure is involved in binding, which is the metal ion “anchored” to the amino group[21]. The chitosan polymer is formed by a chain composed of several dimers of chitin. Similar to the EDTA molecule, the chitin dimer shows two nitrogen atoms with pairs of free electrons responsible for the ionic interaction between the metal and the chelating agent. In an acidic medium, the amino groups present in the bipolymer are protonated, resulting in an overall positive charge ( $-NH_3^+$ ). This form is responsible for the attraction to other molecules in order for adsorption to occur[22].

This study demonstrated that there was no statistically significant difference between Chitosan and EDTA in the smear layer removal. This was in accordance to the study conducted by Silva et al, in

which 0.2% chitosan, 15% EDTA and 10% citric acid were associated with similar smear layer removal patterns[3]. Pimenta et al reported that the 0.2% chitosan solution has a capacity of reducing dentin microhardness similar to that of 15% EDTA[2].

Herbal and natural products have been used for centuries, throughout the world, in every culture. *Morinda citrifolia* is one such product which is gaining importance for natural remedies now a days[23]. This study demonstrated that *Morinda citrifolia* juice has removed smear layer better than the control group. Apart from the bioactive compounds which are responsible for the antibacterial property of *Morinda citrifolia* juice, it also contains organic acids like caproic acid, ursolic acid and caprylic acids[24]. The smear layer removal property of MCJ could be due to the presence of these organic acids.

All the groups showed less or no removal of smear layer at the apical third of the root canal. This is because, the flow ability and backflow of the fluid are poor at the apical third due to the reduced diameter and the increase in depth of the root canal[14].

## CONCLUSIONS

The study concluded that

- The effect of smear layer removal of 0.2% Chitosan is similar to 17% EDTA solution and better than *Morinda citrifolia* juice.
- Natural polysaccharide like Chitosan effectively removes smear layer when compared to EDTA.
- The effect of smear layer removal of *Morinda citrifolia* juice is better than control group.
- The effect of smear layer removal for all groups at different root canal levels: coronal third better than the middle third which is again better than the apical third.

## REFERENCES

1. Violich DR, Chandler NP; The smear layer in endodontics – a review. *Int Endod J*, 2010; 43(1): 2-15.
2. Pimenta JA, Zapparoli D, Pecora JD, Cruz-Filho AM; Chitosan: effect of a new chelating agent on the microhardness of root dentin. *Braz Dent J*, 2012; 23(3): 212-217.
3. Silva PV, Guedes DF, Nakadi FV, Pecora JD, Cruz-Filho AM; Chitosan: a new solution for removal of smear layer after root canal instrumentation. *Int Endod J*, 2013; 46(4): 332-338.
4. Rinaudo M; Chitin and chitosan: Properties and applications. *Prog Polym Sci*, 2006; 31: 603– 632.
5. Silva PV, Guedes DF, Nakadi FV, Pecora JD, da Cruz-Filho AM; Time-dependent effects of Chitosan on dentin structures. *Braz Dent J*, 2012; 23(4): 357-361.

6. Kong M, Chen XG, Xing K, Park HJ; Antimicrobial properties of chitosan and mode of action: A state of the art review. *Int J Food Microbiol*, 2010; 144(1): 51–63.
7. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK *et al*; *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. *Acta Pharmacol Sin*, 2002; 23(12): 1127–1141.
8. Murray PE, Farber RM, Namerow KN, Kuttler S, Garcia-Godoy F; Evaluation of *Morinda citrifolia* as an endodontic irrigant. *J Endod*, 2008; 34(1): 66-70.
9. Bhardwaj A, Velmurugan N, Sumitha, Ballal S; Efficacy of passive ultrasonic irrigation with natural irrigants (*Morinda citrifolia* juice, Aloe Vera and Propolis) in comparison with 1% sodium hypochlorite for removal of *E. faecalis* biofilm: An invitro study. *Indian J Dent Res*, 2013; 24(1): 35-41.
10. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K *et al*; A newsolution for the removal of the smear layer. *J Endod*, 2003; 29(3): 170-175.
11. Dechichi P, Moura CCG; Smear layer: a brief review of general concepts. Part I. Characteristics, compounds, structure, bacteria and sealing. *RFO UPF*, 2006; 11(2): 96-99.
12. Bansal P, Gupta H; Smear layer in Endodontics- A Review. *Indian J Dent Sci*, 2009; 1: 67- 69.
13. Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F; Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. *J Endod*, 2007; 33(2): 96-105.
14. Wu L, Mu Y, Deng X, Zhang S, Zhou D; Comparison of the effect of four decalcifying agents combined with 60 °C 3% Sodium hypochlorite on smear layer removal. *J Endod*, 2012; 38(3): 381-384.
15. Crumpton BJ, Goodell GG, McClanahan SB; Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. *J Endod*, 2005; 31: 536–538.
16. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S; The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. *J Endod*, 2003; 29: 233–239.
17. Tay FR, Gutmann JL, Pashley DH; Microporous, demineralized collagen matrices in intact radicular dentin created by commonly used calcium-depleting endodontic irrigants. *J Endod*, 2007; 33: 1086–1090.
18. 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.
19. Rhazi M, Desbrieres J, Tolaimate A, Rinaudo M, Vottero P, Alagui A *et al*; Influence of the nature of the metal ions on the complexation with chitosan. Application to the treatment of liquid waste. *Eur Polym J*, 2002; 38: 1523-1530.
20. Blair HS, Ho TC; Studies in the adsorption and diffusion of ions in chitosan. *J Chem Technol Biotechnol*, 1981; 31: 6-10.
21. Domard A; pH and c.d. measurements on a fully deacetylated chitosan: application to CuII–polymer interactions. *Int J Biol Macromol*, 1987; 9: 98-104.
22. Shahidi F, Arachchi JKV, You JJ; Food applications of chitin and chitosan. *Trends Food Sci Technol*, 1999; 10: 37-51.
23. Mohd Zin Z, Abdul-Hamid A, Osman A; Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chem*, 2002; 78: 227–231.
24. Blanco Y C, Vaillant F, Perez A M, Reynes M, Brillouet J M, Brat P; The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. *J Food Compo Anal*, 2006; 19: 645-654.