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The Effectiveness of a Chitosan-Citrate Solution to Remove the Smear Layer in Root Canal Treatment-An *in-vitro* study

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Abstract: ***Successful endodontic treatment is based on shaping, cleaning and 3D sealing of the root canal system. Still today in clinical practice is challenging effective removal of smear layer, especially in teeth with complex endodontic anatomy or in retreatment cases. Many solutions are used as irrigants but most of them have disadvantages as for use alone and in combination - sodium hypochlorite, ethylenediaminetetraacetic acid, citric acid, hydrogen peroxide, chlorhexidine, saline solution, distilled water, etc. The endodontic science still looking for the ideal irrigant, the solution which meets all the requirements. In this respect chitosan is quite unique bio-based polymer with different characteristics that make it one of the promising exploitable material in medicine. Now a day as an alternative antimicrobial agent in dentistry was proposed the use of chitosan.***

Keywords: chitosan, chitosan-citrate, dentin tubules, smear layer, scanning electron microscopy.

1. Introduction

Successful endodontic treatment is based on expansive clinical experience, recommendations from guidelines, treatment protocols and current evidence based dental medicine. Accurate debridement of root canals is recommended in most endodontic treatment [13]. Mechanical preparation alone cannot suffice to create a sterile space within the infected root canals due to complex nature of the human root canal systems [15]. As per recent consensus statements on endodontics, both traditional stainless steel hand instruments and the use of nickel-titanium equipment leave nearly half of the root canal walls untreated [15]. Thus, it is critical to have an irrigation system or intervention as part of the conventional root canal treatment. The sole objective of the irrigation is for cleansing that does not take place with mechanical preparation [8].

Based on current evidence, endodontic science recommends use of sodium hypochlorite (NaOCl) as a medium for root canal irrigation. In some cases it is recommended the combination of ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX) which is a better alternative to sodium hypochlorite [3]. Based on a literature review of current endodontic treatment and irrigating interventions, the aforementioned irrigants are most likely recommended owing to their capability to dissolve necrotic pulp and a broad antimicrobial spectrum [11].These irrigants also help in dissolving inactive endotoxins which is critical in endodontic treatment. In some cases, the irrigants are known to prevent or dissolve the smear layer [31]. The conventional root canal irrigants have been associated with serious health disadvantages; for example, NaOCl is associated with cytotoxicity, allergic reaction, unpleasant taste, effects on the periradicular tissues including caustic erosion [8]. Many researchers have been investigating for alternatives to NaOCl due to its severe inflammatory reactions and irrigating properties [3].

In comparison to NaOCl, CHX can be considered as an alternative. However, based on current evidence and a systematic review, 2% CHX is associated with skin irritation and other minor allergic reactions [31].Thus, as per a consensus from major review articles and current evidence, the ideal root canal irrigation systems should be non-caustic, non-toxic, and have low levels of anaphylactic reactions [23]. Latest recommendations are directed to use of citric acid as an ideal alternative to the more reactive EDTA. However, citric acid is associated with calcium ion reactions and has low antibacterial characteristics [20. However, the potential use of citric acid lies within its concentration since, the antibacterial strength is linked with its concentration [24].

Based on current reviews, 10% citric acid is known to have ideal chelating properties for phosphorus (dentin) and calcium ions [11]. High concentration citric acid (25 to 50%) is known to dissolve smear but was associated with high dentin erosion. Thus, citric acid cannot be considered as an ideal irrigant in endodontics [10]. Due to the prevailing issues with conventional irrigating interventions, many researchers were involved in the developed of a mixture of antibiotics and citric acid. Some of the irrigants developed include Biopure MTAD® and Tetraclean® [28]. Based on a few trials, most of the irrigants were known to have enhanced antimicrobial activity while some also helped in smear removal [16,18,19]. However, after a few more trials, it was observed that the newly developed irrigants were associated with bacterial resistance [21]. Thus, based on the review of conventional non-effective irrigating systems, researchers focused on chitosan and chitosan-based mixtures [7,12,23]. Chitosan which is a natural derivative and a polysaccharide is known to have great impact in endodontics due to its bio-adhesion, biodegradability, biocompatibility, and low toxic profile [23]. Chitosan has been shown to have a broad spectrum of antimicrobial properties and also associated with high chelating characteristics with respect to metal ions in extreme acidic conditions [1].Based on current

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research to assess the antibacterial properties of chitosan, researchers combined calcium hydroxide with chitosan and observed activity against E. faecalis [4. The same study also provided evidence on the smear removal and conditioner property of chitosan-acetate solution [4].

In this respect chitosan is quite unique bio-based polymer with different characteristics that make it one of the promising exploitable material in medicine. Now a day as an alternative antimicrobial agent in dentistry was proposed the use of chitosan. The presence of amino groups in the chitosan structure differentiates chitosan from chitin ,and gives to this polymer many exclusive properties. The amino group of the D-glucosamine residues might be protonated and providing solubility in diluted acid (pH < 6) which opens prospects to wide range of application. Due to the amino groups chitosan efficiently complex various species. The chitosan shows an excellent biocompatibility, no toxicity, high bioactivity, biodegradability, selective permeability, antimicrobial activity, adsorption capacity, and last but not least chelation ability [2,9]. The use of chitosan in endodontics is of interest due to its biocompatibility, antimicrobial and chelating properties.

The irrigating solutions should eliminate both organic and inorganic phases of the smear layer and not have an erosive effect on dentin surfaces [27,28].The activities of chitosan should be evaluated at different concentration and application time on smear layer removal and dentin structure.

The aim of this study was to investigate the chelating ability of chitosan, as a chitosan-citrate irrigation solution in different concentrations in endodontics to remove the smear layer.

2. Material and Methods

The study included 30freshly extracted single-rooted human teeth. After extraction, all the teeth were placed in a 2.5% NaOCl solution for 15 min. The tissue and debris remnants on the root surface were then removed and stored in a 0.9% saline solution with thymol. The teeth were free of caries, cracks, root canal treatment and restorations.The selected teeth were divided into four groups according to chemical preparation of radicular dentin.

Root canal preparation

The sound teeth were decoronated at the cementoenamel junction. The working length was established using a K-file #10 (Dentsply Maillefer), which was introduced into the root canal of each tooth up to the point that it was visible at the apex and then returns 1 mm. The canals were enlarged with rotary nickel-titanium instruments - ProTaper Universal(Dentsply Maillefer) - a standard flare was produced by endodontic instruments toF4 (ISO 40)using the crown-down technique according to the manufacture's protocol. It was used different irrigation solutions in each group between files (from SX to F4). In Group I (*n=5*) 3 ml 5.25% NaOCl was used between files, Group II (*n=5*) - 17% EDTA, Group III (*n=5*) - 5.25% NaOCl and 17% EDTA, Group IV (*n=5*) - 0.6 % chitosan-citrate (0.6 mg of the chitosan powder were dissolved in 100 ml of 1% citric acid). In a control Group V (*n=1*) - distilled water was used for irrigation and in a negative Group VI(*n=1*) the root was instrumented without irrigation.

Specimen preparation

The roots were splited longitudinally into two halves: buccal and lingual (palatinal)after the preparationThe specimens were desiccated and fixed on aluminum stands for vacuum-coated with gold dust in an argon medium with cathode sputtering (JEOL JFC-1200 Fine Coater, Tokyo, Japan). The dentin wall was examined using a scanning electron microscope (JEOL JSM-5510, Tokyo, Japan) at × 2 500 magnifications.

SEM evaluation

From each sample, 6 magnified images were taken (2 images from each part of the roots - coronal, middle and apical). The cleaning effect was measured as a percentage according to the ratio of the number of open versus total dentin tubules for evaluation of smear layer removal. It was used a three-point scoring system proposed by Torabinejad et al. [28]: *score 0* - no smear layer (no smear layer on the dentin surface and all tubules are clean and open); *score 1* - moderatesmear layer (no smear layer on the dentin surface, but there are tubules with debris), and *score 2* - have smear layer (smear layer covers dentin and smear plugs are visible in dentin tubules).

All the SEM micrographs of the coronal, middle and apical third of the roots were obtained using a scanning electron microscope. The collected data were analyzed using Kruskal-Wallis and Mann-Whitney U tests. P values were computed and compared with statistical significance at the p=0.05 level.

3. Results

The smear layer removal or dentin wall cleanliness is presented in Table 1. In the limitation of this study our results show a comparability of results in Group III and Group IV.

Table 1: Presentation of statistically analyzed data for cleanliness of the dentin wall among different groups

Average cleanliness Group	
Group I	1.99
Group II	1.79
Group III	1.22
Group IV	1.23
Group V (+)	2
Group VI (-)	0

Specimens treated with 5.25% NaOCl and 17% EDTA (Group III)present surface with open dentin tubules and single area with a smear layer on dentin (Fig.1).Specimens irrigated only with NaOCl or EDTA (Group I and Group II) present a thick smear layer and smear plugs in both the apical and middle third (Fig.2A,B).

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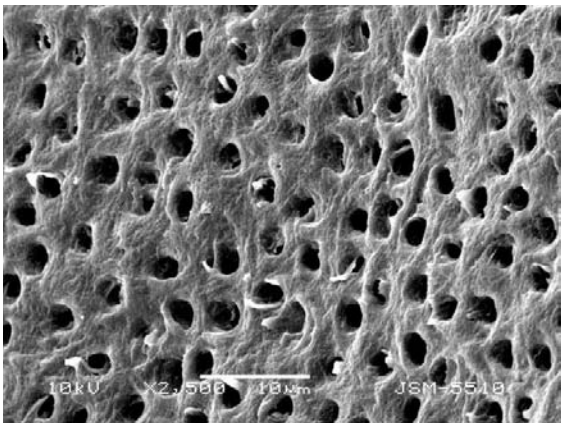

Figure 1: SEM image of dentin surface from specimen of Group III (*apical third*) - treated with 5.25% NaOCl and 17% EDTA

In this study we have not evaluated erosion processes and their degree after treatment but SEM images clearly show the absence of erosion in Group IV (0.6 % chitosan-citrate) (Fig.3). In contrast to Group IV (0.6 % chitosan-citrate) the specimens of Group II (EDTA) show a significant degree of resorption - intertubular and peritubular (Fig.4,5).

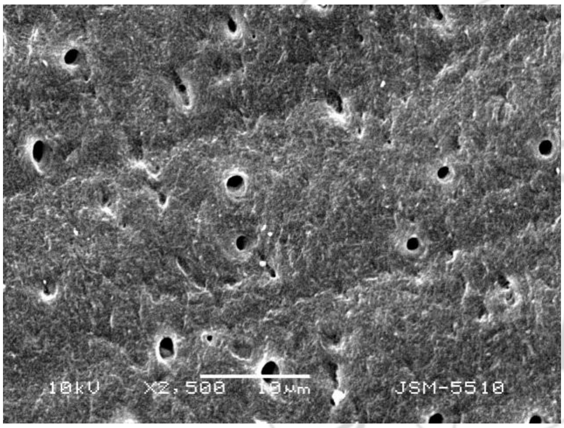

Figure 2 (A): SEM image of dentin surface from specimen of Group I(*apical third*) - treated with 5.25% NaOCl.

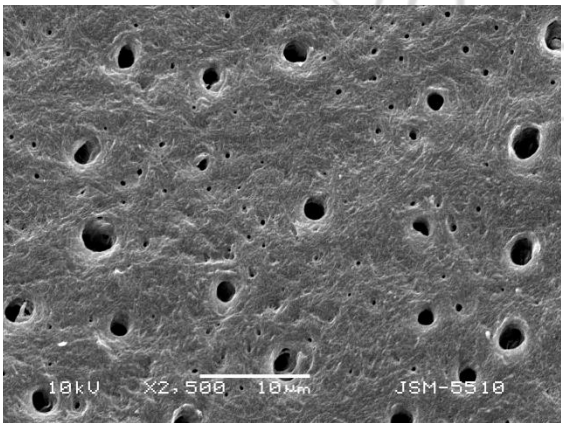

Figure 2 (B): SEM image of dentin surface from specimen of Group II (*apical third*) - treated with 17% EDTA.

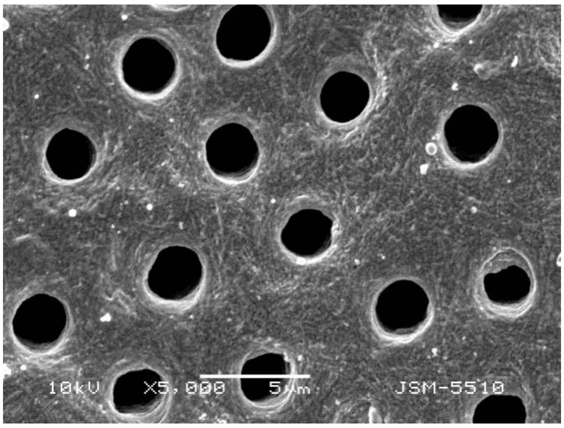

Figure 3: SEM image of dentin surface from specimen of Group III (*apical third*) - treated with 5.25% NaOCl and 17% EDTA

Conditioning dentin surface with 0.6 % chitosan-citrate appears smooth with wide, open dentinal tubules. It was not observed intertubular or peritubular erosion instead of this the dentin surfaces are covered by a film consisting probably of organic substance including collagen (Fig.6). The smooth dentine surfaces should be seen on larger magnification (Fig.7). The differences between treated surfaces are illustrated on Fig.5 and Fig.7.

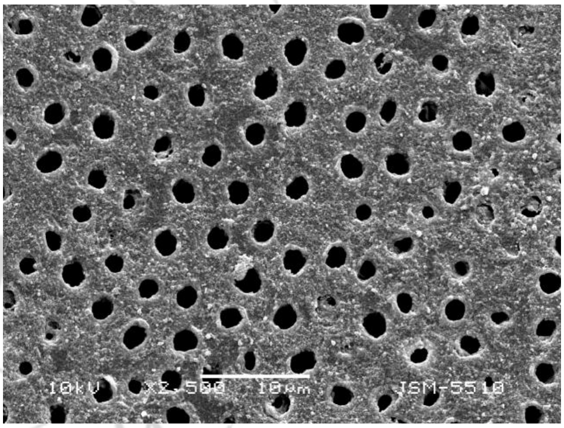

Figure 4: SEM image of dentin surface from specimen of Group II (*apical third*) - 17% EDTA (magnification x 2500).

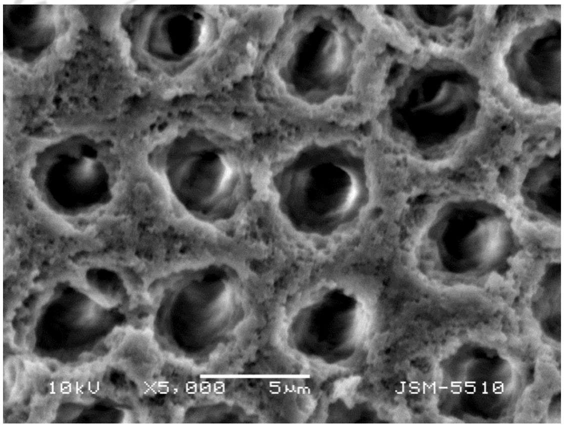

Figure 5: SEM image of dentin surface from specimen of Group II (*apical third*) - 17% EDTA (magnification x 5000).

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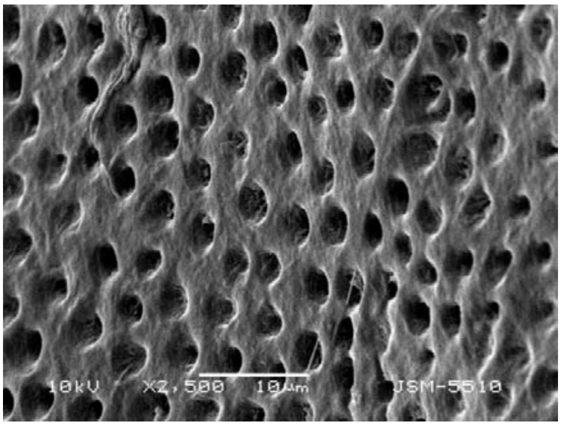

Figure 6: SEM image of dentin surface from specimen of Group IV (*apical third*) - treated with 0.6 % chitosan-citrate (magnification x 2500).

The SEM images showed that the three solutions removed smear layer and plugs from the coronal third of the root canal (Fig.8). There was no significant difference (p>0,05) in smear layer removal based on opened dentin tubules in Group III and Group IV.

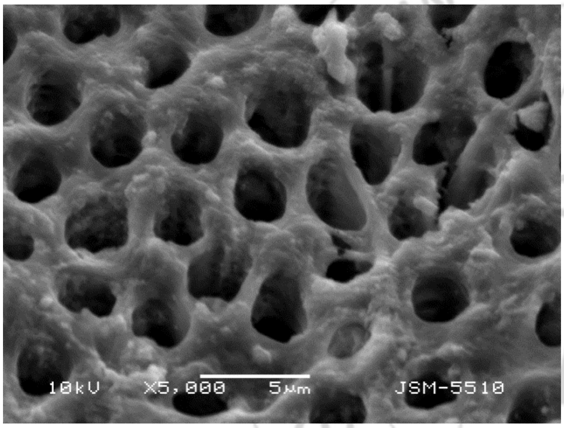

Figure 7: SEM image of dentin surface from specimen of Group IV (*apical third*) - treated with 0.6 % chitosan-citrate (magnification x 5000)

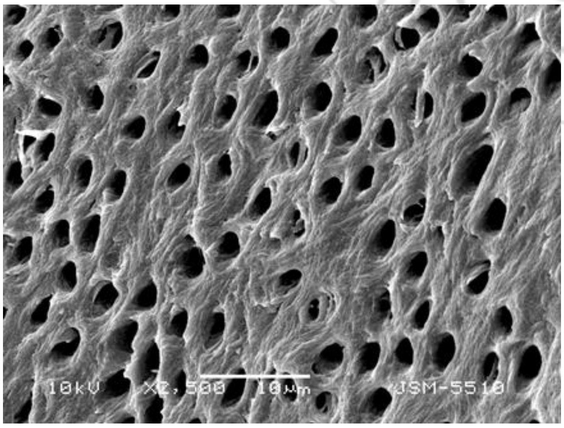

Figure 8: SEM image of dentin surface from specimen of Group III (*coronal third*) - treated with 5.25% NaOCl and 17% EDTA

4. Discussion

Scientific studies have reported different findings on the effectiveness of contemporary endodontic irrigants to remove the smear layer and debris. Most studies reported that these irrigating solutions were ineffective, especially in the apical third [5, 6, 8]. Due to the limitations of current endodontic irrigants, there is great interest in developing new, improved irrigating solutions.

In comparison to 10% citric acid, chitosan-citrate is known to have enhanced smear layer removal capabilities with less dentin erosion (when immersed for 5 mins). Based on a comparative study to assess the chelating and cleansing capabilities, chitosan-citrate solution scored comparatively higher than citric acid. Most of the root canal walls were cleaner with chitosan-citrate than 10% citric acid solution [26]. In a similar study, the properties of chitosan were closely attributed to better and enhanced cleansing and chelating capabilities [14]. A significant difference in dentin erosion was observed between chitosan-citrate and citric acid solution [14].In another study to assess the chelating effects of chitosan, the smear layer removal was enhanced with the combination of chitosan and acetic acid [22]. Furthermore, the smear layer was retained with acetic acid. In an observational study, the immersion time of 5 mins chitosan solution was assessed. The researchers stated that chitosan is a new and improved irrigant for endodontic procedures. In some cases, a shorter duration of immersion (≤ 2 mins) has also shown promising results. Chitosan solution can be compared with most conventional ineffective irrigants. Chitosan solution has also been effective in chelating the third and apical root which is considered difficult to reach for most conventional irrigants.

In this paper we supposed that the chitosan acts in appropriate root canal environment, prepared from citric acid. In this situation after the irrigation with 0.6% chitosan-citrate we could observe chitosan precipitates (grained layer) covered the dentin surfaces. So, in these clinical conditions chitosan can act through its antibacterial properties.

Researchers have hypothesized two theories on the mechanism involved in chelating with chitosan. The first theory is based on the bridge model, where the same metal ion is bound by two or more amino groups on the chitosan chain [25]. The second theory claims that only one of the amino groups on the chitosan chain is anchored to the metal ion [26].Many dimers of chitin combine to form a chitosan polymer. The polymer is similar to that found in the EDTA molecule. The chitin dimer comprises of nitrogen atoms that combine with free electrons which are responsible for the ionic reaction with the metal and the chelating molecule. Based on a chemistry analysis of chitosan, the amino groups in the chitosan chain (in acidic medium) are known to have a positron charge based on bi-polymer protonation. The phenomenon is associated with the attraction of metal ion to the chelating agent [14]. Further analysis revealed that chitosan and metal ion complexes are a result of ion exchange, chelation, and adsorption [14]. The chelating interaction is dependent on the type of interaction, pH of the solution, and the structure of chitosan [30].Based on a recent study, chitosan is known to have conditioning effects on radicular dentin. Researchers revealed that 0.2% chitosan at pH 3.2 is known to have dentin reducing properties [29]. (Varshosaz,&Alinagalri 2005). The chitosan is known to reduce dentin micro-hardness which is similarly observed in

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15% EDTA at pH 7.25. Thus, it can be concluded that chitosan citrate is also an ideal conditioner for radicular dentin [29].

Thus, based on an extensive literature review and a comprehensive overview of conventional dental irrigants, chitosan-citrate can be considered as a new, novel, safe, and effective irrigant and conditioner for radicular dentin in endodontics.

The activities of chitosan should be evaluated at different concentration and application time on smear layer removal and dentin structure.It was demonstrated that 0,2% chtisan for 3 min. removed the smear layer adequately and caused less erosion than EDTA which is demonstrated also in the literature [17] .

5. Conclusion

In conclusion, based on the results presented and the limitation of the study, the following conclusion can be made: 0.6% chitosan and combination ofNaOCl and EDTAshowed similar effects in reducing the smear layer and removing dentin plugs. Conditioning dentin surface with 0.6% chitosan-citrate appears smooth with wide, open dentinal tubules. It was not observed intertubular or peritubular erosion instead of this the dentin surfaces is covered by a film consisting probably of organic substance including collagen.

Based on our results we hypothesize that effectiveness of a chitosan-citrate solution to remove the smear layer in root canal treatment is satisfied and comparable with classical solutions (Group III). The chitosan-citrate solution might be the promisingendodontic irrigation solution in future.

6. Acknowledgements

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