

Comparative Evaluation of Effect of Ultrasonic Agitation on EDTA and Chitosan on Smear Layer Removal from Root Dentin: An in Vitro SEM Study

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Abstract: *This study compared efficacy of ultrasonic agitation with EDTA and Chitosan on smear layer removal from root canal walls. 60 extracted teeth randomly distributed into 5 groups, prepared by using Profile rotary instruments and subjected to different final regimens; group 1 - NaOCl, group 2 – EDTA, group 3 – EDTA + Ultrasonic agitation, group 4 – Chitosan and group 5 – Chitosan + Ultrasonic agitation. Samples were examined under the scanning electron microscope. Statistical analysis showed that group 1 was not able to remove the smear layer. Group 3 and 5 performed significantly better than group 2 and 4. This study advocates that integration of ultrasonics with EDTA and Chitosan might prove beneficial in increasing the ability of EDTA and Chitosan to remove the smear layer.*

Keywords: EDTA, Chitosan, smear layer, ultrasonic agitation, SEM.

1. Introduction

The success of endodontic treatment depends on the root canal system being thoroughly cleansed and disinfected, followed by the three-dimensional obturation of this space. However, after preparation of the root canals, an amorphous, irregular layer is formed on the root canal walls known as smear layer containing remnants of ground dentine; pulp tissue and bacterial toxins. Many studies have demonstrated that canal preparation techniques produces a considerable amount of smear layer, remaining pulp tissue, and inorganic dentin debris [1]. The smear layer has been considered as an important factor in root canal therapy since the report by McComb and Smith [2]. Since then various chemicals, irrigants, ultrasonics, and lasers, in combination or alone, has been studied for the removal of the smear layer from root canal walls with varying degrees of result [1, 3].

Sodium hypochlorite (NaOCl) has been used as a popular root canal irrigant because it has bactericidal potential and has a ability to dissolve organic materials; but it is unable to remove the smear layer [4, 5]. For removal of smear layer many decalcifying solutions such as phosphoric acid, citric acid, maleic acid, ethylenediaminetetraacetic acid (EDTA), and MTAD have been used [6,7].

A new chelating agent and natural polysaccharide which is known as Chitosan, has been introduced. It has biocompatible, biodegradable, and bioadhesive properties [8,9]. It is a non-toxic cationic biopolymer usually obtained

by alkaline deacetylation from chitin, which is the principal component of crustacean exoskeletons [10]. It has become cost-effectively appealing for various applications due to its stacks in nature and low assembly costs [11].

Ultrasonic devices were first introduced in Endodontics by Richman in 1957 [12]. Preparing and debridement of root canals mechanically is the property of ultrasonically activated files. Hence ultrasonically driven files are valuable for the 'irrigation' of root canals. Two types of ultrasonic irrigation have been described in the literature; (1) irrigation with simultaneous ultrasonic instrumentation (UI); (2) irrigation without simultaneous instrumentation, called as passive ultrasonic irrigation (PUI) [13, 14]. In 1980, Weller et al. was the first to describe Passive ultrasonic irrigation [15]. The term 'passive' is related to the 'noncutting' action of the ultrasonically activated file. PUI relies on the transmission of acoustic energy from an oscillating file or smooth wire to an irrigant in the root canal [16, 17].

This study evaluates the efficacy of smear layer removal from the root canals using ultrasonic agitation with EDTA and Chitosan during endodontic therapy.

2. Materials and methods

A total of 60 adult human non-carious mandibular premolars were taken for the study. Inclusion criteria was single-rooted teeth with straight, patent roots, and fully formed apices. Standard radiographs were taken in a buccolingual and

mesiodistal direction of each tooth after being held in a custom made jig to determine whether or not the sampled tooth conforms to the selection criteria adopted for the study.

Sample preparations:

All the teeth were stored in 10% formalin solution till they were used for the study. The root surfaces were cleaned and standardized root length of 12 mm were obtained by decoronating the samples using a diamond disc under water irrigation. Subsequently, #10 K-file (Mani Inc., Japan) was inserted beyond the apex to confirm patency; 1 mm was subtracted from this length to establish the length to which the canals would be instrumented. The canals were enlarged, and a glide path established with hand instruments to a size #15 K-file (Mani Inc., Japan). Apices of the roots were sealed with sticky wax to simulate the clinical conditions and root canal instrumentation was initiated with hand files up to #20 (Mani Inc., Japan) followed by ProTaper rotary files up to size F3 (Dentsply Maillefer, Switzerland). 1ml of 3% NaOCl (Prime Dental Products PVT LTD) was used as an irrigant after every instrumentation. The irrigants were delivered with a disposable syringe and a 30-gauge Max-I-Probe needle placed 1 mm short of the working length. Finally, 3 ml of 3% NaOCl was used to wash out the debris from the root canals followed by a rinse with 5ml of distilled water to cease any action of the solvents remaining in the canal. A constant total volume of 15 ml of NaOCl was used as an irrigant for each root canal during the study.

Grouping of samples

After biomechanical preparation, the samples were divided into five different groups of twelve specimens in each group.

- Group A (NaOCl) – Root canals were irrigated with a final flush of 1 ml of 3% NaOCl for 1 min followed by 3 ml of 3% NaOCl.
- Group B (EDTA) – Root canals were irrigated with a final flush of 1 ml of 17% EDTA (Prevest DenPro) for 1 min, followed by 3 ml of 3% NaOCl
- Group C (ultrasonic + EDTA) - The root canals were irrigated with a final flush of 1 ml of 17% EDTA with passive ultrasonic activation for 1 min with a #30 E ultrasonic i-SuperTip (Integrated Endodontics Pvt Ltd.) placed 1 mm short of the working length, followed by 3 ml of 3% NaOCl.
- Group D (Chitosan) – The root canals were irrigated with a final flush of 1 ml of 0.2% Chitosan for 1 min, followed by 3 ml of 3% NaOCl.
- Group E (ultrasonic + Chitosan) - The root canals were irrigated with a final flush of 1 ml of 0.2% Chitosan with passive ultrasonic activation for 1 min with a #30 E ultrasonic i-SuperTip placed 1 mm short of the working length, followed by 3 ml of 3% NaOCl.

The root canals were finally flushed with 5 ml of distilled water to terminate the action of the irrigating solutions dried and prepared for scanning electron microscope (SEM) examination.

Scanning microscope examination:

The teeth were grooved along the buccal and lingual planes by using a diamond disc at low speed. The roots were then split longitudinally with a bi-beveled chisel and a mallet. One-half of each root was selected depicting the entire root

canal length and prepared for SEM examination. The selected samples were progressively dehydrated using graded concentrations of aqueous ethanol (70%, 80%, 90%, and 100%) for 24 h at each concentration. After dehydration, samples were placed in a vacuum chamber and sputter coated with a 30 nm gold layer. The dentinal wall of the root canals was examined at coronal, middle, and apical thirds at a magnification of $\times 1000$ for the presence or absence of smear layer and patency of dentinal tubules. Photomicrographs of the root canals were taken at coronal, middle, and apical level for scoring individually in a calibrated single-blind manner according to the rating system developed by Gutmann et al. [18] and the results were tabulated.

- Score 1: Little or no smear layer; covering <25% of the specimen; most tubules were visible and patent, or almost complete laser melting
- Score 2: Little to moderate or patchy mounts of smear layer; covering 25–50% of the specimen; many tubules visible and patent, or laser melting
- Score 3: Moderate amounts of scattered of aggregated smear layer; covering 50–75% of the specimen; minimal to no tubule visibility or patency, or scattered laser melting
- Score 4: Heavy smear layer covering >75% of the specimen; no tubule orifices were visible or patent; or no visible laser melting

Data were analyzed using one way ANOVA and Multiple Comparison Tukey Test using SPSS 20.0 version and EPI-INFO 6.0 version and $p < 0.05$ was considered as level of significance.

3. Results

The statistical parameters: mean, standard deviation along with p-value and F-value of smear layer removal scores were obtained for each group as shown in Table 1. The mean for Group C at coronal third site was lowest i.e. 1.00 ± 0.00 while that of Group A at middle and apical third site were highest i.e. 4 ± 0 and 3.83 ± 0.38 respectively.

Table 1: Comparison of smear layer scores at coronal, middle and apical third of root canal in five groups
One way ANOVA

| Group | N | Coronal Site | Middle Site | Apical Site |
|---------|----|-----------------|-----------------|-----------------|
| Group A | 12 | 3.66 ± 0.49 | 4 ± 0 | 3.83 ± 0.38 |
| Group B | 12 | 1.83 ± 0.71 | 1.91 ± 0.79 | 1.91 ± 0.66 |
| Group C | 12 | 1.00 ± 0.00 | 1.08 ± 0.28 | 1.25 ± 0.45 |
| Group D | 12 | 2.16 ± 0.57 | 2.25 ± 0.62 | 3.75 ± 0.45 |
| Group E | 12 | 1.91 ± 0.66 | 2.08 ± 0.66 | 2.83 ± 0.38 |
| F-value | | 36.79 | 44.27 | 66.25 |
| p-value | | 0.0001,S | 0.0001,S | 0.0001,S |

One way ANOVA revealed significant variation in mean smear layer score among all five groups at coronal third ($F=36.79, p=0.0001$), middle third ($F=44.27, p=0.0001$) and apical third ($F=66.25, p=0.0001$) respectively.

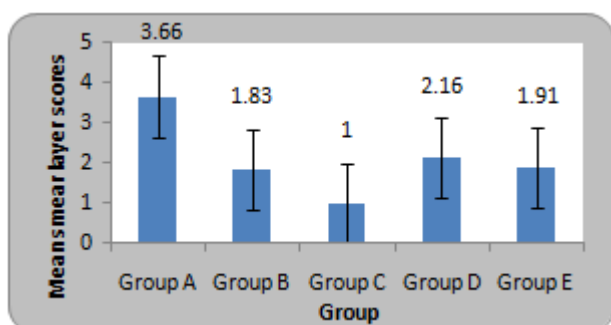
In Table 2, Pairwise Comparison: Tukey Test revealed significant difference in all the five groups except in group B versus group D ($p=0.585$), group B versus group E ($p=0.996$) and group D versus group E ($p=0.804$) which shows

statistically no significant difference at coronal third level (Graph 1). At middle third level significant difference was found in all five groups except in group B versus group D ($p=0.587$), group B versus group E ($p=0.948$) and group D versus group E ($p=0.948$) which shows statistically no

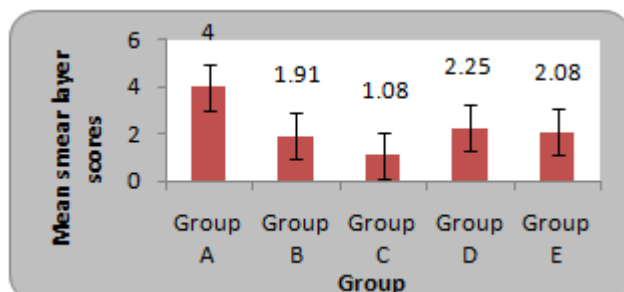
significant difference (Graph 2). At apical third level significant difference was found in all five groups except in group A versus group D ($p=0.993$), which shows statistically no significant difference (Graph 3).

Table 2: Pairwise Comparison: Tukey Test

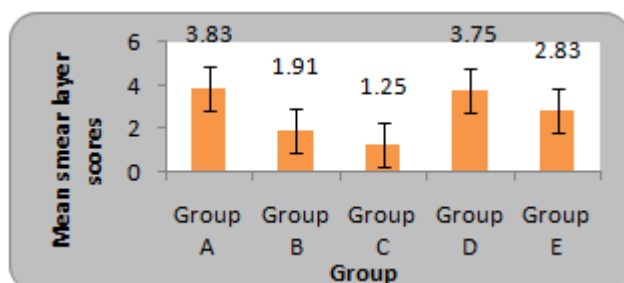
| | | Coronal Site | | Middle Site | | Apical Site | |
|---------|---------|-----------------|----------|-----------------|----------|-----------------|----------|
| Group | | Mean Difference | p-value | Mean Difference | p-value | Mean Difference | p-value |
| Group A | Group B | 1.83 | 0.0001,S | 2.08 | 0.0001,S | 1.91 | 0.0001,S |
| | Group C | 2.66 | 0.0001,S | 2.91 | 0.0001,S | 2.58 | 0.0001,S |
| | Group D | 1.5 | 0.0001,S | 1.75 | 0.0001,S | 0.08 | 0.993,NS |
| | Group E | 1.75 | 0.0001,S | 1.91 | 0.0001,S | 1 | 0.0001,S |
| Group B | Group C | 0.83 | 0.005,S | 0.83 | 0.005,S | 0.66 | 0.011,S |
| | Group D | 0.33 | 0.585,NS | -0.33 | 0.587,NS | -1.83 | 0.0001,S |
| | Group E | 0.08 | 0.996,NS | -0.16 | 0.948,NS | -0.91 | 0.0001,S |
| Group C | Group D | 1.16 | 0.0001,S | -1.16 | 0.0001,S | -2.5 | 0.0001,S |
| | Group E | 0.91 | 0.001,S | -1 | 0.0001,S | -1.58 | 0.0001,S |
| Group D | Group E | 0.25 | 0.804,NS | 0.16 | 0.948,NS | 0.91 | 0.0001,S |



Graph 1: Comparison of smear layer scores at coronal third of root canal in five groups



Graph 2: Comparison of smear layer scores at middle third of root canal in five groups



Graph 3: Comparison of smear layer scores at apical third of root canal in five groups

4. Discussion

Smear layer consist of necrotic tissue, dentin chips, counting leftovers of odontoblastic procedures, micro-organisms and pulp tissue. Within the dentinal tubules, smear layer acts as

barrier and it hinders the penetration of irrigants and root canal sealer [19]. Thus, preference of irrigants should also be based on its capability to remove smear layer.

In the present study, group A showed the presence of heavy smear layer throughout the length of the canals which is similar to previous many studies by Amin et al. [3], Baumgartner and Mader [20], Torabinejad et al. [21] and Gade VJ et al. [22] that showed NaOCl to be ineffective in removing smear layer (Figure 1). In group B, EDTA showed effective smear layer removal from coronal third as well as from middle third and apical third which was statistically significant and in harmony with the results of various studies [19,20]. In the coronal third area, a larger canal diameter exposes the dentin to a higher amount of irrigants, allowing an enhanced flow of the solution and therefore, improving the value of smear layer removal [23] (Figure 2).

In group C, the root canal surfaces were clean and free of smear layer in the coronal and middle third, whereas the apical third showed speckled areas with smear layer. No significant difference in smear layer scores was recorded at the coronal and middle third levels (Figure 3). Lui et al. (2007) [24] found that addition of ultrasonics with EDTA improved smear layer removal from root canals. Similar results were found by Kuah et. al [25] and Khalid et al. [3]. Many studies has shown increased smear layer removal principally from the coronal part of the root canal wall rather than the apical part [26, 27, 28].

In the present study, Chitosan solution was prepared using 1% acetic acid. In group D, Chitosan has demonstrated its chelating behaviour by screening that it acts on the inorganic portion of the smear layer, favouring its removal. Similar result has been found by Silva et al. (2013) [29]. In relation to the cleaning of the coronal and middle thirds, there were no significant differences amongst group B versus group D but EDTA shows better result as compared to Chitosan. At apical third, it has shown significant differences with all other groups (Figure 4).

In present study for the first time, in group E, 0.2% Chitosan was used along with passive ultrasonic activation which has shown better result compared to Chitosan alone (group D).

At coronal and middle third, group E has shown no significant difference with respect to group D (Chitosan alone), but it has shown significant difference with group D at apical third level (Figure 5). This means that 0.2% Chitosan in combination with ultrasonic performed better than Chitosan alone in removal of smear layer.

The addition of ultrasonics to EDTA and Chitosan increases the smear layer removing efficacy of EDTA and Chitosan by enhancing its penetration into the middle and narrow apical regions of the root canal walls [3].

Thus present study advocate that integration of ultrasonics with EDTA and Chitosan might prove beneficial in increasing the ability of EDTA and Chitosan to remove smear layer by enhancing its interaction with the root canal wall.



Figure 1: SEM images of group 1 (NaOCl) at coronal, middle and apical third of root canal

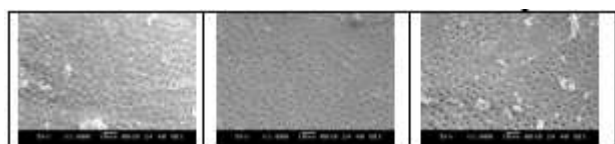


Figure 2: SEM images of group 2 (EDTA) at coronal, middle and apical third of root canal

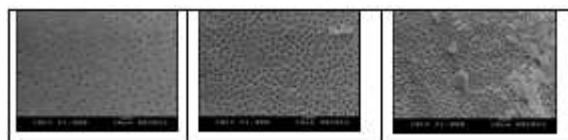


Figure 3: SEM images of group 3 (EDTA + Ultrasonic) at coronal, middle and apical third of root canal

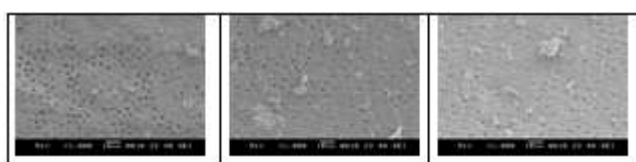


Figure 4: SEM images of group 4 (Chitosan) at coronal, middle and apical third of root canal



Figure 5: SEM images of group 5 (Chitosan + Ultrasonic) at coronal, middle and apical third of root canal

5. Conclusion

Within the limitations of the current study, all the tested groups were able to remove the smear layer from root canals to different degrees except NaOCl. When used in combination with ultrasonic, EDTA and Chitosan performed radically better than EDTA and Chitosan alone.

Ultrasonic could be a good accumulation to the armamentarium used for smear layer removal and could increase the success rate of endodontic therapy.

More research will be required to evaluate the effect of various agitation techniques on chitosan irrigant for the smear layer removal from root canal dentin.

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