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Efficacy of Chitosan as Root Canal Irrigant in Removing Smear Layer in Curved Root Canals Using Endovac Irrigation System (SEM Study)

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ABSTRACT

Purpose: This study aimed to evaluate the efficacy of Chitosan as root canal irrigant with Endovac irrigation system in removing smear layer of curved root canal walls using Scanning Electron Microscope (SEM). **Materials and Methods:** Fifty extracted human first mandibular molars were selected with curved mesiobuccal root (20º-35º). Samples were instrumented with Universal ProTaper rotary files till size F4 then divided into 2 main experimental groups according to the final rinse used; Group I: irrigated with 17 % EDTA Group II: irrigated with 0.2% chitosan acetate and control group, irrigated with sterile saline (10 samples). Each group was further subdivided into 2 subgroups according to the irrigating technique used; Subgroup A: using conventional needle irrigation and Subgroup B: using Endovac irrigation system. Samples were grooved longitudinally and smear layer was evaluated using SEM at the coronal, middle and apical root canal levels. The significance level was set at $P \leq 0.05$. **Results:** There was a statistically significant difference among the tested groups and the control group in the mean scores of smear layer at all root canal levels and no significant difference between irrigants used. Concerning the irrigating devices, there was no statistical significant difference between conventional irrigation and Endovac system at the coronal and apical root canal levels while at the middle level, 0.2% chitosan showed statistical significant difference with Endovac system subgroup than conventional irrigation ($P \leq 0.05$). **Conclusion** Endovac irrigation system was effective in smear layer removal from curved root canals using either 0.2% Chitosan acetate or 17 % EDTA as final rinse.

KEYWORDS

Chitosan,
Irrigating Solution,
Smear Layer,
Endovac irrigating System.

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INTRODUCTION

Successful root canal therapy is attributed to effective chemicomechanical preparation of the root canal system and proper apical and coronal seal. Various instrumentation techniques leave an amorphous, irregular layer known as smear layer, which may prevents the penetration of intracanal medicaments into dentinal tubules and influence the adaptation of filling materials to canal walls [1] . It has been shown that the smear layer may contain and harbor bacteria, preventing the canal from being disinfected, limit the penetration of intracanal disinfectants and sealers into dentinal tubules and interferes with a tight adaptation of root canal sealers to dentin walls. So, the removal of smear layer is crucial for long-term success of root canal treatment [2].

Complete cleaning of the root canal system requires the use of irrigants that dissolve organic and inorganic material and prevent the formation of smear layer during instrumentation and calcification hindering mechanical preparation which are frequently encountered in the canal system. As NaOCl is active only against organic part, other substances must be used to remove the inorganic material. Demineralizing agents such as ethylenediamine tetraacetic acid (EDTA) and citric acid have therefore been recommended as adjuvant in root canal therapy [3].

Irrigation solutions at our disposable have their share of limitations, therefore herbal or natural products have become more popular today due to their high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties [4]. Chitosan is a natural polysaccharide, which has attracted attention in dental research because of its biocompatibility, biodegradability, bioadhesion and lack of toxicity. It has a high chelating ability for various metal ions in acidic conditions and has been applied widely for the removal or recovery of metal ions in different industrial areas [5]. Chitosan is obtained by the deacetylation of chitin which is found in crab and shrimp shells and has become ecologically interesting for various applications because of its abundance in nature and low production costs [6].

A preliminary study evaluated the chelating properties of chitosan as a (natural polymer) in comparison with other irrigating solutions on the middle third of instrumented root canal using scanning electron microscopic showed that 2% chitosan acetate was effective in removing the smear layer as 17% EDTA and the use of 2% chitosan acetate followed by NaOCL produced clean surface. These results suggested that chitosan is a promising chelating agent [7]. The effect of chitosan at different concentrations on the removal of the smear layer and on dentin structure was evaluated. The results showed that 0.2% chitosan for 3 min appeared to be efficient for removing the smear layer causing little erosion of dentin [8].A recent study compared the smear layer removal from root canal dentine subjected to two root canal irrigants; 17% EDTA and 0.2% Chitosan using Scanning Electron Microscope. The results indicates that 0.2% chitosan removed the smear layer with greater efficiency than 17% EDTA at the apical third of the root canal [9].

Although conventional needle irrigation is one of the most commonly used techniques, its efficacy in the removal of the smear layer from the curved root canals especially in the apical third is still questionable. It has been shown that the irrigant doesn't go more than 1 mm beyond the needle tip, so the apical few millimeters are never irrigated. To make the irrigant reach the apical 1-2 mm, needle should go close to the working length, which in turn increases the risk of apical extrusion of irrigant [10]. For successful root canal treatment, a system that delivers the irrigant effectively and passively to the working length (WL) is required. Newer irrigation systems have been introduced to increase the mechanical flushing action of irrigants for better

removal of smear layer, which was not possible with conventional syringe irrigation [11].

The Endovac system is an apical negative pressure irrigation system that has been showcased recently. The aim of this system is to provide safe and effective cleaning within the apical zone of the root canal apex especially when canal curvatures are present and to deliver the irrigant safely in the apical 1-2 mm without any risk of perfusion of irrigant beyond the apex [12, 13].

Several studies evaluated the ability of Endovac system in enabling irrigants to reach the apical third and help overcome the issue of apical vapor lock. The efficacy of Endovac and needle irrigation to debride the apical 3mm of a root canal was compared at 1mm and 3 mm from working length. This study showed significantly better debridement at 1 mm from the working length by using the Endovac compared to needle irrigation [14]. An *in vitro* study compared different endodontic irrigation and activation systems for removal of the intracanal smear layer; syringe and needle irrigation, sonic irrigation, passive ultrasonic irrigation and Endovac irrigation system. Scanning electron microscopic evaluation showed that Endovac system cleaned the apical part of the canal more efficiently than sonic, ultrasonic and syringe and needle irrigation [15].Also, the efficacy of apical negative pressure (ANP), manual dynamic agitation (MDA), passive ultrasonic irrigation (PUI) and needle irrigation (NI) as final irrigation activation techniques was assesed for smear layer removal in curved root canals ranging (25º- 35º). It was concluded that ANP (Endovac system) can be used as the final irrigation activation technique for effective smear layer removal in curved root canals [16]. Therefore, the aim of the present study was directed to evaluate the efficacy of 0.2 % Chitosan acetate as final root canal irrigant with Endovac irrigation system in removing smear layer of curved root canal walls using Scanning Electron Microscope (SEM).

MATERIALS AND METHODS

Teeth selection and preparation:

The mesiobuccal canal of fifty freshly extracted human first mandibular molars were selected, where the mesial roots having 2 separate mesial canals and apical foramina and with mature apices with no evidence of cracks or resorption. The teeth were placed in sodium hypochlorite 2.6% for 1 hour and stored in saline solution until use. The root of each tooth was standardized to be 16 mm. The working length was measured by subtracting 1mm from lengths when the tip of #10 K-files became observed at the apical foramina. CBCT scan was acquired using i-CAT imaging system to determine the degree of root curvature according to Schneider's method [17] to be within an average of (20º-35º). The specimens were mounted in a cast to simulate the clinical situation.

Universal ProTaper Ni-Ti rotary files were used in a crown-down manner for root canal preparation with a 16:1 reduction handpiece that was powered by a torque-controlled electric motor; at a rotational speed of 300 rpm and a torque-control of 2.6N/cm. A set of seven files were used, three shaping files (Sx, S1 and S2) for coronal 2/3 preparation and four finishing files (F1, F2, F3 and F4) for apical finishing used according to the manufacturer instructions to provide adequate space for the microcanula of Endovac irrigating system (Discus Dental, Smart Endodontics, Culver City (CA) to reach the full working length to help ensure a cleaner canal [18]. After each instrument use, irrigation with 2 ml of freshly prepared 2.6% sodium hypochlorite (NaOCl) solution was dispensed through a 31 gauge side vent irrigating needle.

Preparation of chitosan solution:

Preparation of 0.2% chitosan acetate solutions was performed using 0.2g chitosan (Sigma Co., Egypt), diluted in 100ml of 1% acetic acid, and the mixture was stirred for 2 h using a magnetic stirrer[8].

The solution was saved in the refrigerator and used within two weeks after preparation [19].

Samples grouping:

After root canal preparation, the samples were divided into 2 main experimental groups (I and II) according to the final rinse used (20 samples each) and a control group (10 samples). Group I: irrigated with 17 % EDTA. Group II: irrigated with 0.2 % chitosan acetate. Control Group: irrigated with sterile Saline, each group was irrigated using 5ml of the irrigant for 3min. Each group was further subdivided into 2 subgroups (A and B) according to the irrigating device used (10 samples each).

In subgroup A: Samples were irrigated using conventional needle irrigation size 31 gauge side vent irrigating needle, where the needle was inserted 2mm shorter than the working length without binding, then the canals were irrigated with 5 ml distilled water and dried with paper points.

In subgroup B: Samples were irrigated using Endovac irrigating system, one cycle of macroirrigation using 5ml of 2.6% NaOCl was made for 30 sec of active irrigation followed by 60 sec of passive wait. Then three cycles of microirrigation followed, during which the pulp chamber was kept full of irrigant while the micro cannula was moved up and down (2 mm from the working length). In the first cycle 5 ml of NaOCl was used for 30 seconds of active irrigation, then the micro cannula was withdrawn from the canal in the presence of sufficient amount of irrigant in the pulp chamber to insure that the canal remained totally filled with the irrigant and that no air was drawn into the canal space, . The canal filled with irrigant was left undisturbed for 60 seconds, this completed one microirrigation cycle. In the second cycle 5 ml of (17% EDTA, 0.2% chitosan acetate or sterile saline for group I, II and the control group respectively) was used for 30 seconds of active irrigation and 150 seconds left undisturbed in the canal. The third cycle was the same as the first one. At the end of the third microirrigation cycle, the micro cannula was left at the working length without replenishment to move excess fluid. Then the canal was irrigated with 5 ml of sterile distilled water and dried with paper points

Scanning electron microscope preparation and scoring system:

All samples were grooved longitudinally on the external surface (buccal and lingual) with diamond disc, avoiding penetration of the root canals. The samples were then carefully split with a hammer and chisel into two halves. Photomicrographs were taken under magnification (X 4000) for smear layer scores at the coronal, middle and apical thirds of the root canals and analyzed by means of numerical evaluation scores by **Hülsmann et al (1997)** [20] as following:

Score 1: No smear layer and patent dentinal tubules. **Score 2**: Small amount of smear layer, some open dentinal tubules. **Score 3**: Homogenous smear layer covering the root canal wall, only few open dentinal tubules. **Score 4**: The entire root canal wall covered with a homogenous smear layer, no open dentinal tubules. **Score 5**: Heavy, non-homogenous smear layer covering the entire root canal wall.

Statistical Analysis

Data were presented as means and standard deviation (SD) value. Data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). For non-parametric data; Kruskal-Wallis test was used to compare between irrigants. Mann-Whitney U test was used to compare between devices. Friedman's test was used to compare between root levels. Dunn's test was used for pairwise comparisons. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics Version 20 for Windows.

RESULTS

I. Comparison of smear layer scores among the root levels within each group: (figure 1)

In group I (17 % EDTA); the mean scores of smear layer and standard deviation of subgroup (A) (conventional irrigation) at coronal, middle and apical levels were 1.60 ± 0.52, 1.80 ± 0.63 and 2.70 ± 0.48 respectively, and that of subgroup (B) (Endovac irrigation system) at coronal, middle and apical levels were 1.50 ± 0.53, 1.70 ± 0.48 and 2.40 ± 0.84 respectively.

In group II (0.2% chitosan acetate); the mean scores of smear layer and standard deviation of subgroup (A) (conventional irrigation) at coronal, middle and apical levels were 1.90 ± 0.32, 2.20 ± 0.63 and 2.70 ± 0.67 respectively, and that of subgroup (B) (Endovac irrigation system) at coronal, middle and apical levels were 1.60 ± 0.70, 1.50 ± 0.53 and 2.40 ± 0.70 respectively.

In the control group (Saline); the mean scores of smear layer and standard deviation of subgroup (A) (conventional irrigation) at coronal, middle and apical levels were 4.20 ± 0.45, 4.00 ± 0.00 and 4.60 ± 0.55 respectively, and that of control group (B) at coronal, middle, apical levels were 4.60 ± 0.55, 4.40 ± 0.55 and 3.60 ± 0.55 respectively.

Among all the experimental groups there was a statistically significant difference among the three root canal levels in the mean scores of smear layer where the apical level showed the statistically significantly highest mean smear layer score compared to the middle and coronal levels. There was no statistical significant difference between the middle and coronal levels. The control group showed no statistical significant difference among the three root canal levels in the mean scores of smear layer ($P > 0.05$).

II. Comparison of smear layer scores between irrigants at each root level:

In subgroup (A): (Conventional irrigation); the mean scores of smear layer and standard deviation of group I (17 % EDTA), group II (0.2% Chitosan acetate) and control group (saline) at the coronal root level were 1.60 ± 0.52, 1.90 ± 0.32 and 4.20 ± 0.45, at the middle root level were 1.80 ± 0.63, 2.20 ± 0.63 and 4.00 ± 0.00, and at the apical root level 2.70 ± 0.48, 2.70 ± 0.67 and 4.60 ± 0.55 respectively.

In subgroup (B): (Endovac irrigation system); the mean scores of smear layer and standard deviation of group I (17% EDTA), group II (0.2% Chitosan acetate) and control group (saline) at the coronal root level were 1.50 ± 0.53, 1.60 ± 0.70 and 4.60 ± 0.55, at the middle root level were 1.70 ± 0.84, 1.50 ± 0.53, and 4.40 ± 0.55 and at the apical root level were 2.40 ± 0.84, 2.40 ± 0.70 and 3.60 ± 0.55 respectively.

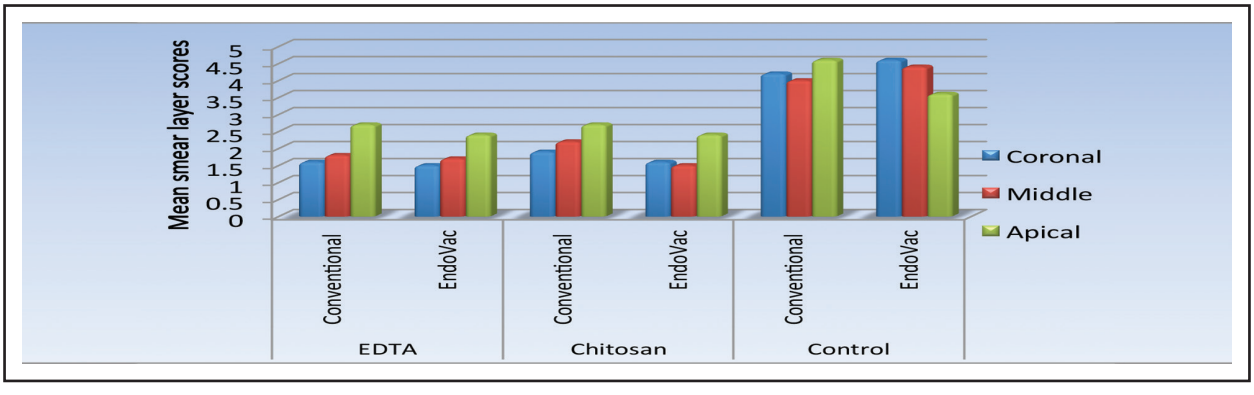

Fig. (1): A bar chart comparing the mean smear layer scores at different root levels within each group.

In both subgroups (A and B), there was a statistically significant difference between the tested irrigants (17 % EDTA and 0.2 % chitosan acetate) and the control group in the mean scores of smear layer ($P \leq 0.05$) where, the control group showed the statistically significant highest mean smear layer score. However, there was no statistical significant difference in the mean smear layer scores produced by 17% EDTA and 0.2% Chitosan acetate in both subgroups at all root levels (coronal, middle, apical).

III. Comparison of smear layer scores between devices within each group at each root level: (Table 1, Figure 2)

In group I (17 % EDTA); the mean scores of smear layer and standard deviation of subgroup (A) (conventional irrigation) and subgroup (B) (Endovac irrigation system) were (1.60 ± 0.52 and 1.50 ± 0.53), (1.80 ± 0.63 and 1.70 ± 0.48) and (2.70 ± 0.48 and 2.40 ± 0.84) at the coronal, middle and apical levels respectively. There was no statistical significant difference between the two irrigating methods (conventional irrigation and Endovac irrigation system) at the coronal, middle and apical levels, ($P > 0.05$).

In group II (0.2 % Chitosan acetate); the mean scores of smear layer and standard deviation of subgroup (A) (conventional irrigation) and subgroup (B) (Endovac irrigation system) were (1.90 ± 0.32 and1.60 ± 0.70), (2.20 ± 0.63 and 1.50 ± 0.53) and (2.70 ± 0.67 and 2.40 ± 0.70) at the coronal, middle and apical levels respectively. There was no statistical significant difference between the two irrigating methods (conventional irrigation and Endovac irrigation system) at the coronal and apical levels as final rinse ($P > 0.05$). However, Endovac irrigation system showed statistically significant lower mean smear layer score compared to conventional irrigation ($P \leq 0.05$).

Table (1): *Mean values and standard deviation (SD) of smear layer scores comparing the different irrigating systems at the coronal, middle and apical root level with the tested irrigants and control group*

Irrigant	Root level	Conventional irrigation		EndoVac irrigation		*P*-value
		Mean	SD	Mean	SD	
Group I (17 % EDTA)	Coronal	1.60	0.52	1.50	0.53	0.739
	Middle	1.80	0.63	1.70	0.48	0.796
	Apical	2.70	0.48	2.40	0.84	0.579
	Total	2.03	0.43	1.87	0.42	0.393
Group II (0.2% Chitosan acetate)	Coronal	1.90	0.32	1.60	0.70	0.247
	Middle	2.20	0.63	1.50	0.53	0.035*
	Apical	2.70	0.67	2.40	0.70	0.315
	Total	2.27	0.44	1.83	0.50	0.043*
Control group (saline)	Coronal	4.20	0.45	4.60	0.55	0.310
	Middle	4.00	0.00	4.40	0.55	0.310
	Apical	4.60	0.55	3.60	0.55	0.056
	Total	4.27	0.28	4.20	0.38	0.690

**: Significant at $P \leq 0.05$*

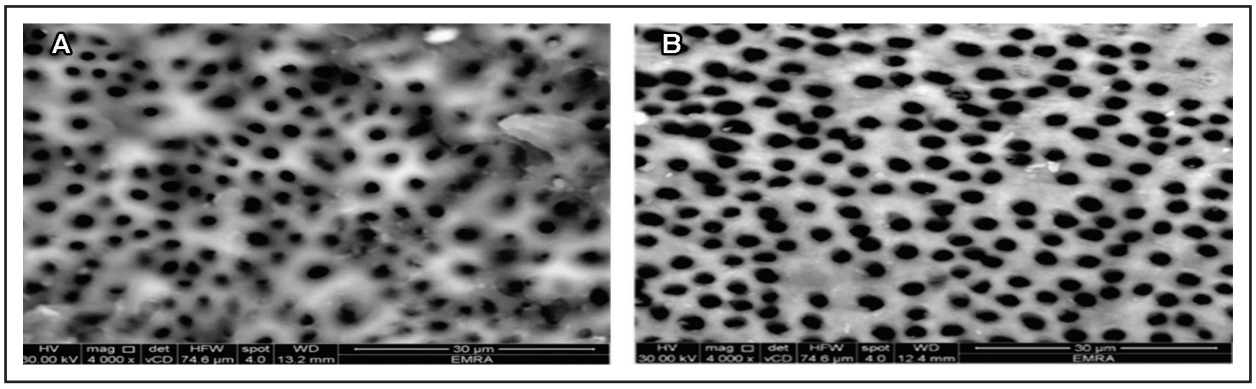

Fig. (2): A scanning photomicrograph of the middle level of a root canal rinsed with 0.2 % Chitosan acetate (a) via conventional irrigation showing most of dentinal tubules opened (score 2, X4000) and (b) Endovac irrigation system showing clean root canal wall (score 1, X4000).

DISCUSSION

The main goal of chemicomechanical preparation is to eliminate bacteria and their byproducts, remove pulp tissue remnants and contaminated organic and inorganic debris from the root canal system. The irrigation solutions at our disposable have their share of limitations and the search for an ideal root canal irrigant continues with the development of newer materials and methods that can help the irrigant to reach the apical third of the canal specially in curved roots. Therefore, the present study was directed to evaluate the efficacy of Chitosan as root canal irrigant using Endovac irrigation system in removing smear layer of curved root canal walls using Scanning Electron Microscope (SEM).

In the present study, 0.2% chitosan acetate showed similar results in removing the smear layer compared to 17% EDTA at all root canal levels (coronal, middle and apical). The coronal and middle levels within **group I** (17% EDTA) as well as **group II** (0.2% chitosan acetate) showed a lower mean scores (more smear layer removal) compared to the apical level This could be attributed to the fact that the access of instruments and chemical solutions to the coronal and middle thirds is easier making efficient removal of the smear layer possible [21].

The efficiency of chitosan for smear layer removal could be attributed to the hydroplilic nature of chitosan which favors its intimate contact with root canal dentin and its adsorption to root canal wall. Additionally, chitosan was dissolved in 1% acetic acid to form the solution. In an acid medium, the amino groups present in the chitosan are protonated (-NH3+), resulting in attraction to other molecules for adsorption to root dentin to occur [22]. However, the incomplete ability of 0.2% Chitosan acetate to remove the smear layer at the apical third could be attributed to its viscosity which may interfere with its flow ability to reach the apical part especially in curved and relatively narrow canals used in the current study. This was confirmed by several researchers who concluded that greater amounts of smear layer were found at the apical third of the canal, however they used straight canals in their studies [23, 24].

EDTA efficiently removed the smear layer due to its chelating property. This might be attributed to formation of stable complex with the calcium ions in dentin. However, it didn't show a pronounced effect in the apical third as compared to middle or coronal thirds of the root canal as the solution not only removes calcium ions but also calcium bonded to noncollagenous proteins of dentin (NCPs). As the content of NCPs decreased at the apical third of the root canal system, accordingly the decalcifying

effect of EDTA at the apical part is low. This was in accordance with previous studies [25, 26].

The similar efficiency of 0.2% chitosan acetate and EDTA to clean the middle third reported in this study corroborated a previous study, which revealed that 0.2% chitosan, 15% EDTA and 10% citric acid solutions removed smear layer efficiently from the middle third of the root canal [27]. However it has been reported that, smear layer removal at the middle third was more effective when final irrigation was performed using 0.2% chitosan solution compared to 17 % EDTA [23]. This could be explained by using EDTA with lower volume and contact time (1 ml for 1 min). The results of the apical level was correlated with a study which reported that 0.2% chitosan have the same ability of EDTA to remove smear layer from the apical third [28]. However, the results of the present study was inconsistent with Kamble and his group who concluded that, 0.2% chitosan removes the smear layer with greater efficiency than 17% EDTA at the apical third of the root canal [9]. This could be attributed to the use of chelating agent for prolonged contact time (5 minutes) than the current study and the use of ultrasonic activation.

The use of Endovac irrigation system in the current study showed more smear layer removal compared to the conventional irrigation without significant difference between them at the coronal, middle and apical levels when 17% EDTA was used as a final rinse. This could be attributed to the advantage of using the microcannula in the Endovac irrigation system that has the ability to reach the full working length where irrigant is pulled into the canal and removed by negative pressure following the use of macrocannula [29].These results were consistent with other studies that were performed on straight root canals [15, 30].

The comparable results between the conventional irrigation and EndoVac irrigation system could be explained by the use of small diameter needle for conventional irrigation (0.25 mm), where the dispersal of the irrigating solution through the side-vent conventional irrigating needle creates an upward turbulent motion around and beyond the end of the needle, which thoroughly irrigates the whole root canal [31]. Other studies reported that Endovac irrigating system showed better results over the conventional irrigation in delivering the irrigant to the working length in curved root canals [16, 32]. This could be related to the large size of Monoject needle used in conventional irrigation group (27 gauge) compared to the apical preparation size.

Using 0.2% chitosan acetate via Endovac irrigation system resulted in more smear layer removal compared to the conventional irrigation without significant difference between them at the coronal and apical levels. However, there was a statistical significant difference in the scores of the middle level where Endovac irrigation system showed lower mean smear layer score compared to conventional irrigation. This could be explained by the placement of macrocannula up to the middle third of the canal. Additionally, the high efficiency of 0.2% Chitosan acetate to clean the middle third could be attributed to its increased viscosity and stagnation of the residual fluid at the site where the macrocannula was inserted.

Regarding the results of Endovac irrigation system using either 17% EDTA or 0.2 % chitosan acetate at the apical root canal level, might be attributed to the increase in the apical preparation size to 0.40 mm by F4 ProTaper file since the Endovac system requires a minimum canal shape at least a #35 instrument with a 4% taper as recommended by the manufacture to allow the microcannula to reach the full working length [18]. It has been reported that, when the apical size increases, the chance of the holes in the microcannula contacting the root canal wall to be blocked are decreased and thus increased the volume of irrigant to the microcannula tip [29].

The results of the current study were in accordance with that shown by **Nielsen (2007)** [14] **and Dua (2015)** [33] who found that the efficiency of Endovac system in removing the smear layer was comparable to that of the conventional irrigation at 3 mm level from the working length. However, other

studies [15, 34] reported that Endovac irrigating system was more effective for smear layer removal than the conventional irrigation, this might be attributed to the use of more volume of the irrigant (10 ml). In addition, both studies were performed on single rooted premolars, which could affect the results, as when canal curvatures are present, effective irrigant delivery becomes even more difficult, especially in the apical third.

CONCLUSION

Within the limitations of this study the following could be concluded:

1. Chitosan acetate (0.2%) as a natural material presented similar smear layer removal efficiency in curved root canal walls compared to 17% EDTA when used as final rinse.
2. Endovac irrigation system was effective in smear layer removal from curved root canals using 0.2% Chitosan acetate or 17 % EDTA as final rinse.

REFERENCES

1. Violich DR, Chandler NP. The smear layer in endodontics: a review. Int Endod J 2010; 43: 2-15.
2. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94:658-66.
3. Hülsmann M, Heckendorf M, Lennon À. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 2003; 36: 810-30.
4. Jaju S, Prashant P. Newer Root Canal Irrigants in Horizon. Int J Dent 2011, 851359:1-9.
5. Kurita K. Chemistry and application of chitin and chitosan. Polyme Degrad Stabil. 1998; 59: 117-20.
6. Jeon YJ, Shahid F, Kin SK. Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. Food Reviews Inter 2000; 16: 159-76.
7. Fawzy MI. Scanning electron microscopic evaluation of the effectiveness of chitosan solution in smear layer removal. Egy Dent J 2011; 57:419-24.
8. Silva PV, Guedes DF, Pécora JD, Cruz-Filho AM. Time-dependent effects of chitosan on dentin structures. Braz Dent J 2012; 23: 357-61.
9. Kamble AB, Abraham S, and Mehta DL. Scanning electron microscopic evaluation of efficacy of 17% ethylenediaminetet-raacetic acid and chitosan for smear layer removal with ultrasonics: an in vitro study. Contemp Clin Dent 2017; 8: 621-26.
10. Ghorbanzadeh A, AminSobhani M, Sohrabi K, Chiniforush N, Ghafari S. Penetration depth of sodium hypochlorite in dentinal tubules after conventional irrigation, passive ultrasonic agitation and Nd: YAG Laser-activated agitation. J Lasers Med Sci 2016; 7: 105-11.
11. Rödig T, Döllmann S, Konietschke F, Drebenstedt S, Hülsmann M. Effectiveness of different irrigant agitation techniques on debris and smear layer removal in curved root canals: a scanning electron microscopy study. J Endod 2010; 36: 1983-7.
12. Boutsioukis C, Psimma Z, van der Sluis LW. Factors affecting irrigant extrusion during root canal irrigation: a systematic review. Int Endod J 2013; 46: 599–618..
13. Gutmann JL, Zehnder M, Levermann VM. Historical perspectives on the roots of the apical negative pressure irrigation technique in endodontics. J History Dent 2014; 62: 32–40
14. Nielsen BA, Baumgartner JC. Comparison of the EndoVac system to needle irrigation of root canals. J Endod 2007; 33: 611-15.
15. Karade P, Chopade R, Anish Kulkarni. Efficiency of different endodontic irrigation and activation systems in removal of the smear layer: A SEM study. Iran Endod J 2017; 12: 414-18.
16. Ahuja P, Nandini S, Ballal S, Velmurugan N. Effectiveness of four different final irrigation activation techniques on smear layer removal in curved root canals: SEM study. J Dent 2014; 11: 1-9.
17. Schneider SW. A comparison of root canal preparations in straight and curved canals. Oral Surg Oral Med Oral Pathol 1971; 32: 271-5.
18. Endovac brochure. Available at https: //www.kerrdental.com
19. Da cruz-Filho AM, De Vito Bordin AR, Souza-Flamini LE, Da costa Guedes DF, Saquy PC, et al. Analysis of the shelf life of chitosan stored in different types of packing, using colorimetry and dentine microhardness. Rest Dent Endod 2017; 42: 87-94.

20. Hülsmann M, Rümmelin C, Schäfers F. Root canal cleanliness after preparation with different endodontic handpieces and hand instruments: a comparative SEM investigation. J Endod. 1997; 23:301-6.

21. Brunson M, Heilborn C, Johnson DJ, Cohenca N. Effect of apical preparation size and preparation taper on irrigant volume delivered by using negative pressure irrigation system. J Endod 2010; 36: 721–4.

22. Rahzi M, Desbriéres J, Tolaimate A, Rinaudo M, Vottero P, et al. Influence of the nature of the metal ions on the complexation with chitosan: Application to the treatment of liquid waste. Euro Polymer J 2002; 38: 1523-30.

23. Darag AB. Effectiveness of different final irrigation solutions on smear layer removal in intraradicular dentin. Tanta Dent J 2014; 11: 93-9.

24. Praveen M, Aarthi G, Karunakaran JV. Comparative evaluation of intraradicular smear removal efficacy of 2% chitosan (low molecular weight), 4% chitosan citrate, and 10% citric acid when used as final rinse in irrigation protocols: A field emission SEM Study. J Pharm Bioallied 2017; 9: S73-S8.

25. De-Deus G, Paciornik S, Pinho Mauricio MH, Prioli R. Real-time atomic force microscopy of root dentine during demineralization when subjected to chelating agents. Int Endod J 2006; 39: 683-92.

26. Rathakrishnan M, Sukumaran VG, Subbiya A. Efficacy of an innovative irrigant on smear layer removal-SEM Analysis. JCDR 2016; 10: 104-6.

27. Pimenta JA, Zaparolli D, Pécora JD, Cruz-Filho AM. Chitosan: effect of a new chelating agent on the microhardness of root dentin. Braz Dent J 2012; 23: 212-21.

28. Silva PV, Guedes DF, Nakadi FV., Pécora JD, da Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int J Endod 2012; 46: 332–8.

29. Schoeffel GJ. The EndoVac method of endodontic irrigation, part 2 efficacy. Dent Today 2008; 48-51.

30. Ahmetoglu F, Keles A, Yalcin M, Simsek N. Effectiveness of different irrigation systems on smear layer removal: A SEM study. Eur J Dent 2014; 8: 53-7.

31. Druttman AC, Stock CJ. An in vitro comparison of ultrasonic and conventional methods of irrigant replacement. Int Endod J 1989; 22:174-8.

32. Munoz HR, Camacho-Cuadra K. In vivo efficacy of three different endodontic irrigation systems for irrigant delivery to working length of mesial canals of mandibular molars. J Endod 2012; 38: 445–8.

33. Dua A. Comparative evaluation of efficacy of EndoVac irrigation system to Max-I probe in removing smear layer in apical 1 mm and 3 mm of root canal: An in vitro SEM study. Dent Res J 2015; 12: 38-43.

34. Suman S, Verma P, and Kumar-Shakya V. A comparative evaluation of smear layer removal using apical negative pressure (EndoVac), sonic irrigation (EndoActivator) and Er: YAG laser -An in vitro SEM Study. Clin Exp Dent 2017; 9: e 981-87.