Acta Scientific DENTAL SCIENCES (ISSN: 2581-4893)

Volume 4 Issue 8 August 2020

Research Article

Role of Xp-Endo Finisher Agitation File with the Use of EDTA and Chitosan, on Smear Layer Removal by SEM and the Use of Flame Atomic Absorption Spectrometry to Quantify the Concentration of Calcium Ions: An *In Vitro* Study

Marwan (Mohammad Shafik) Ashour Seyaj[1]*, Salsabyl Mohamed Ibrahim[2] and Shaimaa Ismail Gawdat[3]

[1]*Masters Student, Faculty of Dentistry, Cairo University, Egypt*

[2]*Professor of Endodontics, Faculty of Dentistry, Cairo University, Egypt*

[3]*Associate Professor of Endodontics, Faculty of Dentistry, Cairo University, Egypt*

***Corresponding Author:** Marwan (Mohammad Shafik) Ashour Seyaj, Masters Student, Faculty of Dentistry, Cairo University, Egypt.

Received: May 20, 2020

Published: July 10, 2020

Abstract

Aim of the work: The aim of this study was to evaluate the effectiveness of the XP-endo Finisher file on smear layer removal in straight root canals after biomechanical instrumentation in comparison to two different chelating solutions (EDTA and Chitosan) by scanning electron microscope and to quantify the concentration of calcium ions on surface of root dentin by using atomic absorption spectrophotometry with flame (AASF).

Materials and Methods: Twenty freshly extracted, permanent, mandibular and maxillary premolars were included in the study. The sound teeth were decoronated at the cemento-enamel junction using a fine tapered diamond stone with round end mounted on high speed hand-piece under water coolant. The root canal of each specimen was mechanically prepared using a BT-Race rotary system (FKG Dentaire) and then divided randomly into two groups, as follows: EDTA group (n = 10) XP-endo Finisher file; 5-mL 17% EDTA. Chitosan group (n = 10) XP-endo Finisher file; 5-mL 0.2% Chitosan. The roots were longitudinally split and specimens were fixed on metallic subs and examined using an environmental SEM for the presence of smear layer and the containers were forwarded for spectrometric determination of calcium ion concentration within the liquid.

Results: Representative SEM images for EDTA group showed efficient smear layer removal with the dentinal tubules open in apical, middle and coronal thirds. On the other hand, chitosan group showed efficient smear layer removal, with coronal and middle thirds having more open dentinal tubules than the apical third. The mean Ca level was 45.58 ± 12.56 in EDTA group which recorded the highest Ca concentration among samples and chelated more calcium ion than others with 69.9 mg/L while the mean Ca level was 23.36 ± 6.38 in Chitosan group which recorded the lowest Ca concentration among samples with 12.9 mg/L. This difference was statistically significant $p < 0.001$, being higher in EDTA group.

Conclusion: Irrigation with 0.2% chitosan solution is as effective in root canal cleaning as 17% EDTA, in terms of smear layer removal. Regardless of the irrigation protocol, the apical third retains more smear layer than the coronal and middle thirds. Irrigation with 0.2% chitosan solution chelated less calcium than 17% EDTA.

Keywords: XP-Endo Finisher; Agitation; Chitosan 0.2%; Smear Layer; Calcium Ion Concentration

Introduction

The biomechanical preparation of root canals with instrumentation produces an amorphous, non-uniform surface layer [1], which was initially named "smeared layer" that caps the dentinal tubules [2]. The smear layer consists of two layers, a superficial sheet that is about 1 - 2-µm thick and a deeper segment plugged in the dentinal tubules that reaches 40-µm in depth and seems to be relatively adhered to the dentinal tubules. This smear layer consists of primarily the inorganic particles and some organic components in the form of necrotic, residual pulp tissue, odontoblastic processes, microbes and blood cells [3].

Retention of smear layer affirms the fact that it plugs the dentinal tubules and reduces permeability of dentin to bacteria and bacterial products [4]. Different materials and techniques have been reported with expanded variation in their efficacy regarding removal of the intra canal smear layer. The most widely used irrigant for this purpose is ethylenediamine-tetra acetic acid (EDTA) in different formulations [5].

It produces canals with patent dentinal tubules. To improve cleanliness, irrigants must be in contact with root canal walls [6]. The conventional irrigation syringe, transports solutions no further than 0 - 1.1 mm beyond the needle tip [7].

Recent research recommended smear layer removal for root canal therapy, in pursuance of enhancing the fluid-tight seal of the system [8], decrease the amount of bacteria in the root canal system [9], facilitated root canal disinfection and improved adaptation of filling material and its adherence to dentinal wall as well as had better long-term treatment outcomes [10,11]. Other researchers have examined the efficacy of techniques associated with different root canal irrigant solutions [12,13]. Recently, a new nickel-titanium rotary finishing file has been developed called the XP-endo Finisher file (FKG Dentaire, Switzerland).

The XP-endo Finisher file is supposed to be used after any root canal instrumentation to accomplish an enhanced cleaning of the root canal while conserving dentin. The XP-endo Finisher has a small core size (ISO 25 in diameter and zero taper) with improved flexibility. The XP endo Finisher file is formed using a proprietary NiTi alloy (MartensiteAustenite Electropolish-FleX). It performs at different temperature and is claimed to have a high flexibility. It has been reported that the curved bulb can expand its extent 6-mm in diameter when the file tip is squeezed or 100-times of a corresponding sized file [14].

EDTA is the most popular broadly used irrigant for smear layer removal and fulfills the cleaning of root canal walls by acting on inorganic part [15,16]. Calcium chelation occurs when EDTA reacts with the calcium ion in dentine and this promotes dentine decalcification at approximate depths of 20 - 30-µm within 5-minutes [17]. However, EDTA causes irritation to the periapical tissues if accidently extruded beyond the apex [18]. It also causes erosion of peritubular and intertubular dentin and reduces the dentin microhardness when used in regular concentrations for extended durations [19].

A new solution which has attracted attention in dental research is Chitosan, a natural polysaccharide and this is due to fact of its biocompatibility biodegradability, bio-adhesion and lack of toxicity [20]. Chitosan is attained by the de-acetylation of chitin, which is found in crab and shrimp shells and has become ecologically interesting for various applications because of its plenitude in nature and low production costs. The properties of chitosan that provide its chelating capacity on canal walls have not been determined, and the prospect for its use as an irrigant in root canal treatment is yet to be investigated [21]. Up till now, the usage of xp-endo finisher with chitosan as chelating agent has not been subjected to adequate investigation; hence came the aim of the study.

Materials and Methods

Experimental teeth

Twenty freshly extracted, permanent, mandibular and maxillary premolars were included in the study. Selected teeth were collected from patients with periodontally affected teeth which were referred for extraction at Oral Surgery Department/faculty of dentistry in Cairo University. Patient's informed consent was obtained according to the recommendations of the ethics committee of Cairo University. Sample size calculation was achieved using PS: Power and sample size calculation software version 3.1.2 (Vanderbilt University, Nashville, Tennessee, USA). The teeth were submerged for 15 minutes in 2.5% NaOCl solution. In 0.9% saline solution mixed with thymol the residual tissue and debris eliminated from the root surface and were then stored. The sound teeth were decoronated at the cemento-enamel junction using a fine tapered diamond stone with round end mounted on high speed hand-piece under water coolant to obtain a uniform 15 mm tooth

length. The working length was established by expressing the file just beyond the apex then withdrawing the file to flush with the apex. This length was recorded as the tooth length and 1-mm was subtracted to establish the working length. Patency of the canals was established using K file #10, and then glide path was established using Scout Race #15/0.02. Mechanical preparation was carried out in all specimens with the aid of VDW. GOLD RECIPROC® motor in a crown-down technique using BT-Race rotary system following the manufacturer's recommendation for the speed (800 RPM) and torque (1.5 N-cm) and were constant for each file as follows: BT1 #10/0.06, BT2 #35/0.00 and BT3 #35/0.04 and all were used to the full working length. During instrumentation, irrigation was done using a 30-gauge, end-vented needle coupled on a 5-mL plastic syringe. After each file use, the canals were irrigated with 3-mL of 2.5% NaOCl. Then, the root canals were irrigated with 10-mL distilled water to prevent the extended effects of the NaOCl solution.

Test irrigating solutions

After biomechanical preparation of the root canals, the specimens were divided randomly into two groups, and were divided as follows: EDTA group (n = 10) XP-endo Finisher file; 5-mL 17% EDTA was delivered using a 30-gauge, end-vented needle coupled on a 5-mL plastic syringe for 1 minute and was agitated with XP-endo Finisher file which set at 800 rpm and advanced to 1mm short of the WL. Slow and gentle 7 - 8 mm lengthwise movements were made for 60 seconds. Chitosan group (n = 10) XP-endo Finisher file; 5-mL 0.2% Chitosan was delivered using a 30-gauge, end-vented needle coupled on a 5-mL plastic syringe for 3 minutes and was agitated with XP-endo Finisher file which set at 800 rpm and advanced to 1mm short of the WL. Slow and gentle 7-8 mm lengthwise movements were made for 60 seconds.

Each tooth was placed in a sterile plastic tube, and the tube lid was perforated in such a way that the tooth could be positioned with the cervical portion outside and the root inside the tube. Then the respective chelating solution was delivered into the root canal passing through the entire root canal. As a final washout, each specimen was then irrigated for 60 seconds with 1-mL 2.5% NaOCl followed by a final rinse with 5- mL sterile saline solution. Achieving a total irrigation volume of 11-mL for each root canal. The root canals were then dried with series size 35 paper points and the specimens were stored in 1.5-mL Eppendorf tubes.

Scanning electron microscopy

The roots were longitudinally split with a chisel and mallet into 2 halves. For each specimen, the half enclosing the most visible part of the apex and the whole canal length was selected and the other half was discarded. Then, each specimen was measured lengthwise with a digital caliper from the apex to the cement-enamel junction for delimitation of the root thirds. Specimens were fixed on metallic subs and examined using an environmental SEM for the presence of smear layer. Micrographs were taken at 3-mm (cervical), 6-mm (middle) and 9-mm (apical) from the apex, while avoiding un-instrumented areas. At each fixed length, one micrograph was taken at 4000-X magnification for smear layer evaluation respectively, making a total of one micrograph per third and 3 micrographs per specimen. Micrographs were coded then assessed following scoring system modified from Takeda., *et al.* [22] for smear layer presence on the root canal walls: (0): no smear layer seen on the surface. Open dentinal tubules, smear layer was completely removed or melted. (1): smear layer removed or melting in some areas; outlines of dentinal tubules observed. (2): Smear layer partially covering few visible tubules. (3): surface and dentinal tubules covered with heavy smear layer.

Concentration of calcium ions (Absorption spectrophotometry with flame)

Eleven-mL of total solution per specimen, the containers were forwarded for spectrometric determination of calcium ion concentration within the liquid. Once all the delutes had been collected, they were analysed for their calcium content using an atomic absorption spectrophotometer with an air-acetylene flame. The dilutions were performed with 1% nitric acid (HNO_3) to these respective proportions: 1-mL for 0.2% chitosan, 1-mL for 17% EDTA group. This dilution was necessary for the correct measurement of the concentration of calcium ions in solutions.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

The used tests were Mann Whitney test for abnormally distributed quantitative variables, to compare between two studied groups. Kruskal Wallis test for abnormally distributed quantitative variables, to compare between more than two studied groups and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. Independent t test for normally distributed of the data, was done for Ca level only.

Results

Regarding smear layer, pairwise comparison between the two groups showed no statistically significant difference among coronal, middle and apical thirds (P-value = 0.139).

The apical third in both groups showed remaining smear layer. Pairwise comparison in EDTA group showed no statistically significant difference in smear layer removal between coronal, middle and apical thirds (P > 0.05), while pairwise comparison in Chitosan group showed no statistically significant difference in smear layer removal between coronal and middle thirds (P > 0.05), however the apical third showed statistically significantly higher scores than each of the coronal and middle (P < 0.05).

	Chitosan (n = 10)		EDTA (n =10)		U	p1
	Median	Min.-Max.	Median	Min.-Max.		
Apical	2.0a	1.0 - 3.0	1.5	0.0 - 3.0	44.0	0.684
Coronal	1.0b	0.0 - 2.0	1.0	0.0 - 1.0	49.5	0.971
Middle	1.0b	1.0 - 2.0	1.0	0.0 - 2.0	29.5	0.123
H(p2)	9.598* (0.008*)		5.924 (0.052)			
Total	1.0	0.0-3.0	1.0	0.0-3.0	362.0	0.139

Table 1: Results of scoring the effect of the selected two chelating agents on smear layer removal in the cervical middle and apical thirds.

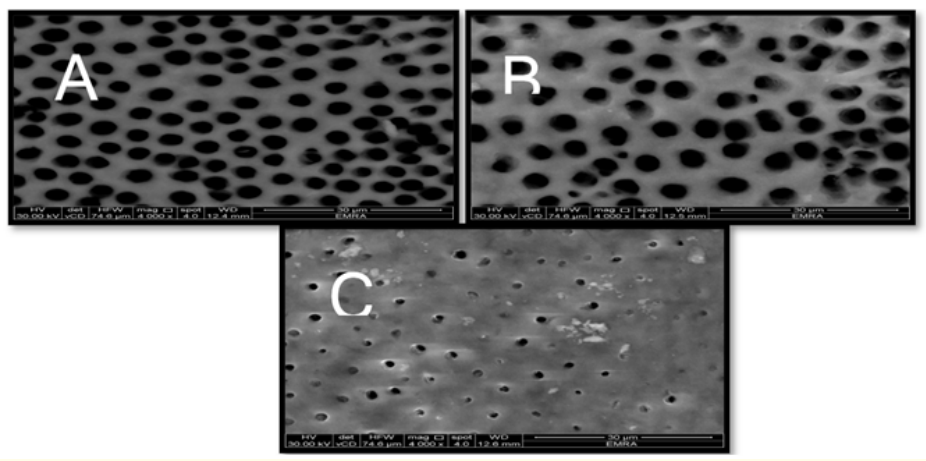

Figure 1: SEM images showing the effect of EDTA at the coronal, middle and apical thirds with (4000-X magnification) on smear layer removal (A, B, C respectively).

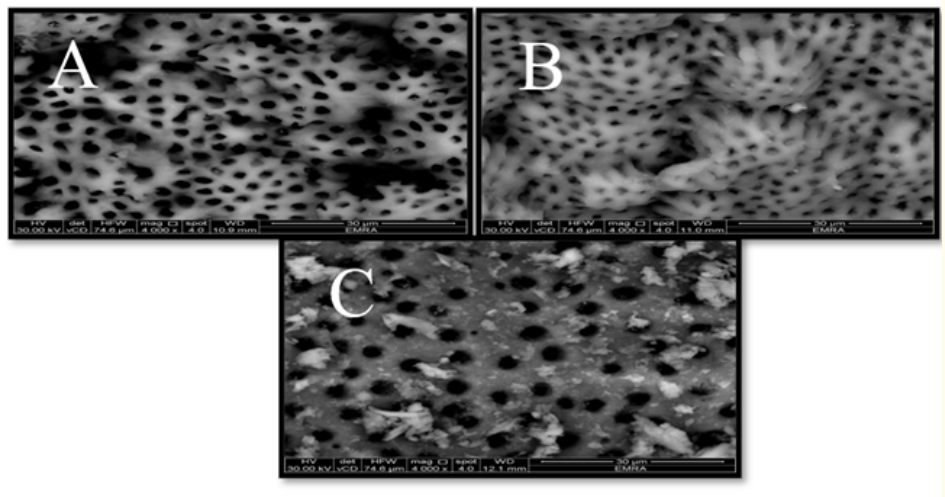

Figure 2: SEM images showing the effect of chitosan at the coronal, middle and apical thirds (4000-X magnification) on smear layer removal (A, B, C respectively).

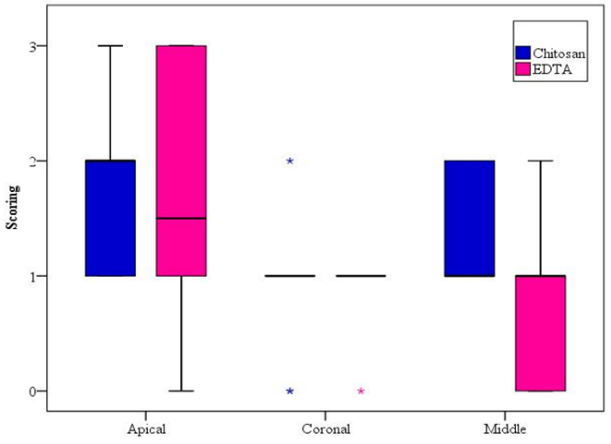

Figure 3: Box plot showing the smear layer scores comparison between the studied groups and in each of the apical, coronal and middle thirds for chitosan and EDTA.

Regarding Ca ion level determination in EDTA and chitosan group**,** the mean Ca level was 45.58 ± 12.56 in EDTA group which recorded the highest Ca concentration among samples and chelated more calcium ion than others with 69.9 mg/L while the mean Ca level was 23.36 ± 6.38 in Chitosan group which recorded the lowest

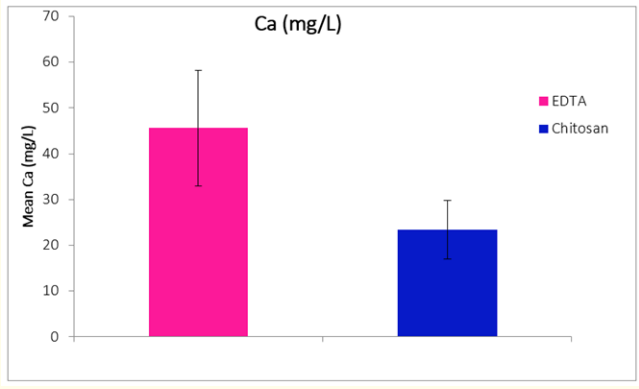

Figure 4: Bar chart represents mean ca level in the studied groups.

	EDTA		Chitosan		
	Mean	SD	Mean	SD	P value
Ca (mg/L)	45.58	12.56	23.36	6.38	< 0.001

Table 2: Mean, SD and independent t test results of Ca level in the studied group.

SD: Standard Deviation, $p \leq 0.05$ is considered statistically significant.

Ca concentration among samples with 12.9 mg/L. This difference was statistically significant $p < 0.001$, being higher in EDTA group.

Discussion

The main goal of endodontic treatment is to instrument and irrigate root canal walls and ensure effective cleaning of endodontic space, including complete removal of necrotic or vital pulp, i.e. all bacteria from the root canal system [23].

Removal of smear layer that forms along the walls during instrumentation is an important clinical parameter for the success of endodontic treatment [24,25]. Currently, several techniques and systems [26]. are available and reported to improve final irrigation before obturation.

The aim of this study was done to evaluate the role of the XP-endo finisher agitation file with the use of EDTA in comparison to Chitosan, on smear layer removal by SEM and the use of Flame Atomic Absorption spectrometry to quantify the concentration of calcium ions.

The choice of single rooted teeth with single canal was because of the oval shape of the selected premolars. Rounded cross-section of endodontic files, with some areas of the main canal bound to remain untouched after instrumentation. Research has shown that these untouched areas can reach up to 35% of total area of the canal walls [27-29]. The elimination of smear layer and eradication of micro-organisms in these areas is fully dependent on the efficacy of the irrigation solution. Accordingly, the role of irrigation along irrigant activation is of utmost importance in the canals.

30-gauge needle with a tip diameter of 0.3 mm was used; along with apical preparation done up to BT Race size 35, whose tip corresponds to ISO # 35 and will lead to a canal diameter of 0.39 and 0.43 at 1 and 2 mm from the apex, respectively. Thus, allowing for better reach and free movement of the need tip at 1 - 2 mm from the apex which in turn should provide better exposure and deeper action of the irrigant especially at the apical third.

The chosen NaOCl concentration was 2.5% because it is the most commonly used concentration in routine endodontic practice [30]. Clinically the chosen concentration is a compromise between the antibacterial activity and cytotoxicity [31,32]. Besides, dentinal erosion was derived from the use of NaOCl at high concentrations (5.25%) [33]. To date, no general agreement exists regarding the concentration that is most efficacious against micro-organisms and still be safe for the patient [34,35].

The selected concentration and application time of chitosan solution was based on the recommendation of Polliana Vilaça Silva., *et al*. [36] who reported that 0.2% chitosan used for 3 minutes as a final flush was efficient in smear layer removal without causing dentin erosion. Therefore, the 3 minutes application time was standardized for the chitosan group throughout the whole study.

Regarding the timing of 17% EDTA, it was previously reported that its usage for 1 minute, which was applied in this study, was effective in smear layer removal without causing dentin erosion [19,37].

XP-Endo Finisher is new endodontic agitation file showed high ability in reaching the inaccessible areas of the canal and untouched areas, thereby, providing better cleaning and enhanced removal of smear layer and bacterial biofilms, in accordance with Živković., *et al*. [38], Bao., *et al*. [39], Sanabria-liviac., *et al*. [40] and ElNaghy., *et al* [41].

For the SEM analysis, the longitudinal grooves were done before chemo-mechanical preparation in order to minimize the introduction of cut debris into the root canal through the orifice and the apical foramen, owing to the smaller canal diameter before preparation. Besides, if accidently introduced, they would be removed during cleaning and shaping. These cut debris if retained on the canal walls, may mislead the results causing underestimation of the cleaning action of the final flush.

Studies used magnifications of 35-x up to 5000-x. A 4000-x magnification was used for smear layer analysis, as proposed by recent study Prabhakaran and Mariswamy [42], because it allowed

for a detailed view of the smear layer, as well as, the orifices of dentinal tubules.

It has been reported by Khademi., *et al*. [43] that the total removal of the smear layer happened only in root canal prepared to an apical diameter of minimum 0.30 mm. Therefore, in the present study, the last file used for the preparation of root canals was BT3 #35/0.04.

There was no significant difference between the two chelating solutions. Smear layer removal occurred without distinction in coronal and middle thirds; however, showed significant difference to apical third in Chitosan group. Mancini., *et al*. [44] found that 17% EDTA and Bio-pure MTAD were not able to remove the smear layer in the apical third. This divergence in results could be explained by the fact that, in the present study, to collect samples of the solutions used as irrigants for spectrometric analysis, the apices of the specimens were patent. Thus, during the act of irrigation, the solution passed through the entire root canal leading directly into the collection tube through the apical foramen. At the same time, the volume of chelating agent that acted in the middle and coronal third was the same as in the apical third.

The results of this study regarding smear layer removal indicated that 0.2% chitosan showed similar cleaning efficacy to 17% EDTA, the gold standard of chelating solutions with no significant difference in middle and coronal third. And this was in consistent with Neha., *et al*. [45] which showed no significant difference between 17% EDTA and 0.2% chitosan in removal of smear layer but 17% EDTA had comparatively better results than 0.2% chitosan at the apical third. On the other hand the results were in contrast with Kamble., *et al*. [46] that showed statistically significant difference between 0.2% chitosan compared to 17% EDTA on smear layer removal using ultrasonic irrigating technique.

In the 0.2% chitosan group there was no significant difference between the smear layer remaining in the middle and coronal thirds. However, there was significant difference in apical third. And this can be also attributed primarily to larger diameter of the coronal and middle part and better tissue-chemical contact during instrumentation and the fact that dentin is exposed to larger volume of irrigant.

Regarding calcium ion concentration, the results were in contrast with Luis., *et al*. [47] who showed high calcium ion concentration in 0.2% chitosan group and PV Silva., *et al*. [48] who demonstrated no significant difference, and this could be because he used 15% EDTA comparing to 0.2% chitosan.

Conclusion

Within the limitation of this *in-vitro* study, the following can be concluded that Irrigation with 0.2% chitosan solution is as effective in root canal cleaning as 17% EDTA, in terms of smear layer removal. Regardless of the irrigation protocol, the apical third retains more smear layer than the coronal and middle thirds. And Irrigation with 0.2% chitosan solution chelated less calcium than 17% EDTA.

Conflict of Interest

The authors deny any conflicts of interest in this study.

Bibliography

1. Peeters Harry Huiz and Ketut Suardita. "Efficacy of Smear Layer Removal at the Root Tip by Using Ethylenediaminetetraacetic Acid and Erbium, Chromium: Yttrium, Scandium, Gallium Garnet Laser". *Journal of Endodontics* 37.11 (2011): 1585-1589.

2. McComb Dorothy and Dennis C. Smith. "A Preliminary Scanning Electron Microscopic Study of Root Canals after Endodontic Procedures". *Journal of Endodontics* 1.7 (1975): 238-242.

3. Mader Carson L., *et al*. "Scanning Electron Microscopic Investigation of the Smeared Layer on Root Canal Walls". *Journal of Endodontics* 10.10 (1984): 477-483.

4. Bogra P., *et al*. "Study of Dimercapto Succinic Acid , Sodium Hypochlorite and Their Combination Used as Irrigant in Root Canals - An *in vitro* Study". *Endodontology* 15.2 (2003): 19-25.

5. Baumgartner J Craig., *et al*. "A Scanning Electron Microscopic Evaluation of Root Canal Debridement Using Saline, Sodium Hypochlorite, and Citric Acid Una Evaluacion de La Biomecanica Del Conducto Usando Solucion Salina, Hipoclorito de Sodio, y Acido Citrico, Con Microscopio de Ba". *Journal of Endodontics* 10.11 (1984): 525-31.

6. Zehnder Matthias. "Root Canal Irrigants". *Journal of Endodontics* 32.5 (2006): 389-398.

7. Munoz Hugo Roberto and Karla Camacho-Cuadra. "*In Vivo* Efficacy of Three Different Endodontic Irrigation Systems for Irrigant Delivery to Working Length of Mesial Canals of Mandibular Molars". *Journal of Endodontics* 38.4 (2012): 445-448.

8. Shahravan Arash., *et al*. "Effect of Smear Layer on Sealing Ability of Canal Obturation: A Systematic Review and Meta-Analysis". *Journal of Endodontics* 33.2 (2007): 96-105.

9. Clark-Holke Deborah., *et al*. "Bacterial Penetration through Canals of Endodontically Treated Teeth in the Presence or Absence of the Smear Layer". *Journal of Dentistry* 31.4 (2003): 275-281.

10. Gengoglu Nimet., *et al*. "Dentinal Wall Adaptation of Thermoplasticized Gutta- Percha in the Absence or Presence of Smear Layer : A Scanning Electron Microscopic Study". *Journal of Endodontics* 19.11 (1993): 558-562.

11. Violich DR and NP Chandler. "The Smear Layer in Endodontics - A Review". *International Endodontic Journal* 43.1 (2010): 2-15.

12. Scelza MF., *et al*. "Efficacy of Final Irrigation--a Scanning Electron Microscopic Evaluation". *Journal of Endodontics* 26.6 (2000): 355-358.

13. Ballal Nidambur Vasudev., *et al*. "Evaluation of Decalcifying Effect of Maleic Acid and EDTA on Root Canal Dentin Using Energy Dispersive Spectrometer". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* 112.2 (2011): e78-84.

14. Silva EJ NL., *et al*. "Effectiveness of XP-Endo Finisher and XP-Endo Finisher R in Removing Root Filling Remnants: A Micro-CT Study". *International Endodontic Journal* 51.1 (2018): 86-91.

15. Marques Andre Augusto Franco., *et al*. "Smear Layer Removal and Chelated Calcium Ion Quantification of Three Irrigating Solutions". *Brazilian Dental Journal* 17.4 (2006): 306-309.

16. Spanó JCE., *et al*. "Atomic Absorption Spectrometry and Scanning Electron Microscopy Evaluation of Concentration of Calcium Ions and Smear Layer Removal With Root Canal Chelators". *Journal of Endodontics* 35.5 (2009): 727-730.

17. Von der Fehr FR and NygaardØstby B. "Effect of EDTAC and sulfuric acid on root canal dentine". *Oral Surgery* 16.2 (1963): 199-205.

18. Gomes-Filho João Eduardo., *et al*. "Comparison of the Biocompatibility of Different Root Canal Irrigants". *Journal of Applied Oral Science* 16.2 (2008): 137-144.

19. Kumar Yogender., *et al*. "Comparative Evaluation of Demineralization of Radicular Dentin with 17% Ethylenediaminetetraacetic Acid, 10% Citric Acid, and MTAD at Different Time Intervals: An *in vitro* Study". *Journal of International Society of Preventive and Community Dentistry* 6.1 (2016): 44.

20. Akncbay H., *et al*. "Application of Chitosan Gel in the Treatment of Chronic Periodontitis". *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 80B.2 (2007): 290-296.

21. Peter MG. "Pure and Applied Chemistry Applications and Environmental Aspects of Chitin and Chitosan applications and environmental aspects". *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry* 32.4 (1995): 629-640.

22. Takeda Frank Hirono., *et al*. "Efficacy of Er:YAG Laser Irradiation in Removing Debris and Smear Layer on Root Canal Walls". *Journal of Endodontics* 24.8 (1998): 548-551.

23. Torabinejad Mahmoud., *et al*. "Endodontics: Principles and Practice". Elsevier Health Sciences (2014).

24. Lester KS and A Boyde. "Scanning Electron Microscopy of Instrumented, Irrigated and Filled Root Canals". *British Dentistry Journal* 143 (1977): 359-367.

25. Torabinejad., *et al*. "Clinical Implications of the Smear Layer in Endodontics: A Review". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 94.6 (2002): 658-666.

26. Poggio Claudio., *et al*. "Effects of NiTi Rotary and Reciprocating Instruments on Debris and Smear Layer Scores: An SEM Evaluation". *Journal of Applied Biomaterials and Functional Materials* 12.3 (2014): 256-62.

27. Paqué Frank., *et al*. "Preparation of Oval-Shaped Root Canals in Mandibular Molars Using Nickel-Titanium Rotary Instruments: A Micro-Computed Tomography Study". *Journal of Endodontics* 36.4 (2010): 703-707.

28. Siqueira Jr., *et al*. "Ability of Chemomechanical Preparation with Either Rotary Instruments or Self-Adjusting File to Disinfect Oval-Shaped Root Canals". *Journal of Endodontics* 36.11 (2010): 1860-1865.

29. Peters Ove A., *et al*. "Root Canal Preparation with a Novel Nickel-Titanium Instrument Evaluated with Micro-Computed Tomography: Canal Surface Preparation over Time". *Journal of Endodontics* 36.6 (2010): 1068-1072.

30. van der Waal, Suzette., *et al*. "Free Available Chlorine Concentration in Sodium Hypochlorite Solutions Obtained from Dental Practices and Intended for Endodontic Irrigation: Are the Expectations True?" *Quintessence International* 45.6 (2014): 467-474.

31. Radcliffe CE., *et al*. "Antimicrobial Activity of Varying Concentrations of Sodium Hypochlorite on the Endodontic Microorganisms *Actinomyces israelii, A. naeslundii, Candida albicans* and *Enterococcus faecalis*". *International Endodontic Journal* 37.7 (2004): 438-446.

32. Siqueira Jr., *et al*. "Chemomechanical Reduction of the Bacterial Population in the Root Canal after Instrumentation and Irrigation with 1%, 2.5%, and 5.25% Sodium Hypochlorite". *Journal of Endodontics* 26.6 (2000): 331-334.

33. Kaya Sadullah., *et al*. "Evaluation of Radicular Dentin Erosion and Smear Layer Removal Capacity of Self-Adjusting File Using Different Concentrations of Sodium Hypochlorite as an Initial Irrigant". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 112.4 (2011): 524-530.

34. Retamozo Bonnie., *et al*. "Minimum Contact Time and Concentration of Sodium Hypochlorite Required to Eliminate *Enterococcus faecalis*". *Journal of Endodontics* 36.3 (2010): 520-523.

35. Berber VB., *et al*. "Efficacy of Various Concentrations of NaOCl and Instrumentation Techniques in Reducing *Enterococcus faecalis* within Root Canals and Dentinal Tubules". *International Endodontic Journal* 39.1 (2006): 10-17.

36. Silva Polliana Vilaça., *et al*. "Time-Dependent Effects of Chitosan on Dentin Structures". *Brazilian Dental Journal* 23.4 (2012): 357-361.

37. Poudyal Sitashi., *et al*. "Efficacy of Solution Form of Ethylenediaminetetraacetic Acid on Removing Smear Layer of Root Canal at Different Exposure Time *In vitro*". *Journal of Huazhong University of Science and Technology* 34.3 (2014): 420-424.

38. Živković Slavoljub., *et al*. "XP-Endo Finisher: A New Solution for Smear Layer Removal". *Serbian Dental Journal* 62.3 (2015): 122-129.

39. Bao Pingping., *et al*. "*In vitro* Efficacy of XP-Endo Finisher with 2 Different Protocols on Biofilm Removal from Apical Root Canals". *Journal of Endodontics* 43.2 (2017): 321-325.

40. Sanabria-liviac Daniel., *et al*. "Comparison of the XP-Endo Finisher File System and Passive Ultrasonic Irrigation (PUI) on Smear Layer Removal after Root Canal Instrumentation Effectiveness of Two Irrigation Methods on Smear Layer Removal". *Journal of Dentistry and Oral Health* 4 (2017).

41. Elnaghy Amr M., *et al*. "Effectiveness of XP-Endo Finisher, EndoActivator, and File Agitation on Debris and Smear Layer Removal in Curved Root Canals: A Comparative Study". *Odontology* 105.2 (2017): 178-183.

42. Prabhakaran Pranitha and Annapoorna Ballagere Mariswamy. "A Scanning Electron Microscope Evaluation of Efficacy of Sodium Hypochlorite and Allium Sativum in Smear Layer Removal in Root Canals with the Use of Modified Evacuation System: An *Ex Vivo* Study". *Journal of Conservative Dentistry: JCD* 21.4 (2018): 401.

43. Khademi Abbasali., *et al*. "Determination of the Minimum Instrumentation Size for Penetration of Irrigants to the Apical Third of Root Canal Systems". *Journal of Endodontics* 32.5 (2006): 417-420.

44. Mancini Manuele., *et al*. "A Comparative Study of Smear Layer Removal and Erosion in Apical Intraradicular Dentine with Three Irrigating Solutions: A Scanning Electron Microscopy Evaluation". *Journal of Endodontics* 35.6 (2009): 900-903.

45. Neha *et al*. "Effect of Different Irrigation Regimens on Smear Layer Removal in Human Root Dentin an *In-Vitro* Study". *Asian Pacific Journal of Health Sciences* 4.2 (2017): 31-37.

46. Kamble AradhanaBabu., *et al*. "Scanning Electron Microscopic Evaluation of Efficacy of 17% Ethylenediaminetetraacetic Acid and Chitosan for Smear Layer Removal with Ultrasonics: An *In vitro* Study". *Contemporary Clinical Dentistry* 8.4 (2017): 621.

47. Luis Fábio., *et al*. "Chelating Agents and Agitation Protocols Assessment of the Amount of Calcium Ions Released after the Use of Different Chelating Agents and Agitation Protocols". *Open Dental Journal* 11.7 (2017): 133-139.

48. Silva PV., *et al*. "Chitosan: A New Solution for Removal of Smear Layer after Root Canal Instrumentation". *International End-*