Integrated Master Degree in Dentistry
2018

Efficacy of smear layer removal using sonic and rotary irrigant activation systems: a SEM evaluation

Rita Balaus Custódio[1], João Paulo Mouta Dias[2], Manuel Marques Ferreira[1,3,4]

[1] Faculty of Medicine, University of Coimbra, Portugal. [2] Pedro Nunes Institute, IPN [3] Coimbra Instituite for Clinical and Biomedical Research, iCBR – Faculty of Medicine, University of Coimbra, Portugal. [4] Center for Neuroscience and Cell Biology, IBILI – Faculty of Medicine, University of Coimbra, Portugal

Abstract

Introduction: Irrigation is a crucial step to ensure the success of endodontic treatments and while conventional needle irrigation is widely accepted, some methods to enhance canal cleanliness have been developed. The purpose of this study was to compare the effectiveness in smear layer removal between the conventional technique and two agitation systems: one sonic and one rotary.

Methods: 30 human single-rooted teeth were collected, decoronated at a standardized length of 15mm and instrumented using the Protaper Universal rotary system up to size F3 while being irrigated solely with 2.5% sodium hypochlorite. The specimens were randomly divided into 3 groups (n = 10) and subjected to different final irrigation protocols: Group CI- conventional irrigation; Group XP- agitation of the irrigant solution using the XP-Endo Finisher file; Group EDDY- agitation of the irrigant solution using the EDDY sonic system. The roots were split longitudinally and examined under a scanning electron microscope (SEM) at 1000x magnification, with which microphotographs were taken of all canal thirds for each sample. The presence of smear layer at 2, 8 and 12mm from the apex was evaluated using a 5-grade scoring system. The data were analysed through the two-factor ANOVA test.

Results: Conventional irrigation showed higher mean smear layer scores, but the difference between groups was not statistically significant ($p = 0.641$). The mean smear layer score was highest for the apical third and lowest for the middle third, with this discrepancy being significant ($p < 0.05$).

Conclusion: No significant difference in canal cleanliness was found between conventional and agitation-assisted irrigation with 2.5% sodium hypochlorite, but XP and EDDY groups showed lower mean smear layer scores.

Keywords: Smear layer, Root canal irrigation, Scanning electron microscope, Sonic activation, XP-Endo Finisher file, Sodium hypochlorite

Introduction

The purpose of endodontic therapy is to achieve canal disinfection in order to prevent reinfection and lesion of periapical tissues (1). For this goal we have at our disposal a variety of instruments such as manual or rotary files, that not only remove the pulp but also the affected dentin from the root canal walls (2) and, in conjunction with irrigant solutions that lubricate and also clean the canal (3), contribute to the long-term success of endodontic treatments.

A major problem during root canal instrumentation is the creation of a smear layer (SL), consisting of organic and inorganic substances such as fragments of tooth structure, microorganisms, necrotic tissue and blood cells (4).

Its appearance is amorphous and irregular and it consists of two separate layers: one that adheres superficially to the dentinal surface and one that penetrates into dentinal tubules up to 40μm (5).

This can potentially create some issues and even compromise the treatment altogether, as the smear layer can act as a harbor and fuel for bacteria (6) and it can prevent proper penetration of intracanalar medication and irrigation solutions and affects the adhesion of sealers. (7). Therefore, to ensure the best results possible, the smear layer should be removed. This, however, has proved to be very challenging, especially in the apical third of root canals (8, 9, 10) and it is believed that irrigant solutions alone are not very effective in removing SL. To counter this shortcoming, multiple agitation methods were developed with the aim of potentiating the effect of said solutions (1).

Sonic activation is an example of such systems, consisting of polymer tips of different sizes activated at sonic frequency (1-6 kHz) by a hand piece and seemingly able to increase the removal of SL and promote the cleanliness and subsequent filling of lateral canals, as stated by the manufacturer. These are generally found to be less effective than passive ultrasonic irrigation (PUI) because they work at a lower frequency. However, the gap between the two might be closed soon with the development of new materials or might already be inexistent, as pointed out by some previous studies (8, 11), with the advantage that sonic tips are more flexible and less likely to damage the canal walls (12). EDDY (VDW, Germany) is a newly introduced system that falls under this category, with tips made of an innovative polymer that promote better cleanliness through a three-dimensional oscillation movement which triggers cavitation and acoustic streaming. It is to be used at a frequency of 5-6 kHz and claims to have similar results in SL removal to PUI, that relies in the same cleansing effects mentioned above.

Other systems rely on innovative rotary instruments to achieve the same goal, like XP-Endo Finisher (FKG, Switzerland) which is a #25 universal nontapered NiTi file made of a patented alloy (Martensite-Austenite Electropolish-Flex) that changes shape according to temperature. When cooled the instrument is straight (M-phase) but when exposed to body temperature it curves (A-phase), increasing its reach while in rotation to a diameter of 6mm while preserving dentin and not leading to overpreparation due to its high flexibility, according to the manufacturer. Its purpose is to activate the irrigant solution, thus contributing to cleaner canal walls.

Considering the wide variety of activation protocols available, the present study aimed to compare the effectiveness in smear layer removal between conventional irrigation and two recent systems of activated irrigation (XP-Endo Finisher and EDDY) when 2.5% NaOCl is used.

The null hypothesis was that there is no significant difference in smear layer removal amongst the three final irrigation protocols.

Methods

Thirty single-root non-carious teeth with a root length of a minimum of 15mm were extracted, cleaned with periodontal scalers to remove remaining soft issue and stored in 0.5% chloramine-T at a temperature of 4ºC until use. Teeth with previous root canal treatment, incomplete root development, more than one canal or foramen, or with a root too short to fit the beforementioned criteria were excluded. The selected teeth were cut transversally using a diamond disk at 15mm from the tip of the root mark for a standardized WL. This WL was confirmed for each tooth inserting a size #10 stainless steel K-file (Dentsply Maillefer, Switzerland) in the canal until the tip of the file protruded from the apical foramen. A small apical plug made out of pink wax was made for each tooth to better emulate the normal clinical conditions and stop the irrigant solution from passing through the apical foramen during irrigation. Root canals were prepared with ProTaper Universal (Dentsply Maillefer, Switzerland) rotary system up to size #30 (F3) at 300rpm and 3.0 Ncm torque. At the start of instrumentation and between each subsequent file 3mL of 2.5% NaOCl was used, delivered by a 27G blunt-tip needle placed in the middle third of the canal and moved back and forth during irrigation procedures. The specimens were randomly divided into three experimental groups (n=10) with different final irrigation protocols as follows (Table I):

1. Conventional irrigation (CI): This group, intended as a negative control, was irrigated with 6mL of 2.5% NaOCl at the end of canal preparation using the same irrigation procedure as before and no activation methods whatsoever.
2. Activated irrigation with XP-Endo Finisher (XP): The file used in this group has specific physical properties that contribute to its effectiveness. Therefore, some extra steps were required to guarantee the validity of results. The file was cooled with chloroethane inside the tube provided by the manufacturer, its tip inserted into the canal and about 1mL of 2.5% NaOCl was used before rotation was turned on at 800rpm and 1 Ncm torque. The file was used for 1 minute with gentle corono-apical and parietal movements and the addition of another 1mL of irrigant during this time to ensure proper canal lubrication. After its removal, the canal was thoroughly irrigated with 4mL of NaOCl to ensure the removal of suspended debris.
3. Activated irrigation with the EDDY sonic system (EDDY): In this group 1mL of 2.5% NaOCl was used to irrigate the canal prior to the insertion of the polymer tip mounted on an air scaler. The activation time was 45 seconds at a frequency of 5000 Hz, moving the tip up and down the length of the canal. Afterwards, another 5mL of irrigant were used to flush the canal and remove debris.

Table I. Final irrigation protocol of the experimental groups.

Group	Irrigant	Activation	Activation time
CI	6mL of 2.5% NaOCl	-	-
XP	6mL of 2.5% NaOCl	Mechanical	60s
EDDY	6mL of 2.5% NaOCl	Sonic	45s

Two parallel grooves were made longitudinally using a diamond disk on the mesial and distal surfaces of each root, in which a sharp chisel was inserted to cleave and thus separate the two halves, taking care not to get debris inside the canal. In some cases the canal wasn't fully exposed by this procedure and additional removal of pericanalar dentin was necessary. Some damage was inflicted on the specimens in this process, and the most intact half of each root was selected to remain in the study.

The samples were dehydrated using a series of graded ethanol solutions (50, 75, 96 and 100%) for one hour each and then stored at 37ºC for 48h in preparation for microscopic analysis.

The specimens were then mounted in stubs and coated with a nanometric gold layer (sputtered on prototype equipment with DC power supply in argon atmosphere) and analysed using a Zeiss Merlin field emission gun high resolution) scanning electron microscope (FEG-SEM with Gemini II column). Three microphotographs were taken from the apical (2mm from the apex), middle (8mm from the apex) and coronal (12mm from the apex) thirds at 1000x magnification.

For each photograph a smear layer score was attributed by two observers, blind to group status, according to the following 5-score index system created by Hulsmann et. al (13):

1. No smear layer, dentinal tubules open.
2. Small amount of smear layer, some dentinal tubules open.
3. Homogenous smear layer covering the root canal wall, only few dentinal tubules open.
4. Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules.
5. Heavy, non-homogenous smear layer covering the complete root canal wall.

Statistical analysis

The descriptive analysis of the results was performed using mean values and standard deviation. Concerning the inferential analysis, the existence of statistically significant differences between experimental groups was evaluated using the two-factor ANOVA test (in relation to irrigation protocol and canal third). To better perceive the distribution of the smear layer scores obtained, dispersion graphics were made for both aspects mentioned above.

Inter-examiner agreement was calculated through the intraclass correlation coefficient, using R *irr* package for this effect.

All statistical analyses were performed with IBM SPSS Statistics v24 software (IBM SPSS Inc, Chicago, IL) and R statistics platform v3.3.2 at a 5% confidence interval ($p < 0.05$).

Results

The inter-examiner agreement proved to be strong ($ICC = 0.915$) and statistically significant ($p < 0.001$).

Tables II, III and VI denote the distribution and frequency of smear layer scores by group at 2, 8 and 12mm respectively.

Table II. Smear layer score distribution between groups at 2mm from the apex.

		2mm				Total
	Scores	2	3	4	5	
Group	CI group	1	1	5	3	10
	EDDY group	0	0	6	4	10
	XP group	0	0	5	5	10
Total		1	1	16	12	30

Table III. Smear layer score distribution between groups at 8mm from the apex.

	Scores	8mm 1	2	3	4	5	Total
Group	CI group	1	2	3	3	1	10
	EDDY group	0	4	2	4	0	10
	XP group	2	2	2	3	1	10
Total		3	8	7	10	2	30

Table VI. Smear layer score distribution between groups at 12mm from the apex.

	Scores	12mm 1	2	3	4	5	Total
Group	CI group	1	1	2	4	2	10
	EDDY group	1	4	2	3	0	10
	XP group	0	3	1	5	1	10
Total		2	8	5	12	3	30

Table V denotes the mean value and standard deviation ($\bar{x} \pm dp$) of smear layer scores observed for each irrigation protocol and relative to canal third.

Table V. Mean scores and standard deviation by protocol and depth of observation.

	2 mm	**8 mm**	**12 mm**	Protocol
CI	4.0 ± 1.05	3.1 ± 1.20	3.5 ± 1.27	3.5 ± 1.20
EDDY	4.2 ± 0.63	3.0 ± 0.94	2.7 ± 1.06	3.3 ± 1.09
XP	3.9 ± 0.74	2.7 ± 1.16	3.4 ± 1.08	3.3 ± 1.10
Depth	4.0 ± 0.81	2.9 ± 1.10	3.20 ± 1.16	3.4 ± 1.12

Said scores are similar between the three experimental groups concerning the technique used and vary more when analysed by distance from the apex.

It is observed that conventional irrigation (CI) has higher mean scores than the remaining groups. However, these differences are not statistically significant ($F(2,90) = 0.447, p = 0.641$).

In the analysis performed by canal third, statistically significant differences were found ($F(2,90) = 9.250, p < 0.001$), with the mean SL score for 2mm being the highest and the one for 8mm being the lowest.

The distribution of the data is visually represented in the following graphics (Figures 1 and 2).

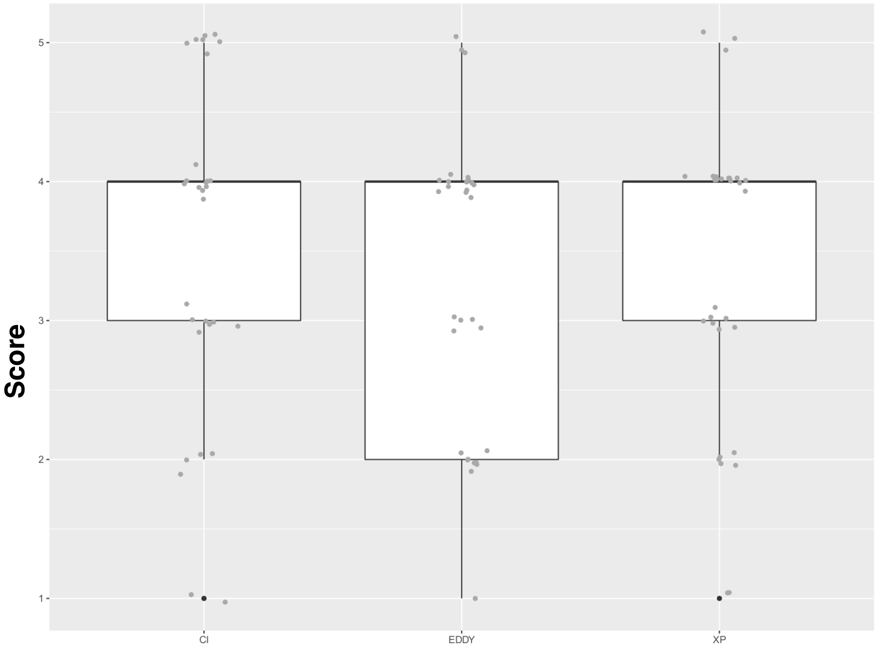

Figure 1. Visual representation of score distribution by irrigation protocol.

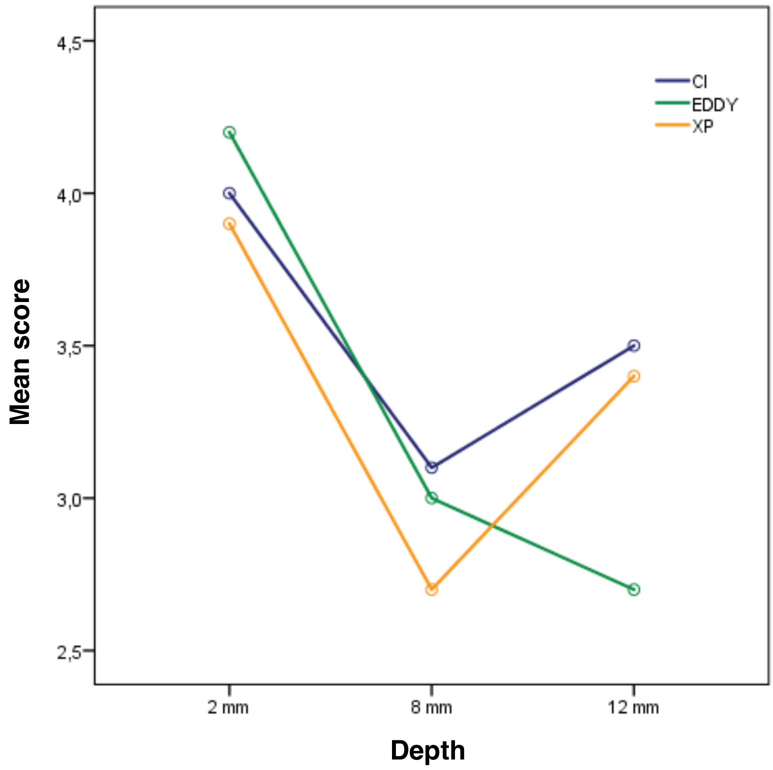

Figure 2. Visual representation of score distribution by canal third.

Figure 3 shows examples of microphotographs taken in the apical, middle and coronal third for all groups.

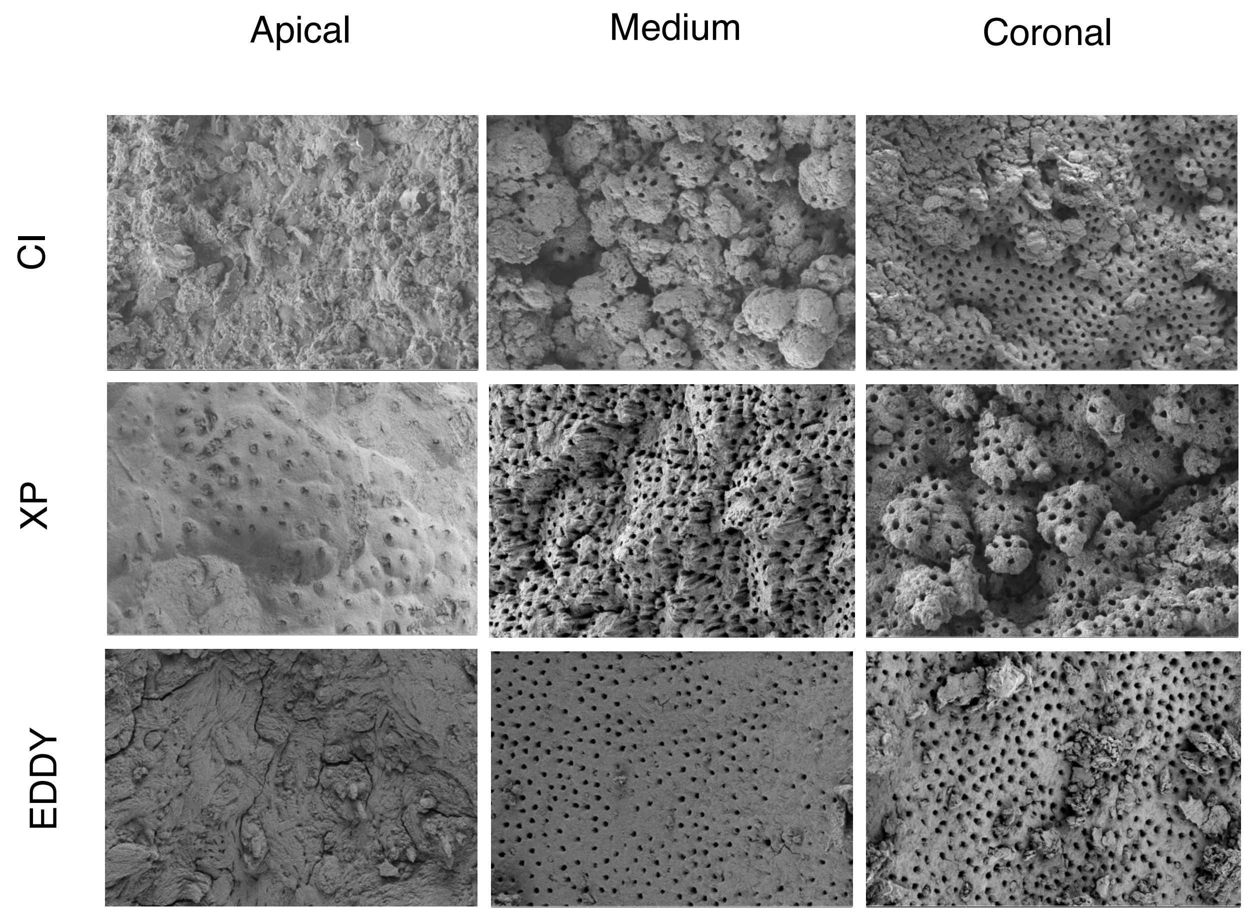

Figure 3. Representative SEM images from all three experimental groups and canal thirds.

Discussion

Disinfecting the root canal system during and after the instrumentation process is the main purpose of the irrigant solutions in endodontics. The shaping of root canals creates a smear layer made up of organic and inorganic substances, including microorganisms, pulp remnants and fragments of odontoblastic processes (4).

This layer can have deleterious effects on the long-term success of endodontic treatments, impeding the penetration of antimicrobial agents (such as the irrigants and intracanalar medications) into the dentinal tubules (14, 7) and compromising the adherence of obturation materials to the canal walls (15, 16). Rotary instrumentation with NiTi files is especially prone to the creation of a smear layer, hence its use in the present study (17).

Previous studies have shown that the conventionally used needle irrigation technique is not very effective in the removal of smear layer (18, 19, 20, 8), since the irrigant solution only penetrates up to 1.1mm beyond the needle's tip (21). For better cleaning effectiveness, the irrigant should be in contact with the root canal wall in its full extent (22), with activation systems playing a significant part in this goal (10, 12).

The aim of this study was to compare the effectiveness in smear layer removal between the conventional irrigation technique and two novel agitation methods, one of them a mechanical rotary system (XP-Endo Finisher) and the other a sonic system (EDDY), when using 2.5% NaOCl as the only irrigant solution.

A closed system was used to better reproduce clinical conditions. In vivo, the root is enclosed in bone, which creates gas entrapment in the apical area and hinders the reposition of irrigants in this region (23, 24).

It has been reported that the minimum apical preparation size for total removal of the smear layer is 0.30 mm (25). The final file used in this study was F3 (30/0.05) from the ProTaper Universal system, and while it is in accordance to the aforementioned study, a larger file could have been used to facilitate the hydrodynamic flow of the irrigant solution to the apical third of the canal.

Although a qualitative scoring method was utilized for the evaluation of smear layer removal (in accordance with previous studies (8-11), it was performed by calibrated observers with a high level of agreement between them, which contributes to the reliability of the results (26). Sample size, however, could have been greater to further cement the validity of the study.

Despite there not being consensus on the volume (27) and time of application (20) of the irrigant solution, this study opted to use a high volume of irrigant between each file during the instrumentation and also in the final irrigation protocols, injecting it at approximately the same speed used clinically in endodontic treatments.

XP-Endo Finisher is a NiTi rotary file that alters its shape at body temperature, being very flexible and able to expand its reach to up to 6mm in diameter, thus contacting areas that would be otherwise inaccessible and contributing to cleaner canal walls, as stated by the manufacturer (FKG Dentaire). There are not many studies available concerning this system, but those conducted so far seem to show promising results (28-31), with only one stating that its ability to reach and clean irregularities in the canal walls did not meet the expectations (32).

EDDY is a sonic-powered activation system with 25.04 size polymer tips that was recently introduced to the market. According to the manufacturer, it is capable of effectively cleaning complex root canal systems with its three-dimensional movement that creates cavitation and acoustic streaming, physical effects previously only attributed to passive ultrasonic irrigation (PUI) systems and that are responsible for their apparent superiority when compared to sonic devices (27, 33, 34). However, recent studies done with EndoActivator (EA), another sonic system, seem to disprove this theory (35, 11), making sonic activation a valid alternative for the improvement of canal cleanliness. Only one study (36) on the efficacy of EDDY in smear layer removal has been published so far, and while it reported results on par with PUI and performed significantly better than the control group, no irrigation was used in the latter, which might explain the positive significance obtained.

Overall, the canal walls were not very clean in the present study, with only 24% of the analysed areas showing clean canal walls (score 1 and 2). Although a lower mean value of smear layer was observed for both the XP and EDDY groups in comparison to the CI group, this difference was not statistically significant. Thus, the null hypothesis that there is no difference between the three final irrigation protocols described in this study was accepted. This can be explained by the fact that the only irrigant solution used was sodium hypochlorite, which is proven to not be effective on its own in smear layer removal (37), affecting only its organic part and having a mainly antibacterial and dissolving pulp tissue remnants and collagen (3). The authors' choice to use this solution alone, not in conjunction with EDTA, was motivated by the desire to evaluate the systems' absolute power of SL removal without resorting to the aforementioned chelating agent. However, from a clinical standpoint, EDTA + NaOCl should be used in final irrigation protocols to ensure ideal smear layer removal (1, 3, 20, 37).

In this study, the results show that SL removal was significantly more effective in the middle third than on the apical third, an outcome supported by previous studies (29,18-20, 35). That can be attributed to the smaller canal diameter, the higher prevalence of tubular sclerosis (39, 40) and the high anatomical variability in the apical section of the canal. In addition, the middle third demonstrated lower SL scores than the coronal third, which can be explained by the fact that the teeth were decoronated, thus lacking a four-wall access cavity to hold a more effective volume of irrigant in this particular section, as pointed out in a previous study (10). The larger canal diameter may also have contributed to this result, given that EDDY's polymer tip comes in only one size, not adapting fully to the canal's taper, which is suggested to affect the efficacy in smear layer removal (41). In the group in which XP-Endo Finisher was used, the file's arched shape when in austenitic phase contacts mostly with the middle third, which might be the explanation for these findings.

Further research must be conducted regarding the activation of irrigant solutions, analyzing new methods and delivery systems through sound methodologies and comparing their effectiveness in canal cleanliness.

Conclusions

Within the limitations of this study, it can be concluded that while the two agitation systems analysed resulted in a lower absolute mean value of smear layer when compared to conventional irrigation, this difference was not significant when using only 2.5% sodium hypochlorite as a final irrigant.

Acknowledgments

The authors thank Pedro Nunes Institute for making the scanning electron microscope available to us for the concretization of this project, as well as Dr. Francisco Caramelo and Dr. Marisa Loureiro for the statistical analysis of the data and assistance with the interpretation of results.

The authors deny any conflict of interest related to this study.

References

1. Plotino G, Cortese T, Grande NM, Leonardi DP, Di Giorgio G, Testarelli L, Gambarini G. New technologies to improve root canal disinfection. Braz Dent J 2016; 27:3–8

2. Hulsmann M, Peters OA, Dummer PMH. Mechanical preparation of root canals: shaping goals, techniques and means. Endodontic Topics 2005; 10, 30–76.

3. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Brit Dent J 2014; 216:299–303

4. Pashley DH. Smear layer: overview of structure and function. Proc Finn Dent Soc 1992;88(Suppl l):215-24.

5. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endod 1984; 10:477-83.

6. Pashley DH. Smear layer: physiological considerations. Oper Dent 1984;suppl 3:13-29.

7. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985; 18:35-40.

8. Mancini M, Cerroni L, Iorio L, Armellin E, Conte G, Cianconi L. Smear layer removal and canal cleanliness using different irrigation systems (EndoActivator, EndoVac, and passive ultrasonic irrigation): Field emission scanning electron microscopic evaluation in an in vitro study. J Endod 2013; 39(11): 1456-60.

9. 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(12): 1983-7.

10. Caron G, Nham K, Bronnec F, Machtou P. Effectiveness of different final irrigant activation protocols on smear layer removal in curved canals. J Endod 2010; 36(8): 1361-6.

11. Schiavotelo T, Coelho M, Rasquin L, Rocha D, Fontana C, Bueno C. Ex-vivo smear layer removal efficacy of two activated irrigation techniques after reciprocating instrumentation in curved canals. The Open Dentistry Journal, 2017; 11,512-519

12. Çapar ID, Aydinbelge HA. Effectiveness of various irrigation activation protocols and the self-adjusting file system on smear layer and debris removal. Scanning. 2014 Jun; 36(6):640–7.

13. Hulsmann M, Rummelin C, Schafers F. Root canal cleanliness after preparation with different endodontic handpieces and hand instruments: a comparative SEM investigation. J Endod 1997; 23:301–306

14. Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990; 6:142-9.

15. Abramovich A, Goldberg F. The relationship of the root canal sealer to the dentine wall. An in vitro study using scanning electron microscope. J Br Endod Soc 1976; 9:81–6.

16. Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F. Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. J Endod 2007; 33:96–105

17. Heard F, Walton RE. Scanning electron microscope study comparing four root canal preparation techniques in small curved canals. Int Endod J 1997; 30:323–31

18. Rödig T, Sedghi M, Konietschke F, Lange K, Ziebolz D, Hülsmann M. Efficacy of syringe irrigation, RinsEndo and passive ultrasonic irrigation in removing debris from irregularities in root canals with different apical sizes. Int Endod J 2010; 43:581-9.

19. 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.

20. Gu LS, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR. Review of contemporary irrigant agitation techniques and devices. J Endod 2009; 35:791-804.

21. Munoz HR, Camacho-Cuandra 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; 38:445–448.

22. Zehnder M. Root canal irrigants. J Endod 2006; 32:389-398.

23. Parente JM, Loushine RJ, Susin L, Gu L, Looney SW, Weller RN et al. Root canal debridement using manual dynamic agitation or the EndoVac for final irrigation in a closed system and an open system. Int Endod J. 2010 Nov 1;43(11):1001-1012.

24. Schmidt TF, Teixeira CS, Felippe MCS, Felippe WT, Pashley DH, Bortoluzzi EA. Effect of ultrasonic activation or irrigants on smear layer removal. J Endod 2015; 41:1359–1363.

25. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. J Endod 2006; 32:417–20.

26. Blank-Goncalves LM, Nabeshima CK, Martins GH, Machado ME. Qualitative analysis of the removal of the smear layer in the apical third of curved roots: conventional irrigation versus activation systems. J Endod. 2011; 37:1268–71.

27. Uroz-Torres D, Gonzales-Rodriguez MP, Ferrer-Luque CM. Effectiveness of the EndoActivator system in removing the smear layer after root canal instrumentation. J Endod 2010; 36:308–11.

28. Leoni GB, Versiani MA, Silva-Sousa YT. Ex vivo evaluation of four final irrigation protocols on the removal of hard-tissue debris from the mesial root canal system of mandibular first molars. Int Endod J 2016; 50(4):398-406.

29. Elnaghy AM, Mandorah A, Elsaka SE. Effectiveness of XP-endo Finisher, EndoActivator, and File agitation on debris and smear layer removal in curved root canals: a comparative study. Odontology 2016 Apr;105(2):178-183.

30. Keskin C, Sariyilmaz E, Sariyilmaz O. Efficacy of XP-endo Finisher file in removing calcium hydroxide from simulated internal resorption cavity. J Endod. 2017 Jan;43(1):126-130.

31. Zand V, Mokhtari H, Reyhani M, Nahavandizadeh N, Azimi S. Smear layer removal evaluation of different protocol of Bio Race file and XP- endo Finisher file in corporation with EDTA 17% and NaOCl. J Clin Exp Dent. 2017 Nov 1;9(11):e1310-e1314.

32. Wigler R, Dvir R, Weisman A. Efficacy of XP-Endo Finisher file in the removal of calcium hydroxide paste from artificial standardized groove in the apical third of oval root canals. Int Endod J 2016 Jul;50(7):700-705.

33. Paragliola R, Franco V, Fabiani C, Mazzoni A, Nato F, Tay FR. Final rinse optimization: influence of different agitation protocols. J Endod 2010; 36:282-285

34. Sabins RA, Johnson JD, Hellstein JW. A comparison of the cleaning efficacy of short-term sonic and ultrasonic passive irrigation after hand instrumentation in molar root canals. J Endod 2003; 29:674-678.

35. Mancini M, Cerroni L, Iorio L, Dall'Asta L. FESEM evaluation of smear layer removal using different irrigant activation methods (EndoActivator, EndoVac, PUI and LAI). An in vitro study. Clin Oral Invest 2018 Mar;22(2):993-999.

36. Urban K, Donnermeyer D, Schäfer E, Bürklein S. Canal cleanliness using different irrigation activation systems: a SEM evaluation. Clin Oral Invest 2017 Dec;21(9):2681-2687.

37. Shahriari S, Kasraei S, Roshanaei G, Karkeabadi H, Davanloo H. Efficacy of sodium hypochlorite activated with laser in intracanal smear layer removal: an SEM study. J Lasers Med Sci. 2017;8(1):36-41.

38. da Costa Lima GA, Aguiar CM, Câmara AC, Alves LC, Dos Santos FA, do Nascimento AE. Comparison of smear layer removal using the Nd:YAG laser, ultrasound, ProTaper Universal system, and CanalBrush methods: an in vitro study. J Endod. 2015; 41:400–4.

39. Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. Int Endod J 2009; 42:335–343).

40. Teixeira CS, Felippe MC, Felippe WT. The effect of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis. Int Endod J 2005; 38: 285–90.

41. Guo X, Miao H, Li L, Zhang S, Zhou D, Lu Y, Wu I. Efficacy of four different irrigation techniques combined with 60°C 3% sodium hypochlorite and 17% EDTA in smear layer removal. BMC Oral Health 2014 Sep 8;14:114.