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Evaluation and effectiveness of antimicrobial effect of endovac system and XP endofinsher on regeneration and apical repair of non-vital immature permanent teeth

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ABSTRACT

Purpose: To evaluate antimicrobial effect of endovac system and XP endofinsher on regeneration and apical repair of non-vital immature permanent teeth

Patients & Methods: A total of 21 Patients with non-vital immature teeth consisting of anterior teeth and (that were selected from the endodontic clinic of the Faculty of Dental Medicine Al-Azhar University, Cairo, Egypt. three groups of root canals with pulp necrosis and apical periodontitis were evaluated according to the disinfection technique: group 1: apical negative pressure irrigation (EndoVac system); , group 2: XP endofinsher group and group 3: apical positive pressure irrigation (conventional irrigation) plus triantibiotic intracanal dressing. The first sample (S1) was collected before disinfection phase and the second sample (S2) was collected after apical negative pressure irrigation (group1) or XP endofinsher (group2) or conventional irrigation/triantibiotic dressing (group 3). All samples were cultured in a culture medium for anaerobic bacteria. Colony-forming unit counts were analysed statistically by the Mann-Whitney test. Post-operative radiographs will be taken, 9 month, and 18 month to evaluate the degree of healing.

Results: The results showed that the amount of bacteria is highly decreased with endovac, followed by XP than with TAP. And there was a significant difference between the three groups. And the amount of healing of radiolucent area was more significant in endovac and XP endofinsher groups.

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Conclusion. In immature teeth with apical periodontitis, use of the EndoVac system and XP endofinsher group can be considered to be a promising disinfection protocol, because it provided more bacterial reduction to that of apical positive pressure irrigation (conventional irrigation) plus intracanal dressing with the triantibiotic paste, and the use of intracanal antibiotics might not be necessary.

Keywords: regeneration, endovac, triple antibiotic paste. XP finisher

Introduction

Regenerative endodontics procedures can be defined as biologically based procedures designed to replace damaged structures including dentin and root structures, as well as cells the pulp- dentin complex[1]. Regenerative endodontic procedures have been proposed to treat non vital immature permanent teeth [2]. The advantages of regenerative endodontics include thickening of the canal walls by deposition of hard tissue, and continued root development[3].

Regenerative endodontic procedures require an appropriate source of stem\ progenitor cells, growth factors and scaffolding in order to control the development of the targeted tissue. Disinfection of the root canal spaces of immature teeth are crucial to create a conducive environment for these three components to effectively act[4].

Several disinfection protocols have been proposed in regenerative endodontics, the most common one is conventional irrigation with sodium hypochlorite (NaOCl) in combination with triple antibiotic paste (TAP) (a mixture of ciprofloxacin, metronidazole, and minocycline) [5] or an intracanal dressing with calcium hydroxide[6] . Although TAP is an established disinfection method, it has its drawbacks. TAP is radiolucent, so the extent of placement of TAP inside the root canal cannot be verified during treatment, an additional appointment is required to removal TAP and re-entry into the tooth to remove TAP introduces a risk of recontamination[7], Furthermore, discoloration due to the presence of minocycline[7] and the potential cytotoxic effects of TAP on the surviving cells around the apical region of the tooth(i.e periodontal ligament cells, stem cells of apical papilla SCAP) which are crucial in success of Regenerative endodontic[8]. To overcome the drawbacks of TAP, the endovac apical negative –pressure system of irrigation has been suggested as a substitute for achieving disinfection of the root canal[9]. EndoVac delivers the irrigating agents to full extent of root canal terminus, thereby removing organic tissue and microbial contaminants effectively ( ). Thus, creating optimum conditions for regenerative endodontics procedures without the use of antibiotics. The XP endo finisher file (Brasseler USA –savannah, Georgia) has been introduced to the market. The XP endo Finisher instrument is a NiTi file No.25 with 0% taper, Below30C, it is in its pliable martensite form and can be straightened or manipulated to any shape. Above 35C (body temperature), it transforms to its austenite phase and is straight until the last 10 mm where it has a spoon shape with a depth of 1.5 mm. it is used after shaping of canals. The action of the tip and the bulb of The XP end Finisher file is to scrape and to disrupt the biofilm and, in addition, to cause turbulence of the irrigant for maximal effect. Very little research has been done to evaluate the anti-microbial effect of EndoVac system and XP endofinisher in regenerative endodontics in human beings.

Patients and Methods

Patients Selection and Informed Consent:

The study population comprised of 21 Patients with non-vital immature teeth consisting of anterior teeth and (that were selected from the endodontic clinic of the Faculty of Dental Medicine Al-Azhar University, Cairo, Egypt. Teeth with developmental anomalies, longitudinal fractures, periodontal affection of grade three mobility and non-restorable teeth were not considered for this study. Furthermore patients with systematic

diseases or history of allergy to any of the materials used in the study were excluded .Following initial examination and acceptance, written informed consent was taken from all patients or from the legal guardians of patients below 18 years of age following a detailed explanation of the procedures involved and the length of the treatment with emphasis on the possible outcomes.

Prior to instrumentation and disinfection of the root canals, administration of local anaesthesia with 2% mepivacaine without a vasoconstrictor and rubber dam application. Then, access cavity preparation was done using a #2 round bur in a high speed hand piece with copious water spray. The tooth was built up using bonded composite resin if it was required to facilitate rubber dam application. The working length was measured radiographically using paralling device and the working length was estimated at 1 mm shorter than the furthest point of the root by placement of a # 80 file in the canal that was stabilized in place using paper points. Minimal mechanical instrumentation was accomplished using circumfrincial filing using a #80 H-file. Irrigation with 1.5% sodium hypochlorite was accomplished using a 27 gauge side vented closed end endodontic irrigation needle attached a 5 ml sterile plastic syringe. The disinfection and irrigation protocol used was as follows:

Group (1): (Apical Negative Pressure irrigation (EndoVac system) fig (1)

The recommended protocol for the use of apical negative pressure irrigation includes 2 main phases: macroirrigation and microirrigation. Because our research model was aimed at testing the disinfection of immature teeth with open apices, the EndoVac protocol recommended by the manufacturer needed to be modified. Canals were irrigated using the macrocannula only after being gauged to fit the apical size of the canal. The macroirrigation was performed to the WL as the open-ended macrocannula was moved up and down in the canal from WL to a point just below the coronal orifice of the canal. During macroirrigation, 10 mL 1.5% sodium hypochlorite (NaOCl) was delivered via the master delivery tip at the access opening. The macrocannula was withdrawn from the canal in the presence of sufficient irrigant in the pulp chamber to ensure that the canal remained totally filled with irrigant and no air was drawn into the canal space. After instrumentation of the canals and phase of disinfection, the second bacterial sample was taken in all groups as follow: a sterile cotton pellet was used to dry the access opening. Then a size #80 sterile paper point was inserted into the root canal and removed. Then, the paper point was placed quickly in a sterile eppendorf tube and sent to the laboratory for bacterial evaluation.

Group (XP): (conventional irrigation with XP endo finisher file) fig (2)

Following instrumentation, Cleaning was accomplished using a combination of NaOCl and the XP endo finisher file. The XP endo finisher file is supplied in a blister pack, each file comes placed in a graduated plastic tube with an attached stopper. The working length. For each canal was determined using the graduated plastic tube and stopper. Then, the access cavity was filled with NaOCl solution. Following this, the file was removed from the plastic tube and inserted into the canal to the full working length. The XP endo finisher file was rotated in the canal for one minute using a slow and gentle in and out movements in the canal. The Xp endo finisher file was rotated at (800 rpm) at 1 Ncm in a 16:1 reduction hand piece in an endodontic motor. Then, the XP endo finisher file was removed from the canal while rotating; The canals were left filled with NaOCl solution for 3 minutes and then irrigated with 10 ml sterile saline solution to remove the rest of the NaOCl. Then final irrigation was accomplished using 1 ml 17% EDTA that was left in the canal for 60 seconds followed by dryness of the canal using size #80 paper points. Following the dryness, the root canal was ready for the regenerative procedures.

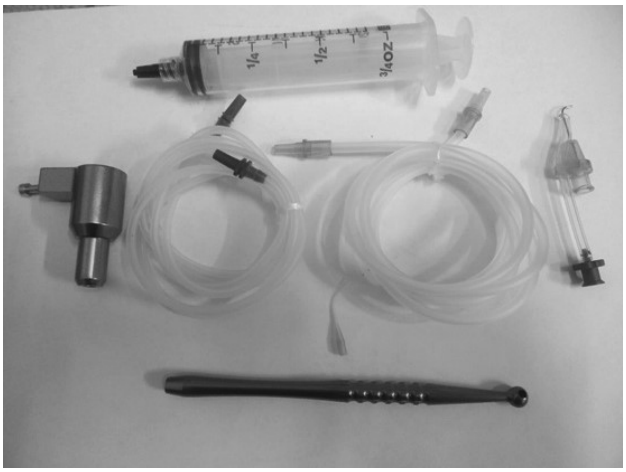
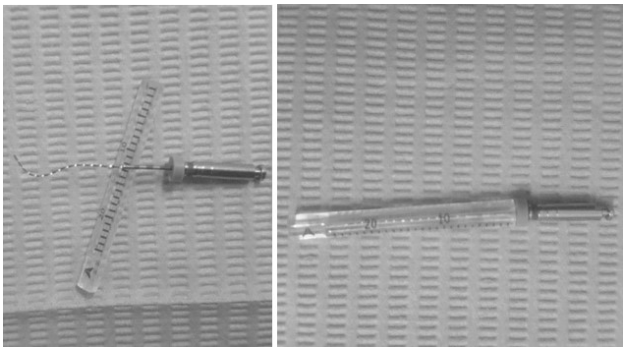


fig (1) a photograph showing components of Endovac system



A **B**

fig (2) A photograph showing the Xp endofinisher file (A), out of plastic sheath & (B) inside the plastic sheath

Group 3: apical positive pressure irrigation plus triantibiotic intracanal dressing:

In this group, apical positive pressure irrigation was performed using a sterile 30-gauge side-vented port needle (Max-i-Probe; Dentsply/Tulsa Dental, York, PA) connected to a syringe. The syringe was filled with 1.5% NaOCl, and the needle was introduced into the canal at the WL. Each canal was irrigated with light pressure with 10 mL of 1.5% NaOCl. Then irrigated again with 2 mL of sterile saline and dried with sterile paper points. A triantibiotic paste was prepared immediately before the treatment by mixing ciprofloxacin, metronidazole, and minocycline with sterile distilled water, at a concentration of 20 mg of each antibiotic, the paste was delivered into the root canals with a 20-gauge needle set at the WL and used with a backfill approach up to the level of the cementoenamel junction. The coronal access was then restored with a double seal of Cavit (3M/Espe) and glass ionomer cement (Vitrabond). The intracanal dressing was left in the canal for a period of 2 weeks. At the second treatment session, all teeth from this group were isolated with a rubber dam. The coronal seal was removed with sterile high-speed burs followed by flushing of the pulp chamber with sterile saline. The triantibiotic intracanal dressing was flushed off the canals with 10 mL sterile saline, and the canals were dried with sterile paper points. After instrumentation of the canals and phase of disinfection, the second bacterial sample was taken in all groups as follow: a sterile cotton pellet was used to dry the access opening. Then a size #80 sterile paper point was inserted into the root canal and removed. Then, the paper point was placed quickly in a sterile eppendorf tube and sent to the laboratory for bacterial evaluation. Following dryness the regenerative procedures were done.

Regenerative procedures:

After the disinfection phase, regenerative procedures were done in all groups as follow:

A pre-curved #25 k-file with a rubber stopper set 2 mm beyond the established working length was used in a rotation motion aiming to initiate bleeding into the canal to the level of the CEJ .A piece of teflon was inserted into the canal orifice and held there for 7 minutes to allow blood clot formation. After that, the piece of teflon was removed and the canal was sealed using a 2mm layer of MTA which was introduced inside the canal using MAP system for 2-3m beyond the CEJ, then the access opening was sealed with resin-modified glass ionmer cement. Then the Patient was recalled for followed up at regular intervals 9 and 18 months.

Results

Data were represented by mean, standard deviation (SD), median (M), with 95% Confidence Interval (95% CI) values.

Repeated measures ANOVA test was used to compare between different groups and sub groups

Post hoc tuckey test was used for comparison between different groups showing significance.

Student t test was used for comparison between means of different groups.

The significance level was set to P ≤ 0, 05. Statistical analysis was performed with IBM®* SPSS®Θ Statistics Version 20 at 95% confidence interval.

Comparison between *CFU* for different ways of treatment

The amount of bacteria is highly decreased in EndoVac group till 0.44 % of the total amount of bacteria followed by XP finisher group till 0.81% and the least decreased amount of bacteria was in TAP group till 1.57%

There was statistically significant difference for Endovac, XP and TAP (p =0.04).

There was statistically significant decrease for Endovac than XP (p=0.023) and TAP (p =0.031). There was statistically significant decrease for XP than TAP (p =0.017)

Table (1), (2) Comparison between CFU for different ways of treatment:

	CFU		
	mean of %	SD	P
XP	2.43%	±1.4	0.04
ENDO VAC	0.36%	± 0.5	
TAP	5.89%	± 3.7	

	CFU			
	before		After	
	NO	%	N0	%
TAP	292600	100%	4600	1.57 %
XP	85900	100%	700	0.81 %
Endo Vac	228200	100%	1000	0.44 %

Figure (3), (4) diagrammatic Chart showing comparison between CFU for different ways of treatment

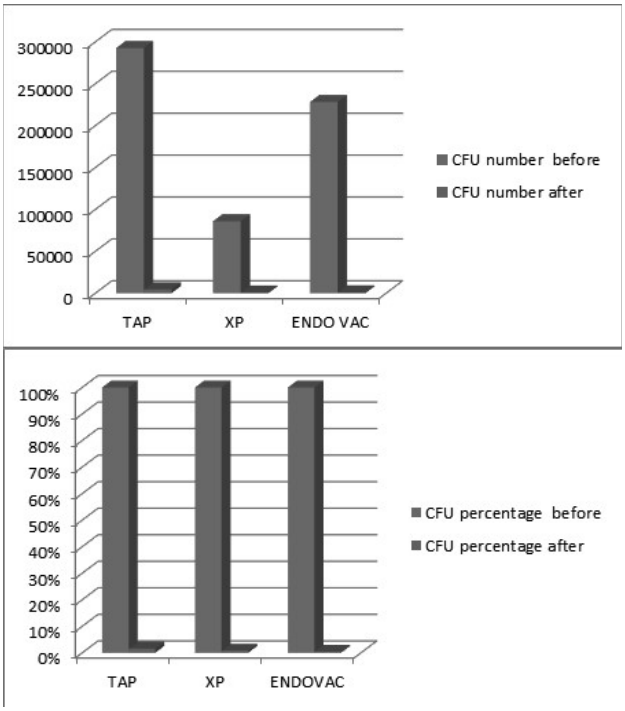


Figure (3), (4) diagrammatic Chart showing comparison between CFU for different ways of treatment

Comparison between size of peri apical radiolucency for different ways of treatment at different periods.

- **Xp Endo Finisher**

There was statistically_significant difference for size of peri apical radiolucency at different periods as p=0.03.

There was statistically_significant difference at pre measures and 9 M as p=0.013 and at 18 M and 9 M as p=0.02 and at pre measures and 18 M as p=0.002.

- **Endovac**

There was statistically_significant difference for size of peri apical radiolucency at different periods as p=0.01.

There was statistically_significant difference at pre measures and at 9 M as p=0.027 and at 18 M and 9 M as p=0.015 and at pre measures and 18 M as p=0.017.

- **TAP**

There was no statistically__significant difference at different periods as p=0.07.

Table (3) comparison between size of peri apical radiolucency for different ways of treatment at different periods

The Size of the Periapical Radiolucency:cm3			
	XP	ENDOVAC	TAP
PRE	0.08±0.002a	0.09±0.001a	0.05±0.0015
9 M	0.04±0.003b	0.04±0.0015b	0.04±0.0017
18 M	0.02±0.003c	0.02±0.002c	0.037±0.002
P	0.03*	0.01*	0.07

* Significance, small letters for significance between the same column p ≦ 0, 05

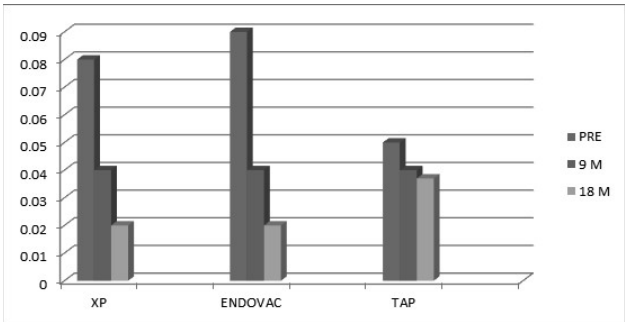


Figure (5) diagrammatic chart representing comparison between sizes of peri apical radiolucency for different ways of treatment at different periods

Discussion

During regenerative procedures, Disinfection of the root canal is a corner stone for success [1,2]. Eradication of latent infection within the canal is a challenge [3,4].

Single rooted anterior teeth were included in this study because they are the most common teeth prone to trauma in the early childhood teeth [5].

Duringinstrumentation,minimalmechanical preparation was done using circumferential filing according to AAE recommendation in revascularization to prevent further weakening of the dentinal walls [6].

With regards to root canal disinfection, during irrigation, a concentration of 1.5 % of NaOCL was used because it has been previously established that it has the least cytotoxic effect on the stem cells (SCAP) compared to other concentrations [7.8,9]

Furthermore, concerning the formulation of TAP, the AAE recommendations for revascularization suggest use of minocycline, metronidazole and ciprofloxacillin, in this research, minocycline was replaced with doxycycline due to lack of minocycline availability and this was similar to other research done in this field [10,11]. The disinfection period was 2 weeks Per the AAE recommendation which was also similar to other studies [12, 13,].

In this study, the EndoVac system was used as an alternative to TAP in disinfection of the root canal. It has been showed to be very effective in bacterial count reduction and in removing smear layer from the surface of root canals [14,15]. In 2010, research was done to evaluate the effectivity of EndoVac as a disinfection method in regeneration in dogs and the research showed very positive results [16]. Furthermore, the XP endofinisher file was also evaluated in this study, previous research has shown that the XP endofinisher file is an effective antibacterial tool and can also remove smear layer from the root canal surface [17,18,19,20,21]

In this study the aim was to evaluate the antimicrobial effect of the Endovac system and XP endo finisher on regeneration and apical repair of non-vital immature permanent teeth. With regards to the antibacterial effect, which was evaluated by calculating the percentage of remaining CFUs in the canals. All disinfection methods showed a dramatic reduction in the number of CFUs after disinfection irrelevant of the disinfection technique. When comparing between the Endovac system, XP endo finisher and TAP, it was found that Endovac had the least amount of remaining bacteria(0.36%± 0.5) followed by XP endo finisher(2.43%±1.4) followed by TAP(5.89%± 3.7) . These difference were significant. It is clear that both endovac and the XPF have significantly less remaining CFUs than TAP. This may be explained that both endovac and the XPF can physically disrupt the biofilm and remove the smear layer within the canals as they are considered active methods of disinfection while TAP is a passive disinfection method that cant disrupt the biofilm or smear layer [22].

Disruption of the biofilm and removing of the smear layer exposes more dentinal tubules allowing the irrigant to penetrate deeper into the dentinal tubules enhancing disinfection[23]. Furthermore, TAP has a high viscosity and penetration into dentinal tubules is limited to a maximum 50-100 micron. These results are in agreement with Previously research which has established that the Endovac and XPF are effective antibacterial tools [24, 25, , ,26 , 27]. When evaluating the size of the periapical radiolucency within each disinfection method over time.it was found that the endovac system and XP endofinisher showed a significant level of healing while TAP did not. This may be attributed to the higher amounts of remnant bacteria in the canal which may hinder healing in the apical area.

Conclusion

Both Endovac and XP finisher are effective as a disinfectant tools in regeneration but TAP is less effective in reduction of bacterial count in regeneration

References

1. langer, R, Vacanti, J: Tissue Engineering " Science: 260: 920-926, 1993

2. Iwaya S, Ikawa M, Kubota M: Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2001; 17:185-7.

3. Jadhav G, Shah N, Logani A. Revascularization with and without Platelet-rich Plasma in Nonvital, Immature, Anterior Teeth: A Pilot Clinical Study. J Endod 2012; 38:1581-7.

4. lovelace T, Henry M, Hargreaves K, Diogenes A. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. J Endod 2011:37:133-38

5. Windley W, Texeiera F, Trope M. Disinfection of immature teeth with a triple antibiotic paste. J Endo 2005; 31:439-43.

6. Thibodeau B, Teixeira F, Yamauchi M, et al. Pulp revascularization of immature dog teeth with apical periodontitis. J Endod. 2007; 33:680-9.

7. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J. 1996; 29:125-30.

8. Kim JH, Kim Y, Shin SJ, et al. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod. 2010; 36:1086-91.

9. Ruparel N, Teixeira F, Ferraz C, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod 2012;38:1372–5

10. Silva LAB, Nelson-Filho P, Silva RAB, Flores DSH, Heilborn C, Johnson JD, et al. Revascularization and periapical repair after endodontic treatment using apical negative pressure irrigation versus conventional irrigation plus triantibioticintracanal dressing in dogs' teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(5):779-87

11. Susin L, Liu Y, Yoon JC, Parente JM, Loushine RJ, Ricucci D, et al : Canal and isthmus debridement efficacies of two irrigant agitation techniques in a closed system. Int Endod J.2010:43:1077-90.

12. Lin M, Shimizu E, Gibbs L, Loghin S, Ricucci D. Histologic and histobacteriologic observations of failed revascularization/ revitalization therapy: a case report. J Endod. 2014; 40: 291–5.

13. Fouad A, Nosrat A. Pulp regeneration in previously infected root canal space. Endod. 2013; 28:24–37

14. Card J, Sigurdsson A, Ørstavik D, Trope M. The effectiveness of increases apical enlargement in reducing intracanal bacteria. Journal of Endodontics. 2002; 28: 779–83.

15. Cvek M, Nord E, Hollender L. Antimicrobial effect of root canal debridement in teeth with immature roots. A clinical and microbiologic study. Odontol Revy 1976; 27:1–10.

16. Bastone B, Freer J, McNamara R. Epidemiology of dental trauma: A review of the Literature. Australian Dental Journal 2000; 45:2-9.

17. American Association of Endodontists. AAE Clinical Considerations for a Regenerative Procedure: American Association of Endodontists; 2016. August [revised 2016 Dec; cited 2017 Dec 2]. [about 10 p.]

18. Martin E, Almeida F, Henry A, Khaing Z, Schmidt E, Teixeira B, et al. Concentration dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod 2014; 40: 51-5.

19. Trevino G, Patwardhan N, Henry A. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod 2011; 37:1109–15.

20. argreaves KM, Diogenes A, Teixeira FB. Treatment options: biological basis of regenerative endodontic procedures. J Endod 2013; 39: S30–43.

21. Tawfik H, Abu-Seida M, Hashem A, Nagy M. Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. International Endodontic Journal. 2013; 46:910-22.

22. Nagy M, Tawfik E, Hashem A, Abu-Seida M. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. J Endod 2014; 40:192–8.

23. homson A, Kahler B. Regenerative endodontics–biologically-based treatment for immature permanent teeth: a case report and review of the literature. Aust Dent J 2010; 55:446–52.

24. Chen Y, Chen L, Chen A. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/ abscess to revascularization procedures. International Endodontic Journal 2012; 45:294–305.

25. Nielsen BA, Baumgartner J. Comparison of the endovac system to needle irrigation of root canals. J Endod 2007; 33:611-5. 30.

26. Hockett L, Dommisch K, Johnson D, Cohenca N. Antimicrobial efficacy of two irrigation techniques in tapered and canal preparations: an in vitro study. J Endod 2008; 34: 1374-7.

27. Cohenca N, Heilborn C, Johnson D, Flores S, Ito Y, Silva A. Apical negative pressure irrigation versus conventional irrigation plus triantibiotic intracanal dressing on root canal disinfection in dog teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 109: e42-6.

28. Trope M, Debelian G. XP-3D Finisher file—the next step in restorative endodontics. Endod Pract US. 2015; 8:22–4.

29. Slavoljub Ž, Jelena N, Milica J, Marijana P, Marija Ž. XP endo finisher: A new solution for smear layer removal. Serbian Dent J 2015; 62:122-6. 24.

30. Elnaghy M, Mandorah A, Elsaka E. Effectiveness of XP-endo finisher, EndoActivator, and file agitation on debris and smear layer removal in curved root canals: A comparative study. Odontology 2017; 105:178-83.

31. Azim A, Aksel H, Zhuang T, Mashtare T, Babu P, Huang T, et al. Efficacy of 4 irrigation protocols in killing bacteria colonized in dentinal tubules examined by a novel confocal laser scanning microscope analysis. J Endod 2016; 42:928-34.

32. Bao P, Shen Y, Lin J, Haapasalo M. In vitro efficacy of XP endo finisher with 2 different protocols on biofilm removal from apical root canals. J Endod 2017; 43:321-5.

33. -Sanabria-Liviac D, Moldauer BI, Garcia-Godoy F, Antonio-Campos A, Casaretto M, Torres-Navarro J, Scalercio JM. Comparison of the XP-Endo Finisher file system and passive ultrasonic irrigation (PUI) on smear layer removal after root canal instrumentation. J Dent Oral Health. 2017; 4:1–7.

34. Spoorthy E, Velmurugan N, Ballal S, Nandini S. Comparison of irrigant penetration up to working length and into simulated lateral canals using various irrigating techniques. Int Endod J. 2013; 46:815–22.

35. Parente M, Loushine J, Susin L, 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; 43:1001–12.

36. Alves FR, Andrade-Junior CV, Marceliano-Alves MF, Pérez AR, Rôças IN, Versiani MA. Adjunctive steps for disinfection of the mandibular molar root canal system: a correlative bacteriologic, micro-computed tomography, and cryopulverization approach. J Endod. 2016; 42:1667–72.

37. Leoni GB, Versiani MA, Silva-Sousa YT, Bruniera JF, Pécora JD, Sousa-Neto MD. 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. 2017; 50:398–406

38. De Gregorio C, Estevez R: Efficacy of different irrigation and activation systems on the penetration of sodium hypochlorite into simulated lateral canals and up to working length: an in vitro study. J Endod 2010: 36, 1216–21.