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The ability of negative pressure irrigation to reduce the count of Enterococcus faecalis bacteria in minimally, prepared root canals: in vitro study

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ABSTRACT

This study aimed to evaluate the ability of negative pressure irrigation technique (NPI) compared to the positive pressure irrigation technique (PPI) in eradication of Enterococcus faecalis inside infected and minimally prepared root canals. 40 extracted premolars were decoronated, instrumented up to size #20 with a taper of 0.04 (minimal preparation), autoclaved and inoculated with Enterococcus faecalis for 15 days, divided into two equal groups according to the irrigation method used: Group 1 (Experminteal, n=20): irrigation by NPI, and Group 2 (Control, n=20): irrigation by PPI. After irrigation, microbial samples were collected, transferred to nutrient agar and incubated for counting of bacterial colony forming units (CFUs) Data were analyzed using dependent and independent sample t-test. The results showed a significant decrease in the mean number of CFUs by both NPI and PPI methods (p=0.000), and the mean number of CFUs after irrigation with NPI was lower than PPI method (p=0.043). Both irrigation methods were effective in reducing the bacterial count, but NPI shows greater effectiveness in reducing the bacterial count in the minimally prepared root canals and infected with Enterococcus faecalis.

1. Introduction

The Microorganisms and their products play a crucial role in inducing pulpal and periapical necrosis. The aim of root canal treatment is to eliminate residues, microorganisms, and their products to achieve successful and long-term treatment outcomes [1]. The canal space is filled with organic debris and tissue residues that interfere with the sealing of the root canal filling material and the internal walls of the canal [2].

The success of root canal treatment is threatened by the presence of untouched areas of the root canal walls by the mechanical preparation instruments [3], [4]. Therefore, irrigation is a critical and important part of

root canal treatment, as it ensures the cleaning and disinfection of untouched areas in the root canal through mechanical preparation instruments [4- 6].

The traditional irrigation method using a syringe with a needle is the most common method among general dentists and endodontists [7], [8]. The irrigants are delivered into the root canal system using a plastic syringe with a needle that reaches the working length (WL) at the apex of the root canal, and flows towards the root canal orifice to be aspirated by a suction tip [9].

This method is commonly known as positive pressure irrigation due to the formation of positive pressure in the coronal third of the root canal system [9]. Despite its widespread use, this method seems to be unable to achieve sufficient cleaning and disinfection in the extreme areas of the root canal system beyond the main root canal [10], [11]. Moreover, many incidents can be occurred such as extrusion beyond the apical foramen [12].

Negative pressure irrigation is an alternative method for delivering irrigants into the root canal system to reducing the risk of irrigants extrusion beyond the apical foramen [13], [14]. Irrigants are delivered using a syringe with a needle tip placed in the pulp chamber and a precise suction tip positioned near the working length at the apex, which generates the necessary negative pressure to draw the irrigants into the root canal system [14], [15].

Several in-vitro studies have compared this method to traditional positive pressure irrigation and have demonstrated the ability of negative pressure irrigation to prevent the extrusion of irrigants beyond the apical foramen [16]. However, conflicting results have been reported regarding the ability of negative pressure irrigation to achieve sufficient cleaning and disinfecting the root canal system [17- 19], which highlights the need for further studies to investigate the potential and effectiveness of negative pressure irrigation in cleaning and disinfecting the root canal system.

2. Materials and Methods

2.1 Ethical consideration and sample size determination

A comparative in vitro study was proposed to evaluate the effectiveness of NPI irrigation method compared to PPI irrigation method. The study protocol was approved by the Scientific Research and Postgraduate Board of Damascus University Ethics Committee of Damascus University, Damascus, Syria (IRB number: UDDS-188-09072020/SRC-1500).

The sample size was determined using a sample size calculation program (PS Power and Sample Size Calculation Program, version 3.0.43). The sample size calculation produced a required sample size of 40 teeth to detect a significant difference (90% power and two-sided 5% significance level).

2.2 Randomisation

Each of the studied teeth was given a number between 1 and 40. Afterward, the teeth were divided randomly through randomization.com into the following four groups: group 1 (Experminteal, n=20): irrigation by NPI, and Group 2 (Control, n=20): irrigation by PPI. Single-blinded trials were adopted in this study so that the assessors would not know which irrigation methods were used.

2.3 Inclusion criteria

The inclusion criteria were newly extracted premolars for orthodontic reasons with a single root canal

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(confirmed by periapical radiographic), a straight root canal, intact roots that were not resorbed or infected with caries, no calcifications or root fractures, teeth that were not previously treated endodontically, and apex size of no more than #20 k-file.

2.4 Sample Preparation

The study was conducted at Damascus University, Faculty of Dentistry, Department of Endodontics. First, the calculus and the attached soft tissues were removed, and the dental radiographs were taken in the buccolingual and mesiodistal directions to ensure that the root canal system was free of any abnormalities and internal resorption. Then, it was cleaned and preserved in 0.5% chloramine T for a week and then kept in saline until the start of the study).

After completing the sample collection, the crowns were cut using diamond discs and standardized to a working length of 16 mm. Subsequently, the pulp chamber was accessed using a high-speed diamond bur with a water-cooled handpiece, and the orifices of the canals were explored using DG-16 (Hu-Friedy, Chicago, IL, USA). Moreover, the pulp chamber access was refined using Endo-z (Dentsply Maillefer, Ballaigues, Switzerland).

Afterward, a glide path was established using #10 K-file (Dentsply Maillefer) and the root canals were prepared using K-files up to size #20 while irrigating with saline solution. Then, the root canals were prepared minimally using rotary ProTaper files [(up to F1 (tip size #20 and 4% taper)] (PTU; Dentsply Maillefer, Ballaigues, Switzerland) through an electric motor (VDW, Munich, Germany) at 300 rpm and 2.5 Ncm as well as the irrigation of the canal with 1 mL of 5.25% sodium hypochlorite solution between each file.

After that, the teeth were coated with two layers of nail polish, to prevent to prevent microbial leakage through the outer surface of the tooth. The apex was closed with resin composite, and the teeth were placed in in acrylic molds after sealing the orifices with cotton pellets and temporary filling. (Each tooth was given a number from 1-40).

After the hardening of acryl was completed, the temporary restoration and cotton ball were removed, and the samples were entered into autoclaved at 121°C for 15 minutes. After sterilization, seven samples were taken randomly from the total sample, and bacterial smears were taken from them to ensure the samples' sterility and that they were cultured negative.

2.5 Preparation of Bacterial Suspension and Sample Contamination

Enterococcus faecalis bacteria was isolated from infected canals, identified, and cultured until the appropriate concentration was reached. The bacterial sample was transferred to a petri dish and sent to the Phoenix Automated System (BD Diagnostic, Sparks, MD) device to confirm the identity of the bacterial sample with high accuracy. The results showed that the sample was Enterococcus faecalis with a confidence level (97%).

The bacterial suspension was prepared and the teeth were contaminated with equal amounts of the suspension (30 microliters in each canal) using a micropipette. The teeth were then incubated at 37°C and left for 15 days to allow the bacteria penetration the and formation a biofilm.

2.6 Initial enumeration of Colony-Forming Unit (CFU)

After the incubation period, the primary bacterial smear was taken from each of the canals as follows: the

canal was filled with saline, and a circumferential preparation was performed with H-file. The smear was taken using a paper point compatible with the measurement of the final preparation file, and it was left for 10 seconds and then transferred to a sterile Eppendorf containing 1 ml of saline. The smear was repeated three times to obtain an accurate bacterial count. Then the tube containing the paper points was shaken for 1 minute with a vortexed for one minute using a Biovortex device to ensure homogeneity of the solution. Three dilutions were performed to determine the appropriate countable dilution as follows:
Dilution 0: A 50 µm of the solution in the Eppendorf tube was taken by a micropipette and cultured on a pre-equipped sectioned Petri dish.

Dilution 1: A 10:1 dilution was performed to change the volume from 1.0 mL to 10 mL by adding 1.0 bacterial suspension to 9.0 mL of serum in a sterile glass tube. A total of 50 µm of the solution in the diluted glass tube was taken and cultured in a sectioned Petri dish.

Dilution 2: A 50:1 dilution was performed by adding 0.5 ml of the 10:1 solution to 9.5 ml of serum in a sterile glass tube. A total of 50 µm of the solution was taken from the diluted 100:1 glass tube and cultured in the same Petri dish.

After 48 hours, the dishes were taken out of the incubator, and the microbial units were counted using a colony counting device. This number indicates the bacterial count in the root canal.

CFU/ml = No. of colonies * dilution factor/1m

These extensions were performed to know the countable bacterial counts of each canal before applying the irrigation protocol to them where dilution No. 2 has been approved (Figure 1).

Figure 1. Initial bacterial colonies

2.7 Irrigation protocol

In NPI method: The samples in this group were irrigated for 1 minute with 10 ml of 5.25% sodium hypochlorite solution at a negative pressure (520 mmHg) in room temperature using a 30-gauge NaviTip needle attached to a syringe with a suction head. The needle was inserted into the canal 1-2 mm short of the apex, and the entire assembly was placed in the tooth chamber and sealed tightly (Endo JT device) (Figure 2). This was followed by irrigation with 5 ml of saline and 3 ml of 17% EDTA solution, before the final irrigation with 5 ml of sterile saline solution to remove any residual of hypochlorite solution from the root canal.

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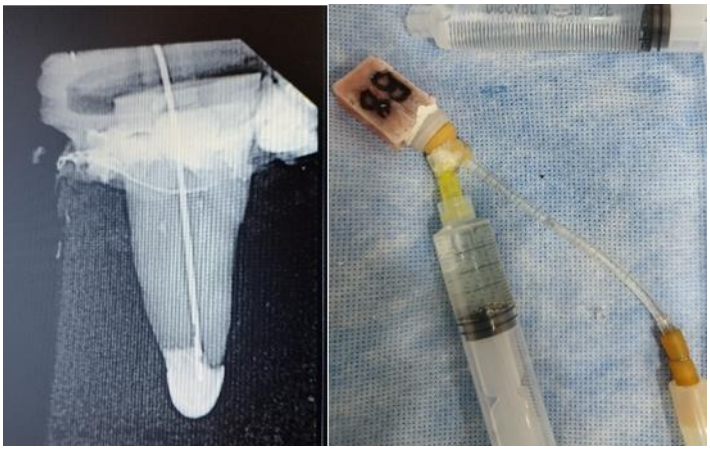

Figure 2. Endo JT NPI system

In PPI method: The samples in this group were irrigated for 1 minute with 10 ml of 5.25% sodium hypochlorite solution at a pressure (0.25 Mpa) in room temperature using a 30-gauge NaviTip needle attached to a syringe with a suction head (Endo JT device) (Figure 3). The needle was inserted into the canal 1-2 mm short of the apex. This was followed by irrigation with 5 ml of sterile saline solution and 3 ml of 17% EDTA solution, before the final irrigation with 5 ml of sterile saline solution to remove any residual of hypochlorite solution from the root canal.

Finally, the root canals were filled with saline, and a circumferential preparation was performed with H-file. The smear was then taken using a paper point compatible with the measurement of the final preparation file, and it was left for 10 seconds and then transferred to a sterile Eppendorf containing 1 ml of saline. The smear was repeated three times to obtain an accurate bacterial count. Then the tube containing the paper points was shaken for one minute with a Biovortex device to ensure homogeneity of the solution and cultured on nutrient agar dishes. The dishes were then incubated at 37°C for 48 hours. The bacterial colonies were counted using the same method as for the primary swabs

Figure 3. Endo JT PPI system

3. Results

The sample consisted of 40 extracted mandibular premolars, divided into two equal main groups (50%) according to the irrigation method (NPI and PPI irrigation methods). In addition, descriptive statistics were conducted for the decimal logarithm of the number of bacteria, which included the arithmetic mean, standard deviation (SD), maximum and minimum values for each group before and after irrigation, as shown in Table 1.

Dependent sample t-test was conducted to study the significance of the differences in the mean decimal logarithm of the number of bacteria between (before irrigation, and after irrigation) as shown in Table 1.

Table 1. Basic sample characteristics and dependent sample t-test

irrigation methods	Studied stage	N	Mean	SD	Min	Max	Differences between two means	t-value	P-value
NPI	Before Irrigation	20	4.97	0.12	4.77	5.17	-1.88	-49.296	**0.000**
	After Irrigation	20	3.09	0.11	2.85	3.20			
PPI	Before Irrigation	20	5.04	0.21	4.49	5.33	-1.72	-19.040	**0.000**
	After Irrigation	20	3.32	0.37	2.70	4.18			

The mean decimal logarithm of CFUs units after irrigation were smaller than and before irrigation, in each of NPI group (p=0.000) and PPI group (p=0.000). Independent sample t-test was conducted to pairwise comparison the mean decimal logarithm of the number of bacteria between (NPI and PPI groups) as shown in Table 2.

Table 2. independent sample t-test

Studied stage	irrigation methods	N	Mean	SD	Min	Max	Differences between two means	t-value	P-value
Before Irrigation	NPI	20	4.97	0.12	4.77	5.17	-0.16	-1.666	0.104
	PPI	20	5.04	0.21	4.49	5.33			
After Irrigation	NPI	20	3.09	0.11	2.85	3.20	-3.79	-2.095	**0.043**
	PPI	20	3.32	0.37	2.70	4.18			

Table 2 shows that no statistically significant differences in decimal logarithm of CFUs units before irrigation between NPI and PPI groups (p=0.104), and a statistically significant differences were found in decimal logarithm of CFUs units after irrigation between NPI and PPI groups (p=0.043) as CFUs units in NPI were smaller than PPI group.

4. Discussion

The gram-positive bacteria Enterococcus faecalis are cocci that are resistant to harsh environments and are commonly found in the human gut [20]. These bacteria have been implicated in the development of endodontic infections [21], [22], so that understanding the microbiology of these organisms is very important for successful treatment procedures [23]. Additionally, studies have focused on the use of antimicrobial agents and disinfection methods for the elimination of these bacteria from root canals system [24], [25].

E. faecalis is one of the most resistant and persistent bacterial species in the oral microbiome, making it difficult to eradicate from infected tissues, especially in the presence of the biofilm matrix [26]. Moreover, several studies have investigated the potential of certain plant extracts and natural compounds for the management of E. faecalis and other bacteria in endodontic infections [23]. Therefore, it has been suggested that the characteristics of the gram-positive E. faecalis should be considered when developing effective treatment strategies.

Considering that the anatomy of the root canals can affect the outcome of the bacterial count, lower premolars were used to standardize the samples, as the study sample consisted of 40 closed-apex lower premolars with single root canal which was confirmed by periapical radiographs [27].

The lengths of the teeth were standardized in the studied sample to fix the length of the surface being

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prepared, thereby ensuring greater uniformity in the study sample. Additionally, the crowns were cut to achieve this goal, thereby obtaining a fixed reference point for the working length [28].

The apical foramen of teeth was closed with composite to transform the inner canal space into a closed system to simulate clinical conditions, as the root canal in the presence of the surrounding tissues tends to be a closed-ended system [29].

Irrigation protocol was standardized in terms of irrigation time and volume because they are important parameters that can influence the antibacterial capacity [30]. Closed-ended NaviTip irrigation tips were used with double lateral openings (30 gauge) to better contact between the surface of the irrigation solution and the dentin, in addition to improve the dynamic movement of the irrigation solution [18].

The results of this study revealed that there were no statistically significant differences in bacterial count before the application of the two irrigation methods. The results also showed a clear decrease in the bacterial count in both the negative and positive pressure irrigation groups after irrigation, which explains the effectiveness of both methods in reducing the bacterial count.

The mean bacterial CFUs count after negative pressure irrigation was lower compared to positive pressure irrigation, negative pressure irrigation showed greater effectiveness in reducing the bacterial count which confirm its superiority.

The results of this study were consistent with [31] study, which showed the superiority of negative pressure irrigation using the EndoVac system in reducing the bacterial count of Enterococcus faecalis compared to traditional positive pressure irrigation. This study also agreed with in-vitro study conducted by [32], which showed that the EndoVac system was more effective than positive pressure irrigation in eliminating Enterococcus faecalis.

However, this study differed with [33] study, which showed no significant difference between the negative and positive pressure irrigation in reducing Enterococcus faecalis, when using 2.5% sodium hypochlorite as irrigants. This study also differed with the clinical study conducted by [34], which showed no significant difference between negative and positive pressure irrigation in reducing Enterococcus faecalis when using 0.5% sodium hypochlorite as irrigants. These differences can be attributed to use lower concentrations of sodium hypochlorite in the previous studies compared to the concentration used in this study, which was 5.25%.

There was a large variation in the protocols used for positive pressure irrigation (traditional method), which may be attributed to the absence of a standardized or recommended protocol for positive pressure irrigation., while the protocols used for negative pressure irrigation were more standardized because of manufacturer recommendations, and this is the major limitation of this study.

5. Conclusion

Within the limitations of this study, it can be concluded that both positive pressure and negative pressure irrigation techniques are effective in reducing Enterococcus faecalis count within the infected root canals. Negative pressure irrigation method showed a promising result in reducing the bacterial count in minimally prepared root canals

6. References

[1] Siqueira Jr, J.F., et al., Clinical outcome of the endodontic treatment of teeth with apical periodontitis using an antimicrobial protocol. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 2008. 106(5): p. 757-762.

[2] Ardila, C., M.K. Wu, and P. Wesselink, Percentage of filled canal area in mandibular molars after conventional root-canal instrumentation and after a noninstrumentation technique (NIT). International Endodontic Journal, 2003. 36(9): p. 591-598.

[3] Peters, O.A., Current challenges and concepts in the preparation of root canal systems: a review. Journal of endodontics, 2004. 30(8): p. 559-567.

[4] Peters, O.A., K. Schönenberger, and A. Laib, Effects of four Ni–Ti preparation techniques on root canal geometry assessed by micro computed tomography. International endodontic journal, 2001. 34(3): p. 221-230.

[5] Gulabivala, K., et al., Effects of mechanical and chemical procedures on root canal surfaces. Endodontic topics, 2005. 10(1): p. 103-122.

[6] Paqué, F., et al., Preparation of oval-shaped root canals in mandibular molars using nickel-titanium rotary instruments: a micro-computed tomography study. Journal of endodontics, 2010. 36(4): p. 703-707.

[7] Dutner, J., P. Mines, and A. Anderson, Irrigation trends among American Association of Endodontists members: a web-based survey. Journal of endodontics, 2012. 38(1): p. 37-40.

[8] Willershausen, I., et al., Survey of root canal irrigating solutions used in dental practices within Germany. International Endodontic Journal, 2015. 48(7): p. 654-660.

[9] Boutsioukis, C. and L.W. van der Sluis, Syringe irrigation: blending endodontics and fluid dynamics. Endodontic irrigation: chemical disinfection of the root canal system, 2015: p. 45-64.

[10] Paqué, F., C. Boessler, and M. Zehnder, Accumulated hard tissue debris levels in mesial roots of mandibular molars after sequential irrigation steps. International endodontic journal, 2011. 44(2): p. 148-153.

[11] Jiang, L.-M., et al., Comparison of the cleaning efficacy of different final irrigation techniques. Journal of endodontics, 2012. 38(6): p. 838-841.

[12] HÜLSMANN, M., T. RÖDIG, and S. Nordmeyer, Complications during root canal irrigation. Endodontic topics, 2007. 16(1): p. 27-63.

[13] Gutmann, J.L., M. Zehnder, and V.M. Levermann, Historical perspectives on the roots of the apical negative pressure irrigation technique in endodontics. J Hist Dent, 2014. 62(1): p. 32-40.

[14] Nielsen, B.A. and J.C. Baumgartner, Comparison of the EndoVac system to needle irrigation of root canals. Journal of endodontics, 2007. 33(5): p. 611-615.

ISSN: 0005-2523
Volume 63, Issue 08, August, 2023

[15] Adorno, C., et al., Comparison of two negative pressure systems and syringe irrigation for root canal irrigation: an ex vivo study. International endodontic journal, 2016. 49(2): p. 174-183.

[16] Boutsioukis, C., Z. Psimma, and L. Van der Sluis, Factors affecting irrigant extrusion during root canal irrigation: a systematic review. International endodontic journal, 2013. 46(7): p. 599-618.

[17] Hockett, J.L., et al., Antimicrobial efficacy of two irrigation techniques in tapered and nontapered canal preparations: an in vitro study. Journal of Endodontics, 2008. 34(11): p. 1374-1377.

[18] Brito, P.R., et al., Comparison of the effectiveness of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: an in vitro study. Journal of endodontics, 2009. 35(10): p. 1422-1427.

[19] Versiani, M.A., et al., Micro-CT evaluation of the efficacy of hard-tissue removal from the root canal and isthmus area by positive and negative pressure irrigation systems. International endodontic journal, 2016. 49(11): p. 1079-1087.

[20] Siqueira Jr, J.F., et al., Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. Journal of Endodontics, 1999. 25(5): p. 332-335.

[21] Peters, L., P. Wesselink, and W. Moorer, Penetration of bacteria in bovine root dentine in vitro. International Endodontic Journal, 2000. 33(1): p. 28-36.

[22] Dametto, F.R., et al., In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against Enterococcus faecalis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 2005. 99(6): p. 768-772.

[23] George, S., A. Kishen, and P. Song, The role of environmental changes on monospecies biofilm formation on root canal wall by Enterococcus faecalis. Journal of endodontics, 2005. 31(12): p. 867-872.

[24] Pinheiro, E., et al., Microorganisms from canals of root-filled teeth with periapical lesions. International endodontic journal, 2003. 36(1): p. 1-11.

[25] Stuart, C.H., et al., Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics, 2006. 32(2): p. 93-98.

[26] Siqueira Jr, J.F., et al., Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. Journal of endodontics, 2002. 28(3): p. 181-184.

[27] Brunson, M., et al., Effect of apical preparation size and preparation taper on irrigant volume delivered by using negative pressure irrigation system. Journal of endodontics, 2010. 36(4): p. 721-724.

[28] Sirtes, G., et al., The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. Journal of Endodontics, 2005. 31(9): p. 669-671.

[29] Jamleh, A., H. Suda, and C.G. Adorno, Irrigation effectiveness of continuous ultrasonic irrigation system: An ex vivo study. Dental materials journal, 2018. 37(1): p. 1-5.

[30] Ulusoy, Ö., et al., The effectiveness of various irrigation protocols on organic tissue removal from simulated internal resorption defects. International endodontic journal, 2018. 51(9): p. 1030-1036.

[31] Thulaseedharan, S., et al., In vitro comparative evaluation of effectiveness of sodium hypochlorite with conventional irrigation method versus EndoVac and ultrasonic irrigation in the elimination of Enterococcus faecalis from root canals. Journal of Pharmacy & Bioallied Sciences, 2020. 12(Suppl 1): p. S105.

[32] Kumar, T., et al., An in vitro comparison of the antimicrobial efficacy of positive pressure and negative pressure irrigation techniques in root canals infected with Enterococcus faecalis. Journal of conservative dentistry: JCD, 2018. 21(4): p. 438.

[33] de Alencar, A.H.G. and R. de Araújo Estrela, Antibacterial potential of 2.5% sodium hypochlorite in distinct irrigation protocols on Enterococcus faecalis biofilm. The Journal of Contemporary Dental Practice, 2015. 16(5): p. 340-346.

[34] Pawar, R., et al., Influence of an apical negative pressure irrigation system on bacterial elimination during endodontic therapy: a prospective randomized clinical study. Journal of endodontics, 2012. 38(9): p. 1177-1181.