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Research Article

Evaluation of The Interaction Between Alternative Endodontic Irrigation Solutions

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Abstract

Statement of the problem: For successful endodontic treatment, mechanical cleaning and chemical irrigation can be used together. Since there is not yet a single solution sufficient for both root canal cleaning alone, combined use of irrigants is recommended for the synergistic effect. Polyhexanide (PHMB), boric acid, phytic acid, peracetic acid, and hypochlorous acid are relatively new alternative endodontic irrigation solutions and there are no studies in the literature on the combined use of alternative solutions.

Objective: This study aimed to evaluate the interactions that would result from applying PHMB as a substitute for CHX following alternative intermediate irrigation solutions that might be utili-zed with NaOCl.

Materials & Methods: Eight microtubes were used for this study. 0.5ml of 5%NaOCl was placed in each microtube first, the microtubes were divided into two groups as the final product CHX and Polyhexanide, and the two groups were divided into four groups with different irrigants in themselves. The study was conducted at room temperature and the images of the mixtures were photographed at 1 min, 10 min, 20 min, 30 min, and 1 hour. Groups; G1: NaOCl+ 1%Phytic acid +0.1%Polyhexanide, G2: NaOCl+ 0.02%Hypochlorous acid +0.1%Polyhexanide, G3: NaOCl+ 2% Peracetic acid +0.1%Polyhexanide, G4: NaOCl+ 5% Boric acid +0.1%Polyhexanide, G5: NaOCl+ Phytic acid+ 2%CHX, G6: NaOCl+ 0.02%Hypochlorous acid +2%CHX, G7: NaOCl+ 2% Peracetic acid+2%CHX, G8: NaOCl+ 5% Boric acid + 2%CHX.

Results: White or brown precipitates occurred as soon as the solutions were applied to all of the CHX groups; milky-white precipitate was only seen in the Polyhexanide group's Phytic acid-added group. After 5 minutes, air bubbles were seen in the Polyhexanide group that had Peracetic acid added, whereas the CHX group had an increase in brown precipitates.

Conclusions: Considering the current research conditions, In clinical practice, the administration of Polyhexanite as the last irrigant and the combination of NaOCl with boric acid or hypochlorous acid can be advised.

Keywords: Endodontic Irrigant, Polyhexamethylene Biguanide, Brown-Orange Precipitate, Peracetic Acid, Hypochlorous Acid

Introduction

Bacteria and their metabolites are the primary etiological factors for pulp and periapical disorders, by invading the root canal System.[1] Because of this, root canal therapy aims to thoroughly eradicate germs and stop re-infection in the affected root canal system and periapical tissues.[2] Mechanical instrumentation and chemical irrigation can be used in combination to achieve this. Mechanical instrumentation during root canal treatment significantly reduces the number of microorganisms but remains inadequate due to the anatomical complexity of the root canal system and the presence of lateral canals.[3,4] Therefore, in addition to mechanical cleaning, a variety of irrigation solutions must be employed to remove bacteria and their products, tissue remnants, and smear layer from root canals and dentinal tubules.[5]

Because of its antibacterial[6] and tissue-dissolving capabilities[7], sodium hypochlorite (NaOCl) is the most frequently used irrigant during endodontic therapy. The most common concentration of NaOCl, 2.5%, was discovered in the endodontic literature, along with others ranging from 0.5% to 5.25 percent. However, although the antimicrobial activity is enhanced at high concentrations of NaOCl, at these doses it is cytotoxic[8] and may cause periapical tissue irritation.[9] After chemo-mechanical activities using NaOCl as the irrigant, studies have shown that 40-60% of root canals still contain detectable quantities of cultivable bacteria.[10]

Another agent frequently used in endodontics is chlorhexidine digluconate (CHX); at low doses, it has a bacteriostatic action, and at high quantities, a bactericidal effect[11], is a broad-spectrum antimicrobial agent[12], and can remain on the root dentin surface exposed to CHX for a long time and its antimicrobial effect can continue, less toxic than NaOCl. However, it cannot fully replace NaOCl due to its lack of tissue dissolving feature.

There is not a single chemical agent that can remove the smear layer with its tissue-dissolving effect, disinfect the root canal system and show antimicrobial properties for a long time. Therefore, the combined use of irrigants is recommended. Different irrigants are utilized in combination to increase irrigation effectiveness because of their beneficial synergistic effects on antibacterial characteristics.[12,13] Because CHX continues to have an antibacterial impact on root canal walls, it can be utilized as a final irrigant.[13]

Acid-base reaction results in the formation of an orange-brown precipitate when used in combination with chlorhexidine and NaOCl.[14] Some investigations have indicated that this brown precipitate contains para-chloroaniline[15,16], whereas other studies have indicated that it

does not.[17] Also, it is difficult to remove this brown precipitate because it plugs the dentinal tubules and lateral canals. In addition to potentially discoloring teeth, it can also compromise the root canal filling's ability to seal.[18] Intermediary irrigant substances have been recommended to inactivate NaOCl residues before applying CHX in order to avoid the occurrence of the brown-colored precipitate.[19] Given these issues, there is still a need to explore new irrigation solutions and techniques.

The biocide, Polyhexamethylene biguanide or Polyhexanide (PHMB), which belongs to the bisbiguanide family, has a wide range of applications as a disinfectant for surfaces, objects, and instruments. Additionally, it is utilized in mouthwash formulations[20], soft lens care solutions[21], and wound therapies.[22] It is effective against Gram-positive (S.epidermidis, S.aureus, and E.faecalis) and Gram-negative (E.coli) bacteria. According to studies, PHMB promotes faster wound healing and has a wide antibacterial spectrum, a low risk of contact hypersensitivity, and good tissue and cell tolerance.[22] PHMB has recently been on the agents as an alternative solution to CHX.[23] PHMB, boric acid, phytic acid, peracetic acid, and hypochlorous acid are relatively new alternative endodontic irrigation solutions and there are no studies in the literature on the combined use of alternative solutions. This study aimed to evaluate the interactions that would result from applying PHMB as a substitute for CHX following alternative intermediate irrigation solutions that might be utilized with NaOCl.

Materials & Methods

Solutions

Different combinations of irrigation solutions were used in this study. 2% CHX (Cerkamed, Stalowa Wola, Poland) solution and its alternative irrigant 0.1% Polyhexamethylene Biguanide (PHMB; Actolind® w Solution, ACTO Pharma, Germany) were combined with different solutions as follows: 5 % NaOCl (Acquafarma; Niteroi, RJ, Brazil), 1% Phytic Acid (Sigma Aldrich, USA), 0.02% (200 ppm) hypochlorous acid (HOCl; Crystalin, Natural Health Products-NHP, Turkiye), 2% Peracetic acid (PAA; Sigma Aldrich, USA), 5% Boric acid (Sigma Aldrich, Darmstadt, Germany).

Mixture of solutions

Eight flat-top 2.0 ml polypropylene microtubes were used in this study. All eight microtubes contained 0.5 ml of 5% NaOCl as the initial irrigation solution. Then, the microtubes

were divided into four main groups (n=2), and 0.5 ml of different irrigants (1% Phytic acid, 0.02% HOCl, 2% Peracetic acid, and 5% Boric acid) were added. No reaction was observed in all groups. After 5 minutes, the main groups were divided into two groups as the final products, CHX and Polyhexanide. The scheme of the working groups is shown in Table 1. The mixing process was carried out at room temperature and the color change was visually inspected. The images of the mixtures were photographed at the 1st minute, 10th minute, 20th minute, 30th minute, and 1st hour.

Results

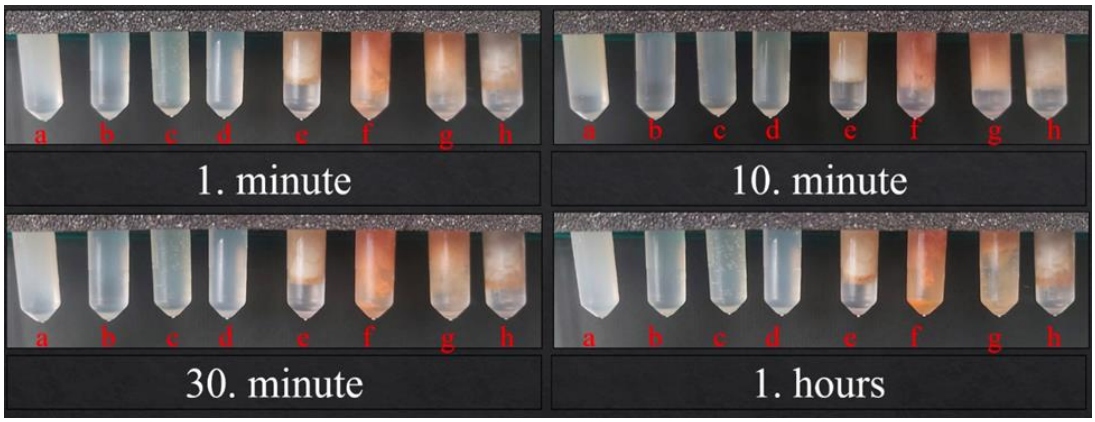
The colored precipitate that resulted from the combination of solutions is presented in Table 1.

Table 1. Irrigant solution associations and their products' visual characteristics.

Solution 1	Solution 2	Final Solution	Appearance
NaOCl	1% Phytic acid	**0.1%Polyhexanide**	Milky-white precipitate
NaOCl	0.02 %Hypochlorous acid	**0.1%Polyhexanide**	No color change
NaOCl	2% Peracetic acid	**0.1%Polyhexanide**	No color change
NaOCl	5% Boric acid	**0.1%Polyhexanide**	No color change
NaOCl	1% Phytic acid	**2%CHX**	Brown-orange precipitate
NaOCl	0.02 %Hypochlorous acid	**2%CHX**	Brown-orange precipitate
NaOCl	2% Peracetic acid	**2%CHX**	Brown-orange precipitate
NaOCl	5% Boric acid	**2%CHX**	Brown-orange precipitate

The color change after interaction between solutions is shown in Figure 1. In all of the CHX groups, white or brown precipitates started to form rapidly after the solutions were added, as seen in Figure 1-e, f, g, h. The milky-white precipitate observed was only noticed in the group that received Phytic acid (Figure 1-a) in the PHMB group. Air bubbles were seen in the group with peracetic acid added in the PHMB group after 5 minutes, while the CHX group showed an increase in brown precipitates.

Figure 1: a; NaOCl+Phytic acid+PHBM b; NaOCl+HOCl+PHBM c; NaOCl+PAA+PHBM d; NaOCl+Boric acid+PHBM, e; NaOCl+Phytic acid+CHX f; NaOCl+HOCl+CHX g; NaOCl+PAA+CHX h; NaOCl+Boric acid+CHX.



Discussion

It is well known that NaOCl and CHX, which are most commonly used clinically as endodontic solutions, interact to form an insoluble and toxic brown precipitate. It is important to investigate the brown precipitate formation prevention protocol. It has been suggested to use intermediate irrigants to neutralize NaOCl and prevent brown precipitate formation.[24-26] However, despite the use of an intermediate irrigant, a brown precipitate may still form in the root canals.[27] Therefore, this study, it was aimed to examine the interactions of the use of four different intermediate irrigants before CHX with NaOCl and the use of CHX as the final product or the use of PHMB as an alternative.

One of the gels used as an intermediate irrigant is Peracetic acid. Peracetic acid (PAA) is a powerful disinfectant with a broad antimicrobial spectrum, even at low concentrations. EDTA and Citric Acid, which act as decalcifying agents, do not have antimicrobial properties. The use of PA instead of EDTA has recently attracted attention due to its potential to improve the disinfection of root canals.[28,29] According to Cord et al[28], irrigations using PAA and 17% EDTA in combination with 2.5% sodium hypochlorite had equal bactericidal effects. Another benefit of PAA is that it has low cytotoxicity and is unaffected by the presence of organic debris, according to Teixeira et al.[30,31]

Guerreiro-Tanomaru et al[32] showed that 1% PAA reduced the E.faecalis count to 86% after 3 minutes and eliminated it after 10 minutes, while 2% CHX and 2.5% NaOCl acted within 30 seconds. The cytotoxic effect of endodontic irrigation solutions is being studied more and more.[33,34] In a study comparing 2% PAA, NaOCl 5.25%, and CHX 2%, on cell viability, it was reported that PAA showed higher viability.[35] similarly, another study reported that 1% PAA showed less cytotoxic effect than 2.5% NaOCl and 17% EDTA.[29] PAA has the advantages of

being less cytotoxic and the capacity to clear the smear layer. In addition, it is not recommended to be used as a final irrigant, as it will not improve disinfection due to its low antibacterial effect on E.faecalis compared to NaOCl and CHX.[35] In this study, air bubbles were observed when the final irrigant was polyhexanidine in the group using PAA as the intermediate irrigant, while there was an increase in brown precipitate when the final irrigant was CHX.

It has been reported that Hypochlorous Acid (HOCl) is effective on the smear layer and effectively cleans the root canal walls, so it has been suggested as an alternative irrigation solution to NaOCl.[36] At the same time, a study investigating the interaction of HOCl with dental materials found that HOCl increased the bond strength between dentin and the self-adhesive resin cement.[37] HOCl is a biocompatible substance with antibacterial action that the immune system of humans naturally produces to fight against infection[38] and has been shown to have substantially lower toxicity than NaOCl while still exhibiting equal antibacterial efficacy against E.faecalis.[39] In this study, no color change was observed when HOCl was used as the intermediate irrigant in the group where PHMB was the final solution. For this reason, its use with NaOCl may be effective to achieve a synergistic effect.

A promising natural organic substance that is isolated from rice bran is phytic acid. It may effectively chelate multivalent cations like calcium (Ca+2), magnesium, and iron due to its numerous negative charges. In addition, the low probability of phytic acid also has an antibiotic effect against E.faecalis. According to Nassar et al[31], it is an alternative to EDTA because of its effectiveness in removing the smear layer and biocompatibility to periapical tissues.

By controlling the tissue's oxidant-antioxidant balance, the ubiquitous mineral known as boric acid and borate exert anti-inflammatory actions.[40,41] Boron applied topically is beneficial in treating periodontal disease by Luan et al.[42] Additionally, boric acid has strong antibacterial properties.[43] Turk et al[27] suggested that boric acid is insufficient to remove the smear layer, but combined use to benefit from its antibacterial effect. We also used this solution as an intermediate irrigant after NaOCl in our study. Boric acid did not prevent the brown precipitate formation in the group using CHX as the pre-product, but no color change was observed in the group using Polyhexanide instead of CHX.

Recently, the use of PHMB in dentistry is particularly interesting. Santos et al performed a systemic review on PHMB and CHX and reported that PHMB can be used as an alternative

to CHX.[23] Mikic et al[44] evaluated PHMB on E.faecalis, C.albicans, and S.epidermidis and reported that PHMB was more effective than NaOCl and CHX in reducing the number of microorganisms.

Han et al. evaluated the effect of different irrigants on intraradicular mature polymicrobial biofilm and reported that PHBM and CHX had similar antibiofilm effects, but that PHBM had a more permanent antibiofilm effect than CHX.[45] Similar results were reached by Chandki et al[46] that compared the substantivity of 0.2% PHMB and 2% CHX on root canal dentin at 1 h, 24 h, 7 days, and 21 days.

In this study, PHBM interacted only with Phytic acid in the groups used as the final product, did not interact with the other three irrigants, and no color change was observed. There are still limited studies on the use of this substance as an irrigant. There is a lack of information about the combination of PHMB and CHX with phytic acid, boric acid, peracetic acid, and hypochlorous acid. Therefore this study is unique in its area to the best of our knowledge.

Conclusion

Within the limits of this study, the use of NaOCl together with boric acid or hypochlorous acid in clinical practice and the application of Polyhexanite as the final irrigant can be recommended.

References

1.Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.

2.Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-28.

3.Peters OA, Laib A, Göhring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod 2001;27:1-6.

4. Ahmed HM, Versiani MA, De-Deus G, Dummer PM. A new system for classifying root and root canal morphology. Int Endod J 2017;50:761-70.

5.Siqueira JF, Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-301.

6.Clarkson RM, Kidd B, Evans GE, Moule AJ. The effect of surfac- tant on the dissolution of porcine pulpal tissue by sodium hypochlorite solutions. J Endod 2012;38:1257-60.

7.Sassone LM, Fidel RAS, Murad CF, Fidel ST, et al. Antimicrobial activity of sodium hypochlorite and chlorhexidine by two different tests. Aust Endod J 2008;34:19-24.

8.Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. J Endod 1985;11:525-28.

9.Gatot A, Arbelle J, Leiberman A, Yanai-Inbar I. Effects of sodium hypochlorite on soft tissues after its inadvertent injection beyond the root apex. J Endod 1991;17:573-74.

10.Rocas IN, Siqueira JF. In vivo antimicrobial effects of endodontic treatment procedures as assesed by molecular microbiological techniques. J Endod 2011;37:304-10.

11.Vineet SA, Rajesh M, Sonali K, Mukesh PA. Contemporary overview of endodontic irrigants – A review. J Dent App 2014;1:105-15.

12.Ohara P, Torabinejad M, Kettering JD. Antibacterial effects of various endodontic irrigants on selected anaerobic bacteria. Endod Dent Traumatol 1993;9:95-100.

13.Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod 1998;24:472-76.

14.Zehnder M. Root canal irrigants. J Endod 2006;32:389-98.

15.Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod 2008;34:181-85.

16.Basrani BR, Manek S, Sodhi RN, Fillery E, et al. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod 2007;33:966-69.

17.Thomas JE, Sem DS. An in vitro spectroscopic analysis to determine whether para- chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine. J Endod 2010;36:315-17.

18.Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod 2008;34:181-85.

19.Akisue E, Tomita VS, Gavini G, JAP de Figueiredo. Effect of the combination of sodium hypochlorite and chlorhexidine on dentinal permeability and scanning electron microscopy precipitate observation. J Endod 2010;36:847-50.

20.Rosin M, Welk A, Kocher T, Majic-Todt A, et al. The effect of a polyhexamethylene biguanide mouthrinse compared to an essential oil rinse and a chlorhexidine rinse on bacterial counts and 4-day plaque regrowth. J Clin Periodontol 2002;29:392-99.

21.Elzinga G, van Doorn J, Wiersema AM, Klicks RJ, et al. Clinical evaluation of a PHMB-impregnated biocellulose dressing on paediatric lacerations. J Wound Care 2011;20:280-84.

22.Guimarães NLSL, Otoch HM, de Andrade LC, Ferreira CM, et al. Microbiological evaluation of infected root canals and their correlation with pain. Sul-Brasileira de Odontologia 2012;9:31-7.

23.Santos DS, Peralta-Mamani M, Brandão, FS, Andrade FB, et al. Could polyhexanide and chlorine dioxide be used as an alternative to chlorhexidine? A systematic review. Sao Paulo Med J 2021;140:42-55.

24.Chhabra N, Gangaramani S, Singbal KP, Desai K, et al. Efficacy of various solutions in preventing orange-brown precipitate formed during alternate use of sodium hypochlorite and chlorhexidine: An in vitro study. J Conserv Dent 2018;21:428-32.

25.Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine. J Endod 2010;36:1154-7.

26.Mortenson D, Sadilek M, Flake NM, Paranjpe A, et al. The effect of using an alternative irrigant between sodium hypochlorite and chlorhexidine to prevent the formation of para-chloroaniline within the root canal system. Int Endod J 2012;45:878-82.

27.Turk T, Kaval ME, Şen BH. Evaluation of the smear layer removal and erosive capacity of EDTA, boric acid, citric acid and desy clean solutions: an in vitro study. BMC Oral Health 2015;15:104.

28.Cord CB, Velasco RVC, Lima LFRM, Rocha DGP, et al. Effective analysis of the use of peracetic acid after instrumentation of root canals contaminated with Enterococcus faecalis. J Endod 2014;40:1145-8.

29.Hartmann RC, Neuvald L, Barth V, de Figueiredo JAP, et al. Antimicrobial efficacy of 0 5% peracetic acid and EDTA with passive ultrasonic or manual agitation in an Enterococcus faecalis biofilm model. Aust Endod J 2018;45:57-63.

30.Teixeira PA, Coelho MS, Kato AS, Fontana CE, et al. Cytotoxicity assessment of 1% peracetic acid, 2 5% sodium hypochlorite and 17% EDTA on FG11 and FG15 human fibroblasts. Acta Odontol Latinoam 2018;31:11-5.

31.Nassar M, Hiraishi N, Tamura Y, Otsuki M, et al. Phytic acid: an alternative root canal chelating agent. J Endod 2015;41:242-7.

32.Guerreiro-Tanomaru JM, Morgental RD, Faria-Junior NB, Berbert FL, et al. Antibacterial effectiveness of peracetic acid and conventional endodontic irrigants. Braz Dent J 2011;22:285-7.

33.Scott II MB, Zilinski GS, Kirkpatrick TC, Himel VT, et al. The effects of irrigants on the survival of human stem cells of the apical papilla, including endocyn. J Endod 2018;44:263-8.

34.Keine KC, Kuga MC, Tormin FBC, Venção AC, et al. Effect of peracetic acid used as single irrigant on the smear layer, adhesion, and penetrability of AH Plus. Braz Oral Res 2019;33:e057.

35.Brandão-Neto DO, Mello JVZ, Marceliano-Alves MFV, Coutinho TMC, et al. Final endodontic irrigation with 2% Peracetic acid: Antimicrobial activity and cytotoxicity. Eur J Dent 2021;15:533-8.

36.Solovyeva AM, Dummer PMH. Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study. Inter Endod J 2000;33:494-504.

37.Totaro M, Badalucco F, Papini F, Grassi N, et al. Effectiveness of a water disinfection method based on osmosis and chlorine dioxide for the prevention of microbial contamination in dental practices. Int J Environ Res Public Health 2022;19:10562.

38.Zoni R, Zanelli R, Riboldi E, Bigliardi L, et al. Investigation on virucidal activity of chlorine dioxide. experimental data on feline calicivirus, HAV and Coxsackie B5. J Prev Med Hyg 2007;48:91-5.

39.Travis BJ, Elste J, Gao F, Joo BY, et al. Significance of chlorine-dioxide-based oral rinses in preventing SARS-CoV-2 cell entry. Oral Dis 2022;28:2481-91.

40.Demirer S, Kara Mİ, Erciyas K, Özdemir H, et al. Effects of boric acid on experimental periodontitis and alveolar bone loss in rats. Arch Oral Biol 2012;57:60-5.

41.Ince S, Kucukkurt I, Cigerci IH, Fidan AF, et al. The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol 2010;24:161-4.

42.Luan Q, Desta T, Chehab L, Sanders VJ, et al. Inhibition of experimental periodontitis by a topical boron-based antimicrobial. J Dent Res 2008;87:148-52.

43.Schmidt M, Schaumberg JZ, Steen CM, Boyer MP. Boric acid disturbs cell wall synthesis in Saccharomyces cerevisiae. Int J Microbiol 2010;2010:930465.

44.Mikić IM, Cigić L, Kero D, Govorko DK, et al. Antimicrobial effectiveness of polyhexamethylene biguanide on Enterococcus faecalis, Staphylococcus epidermidis and Candida albicans. Med Glas (Zenica) 2018;15:132-8.

45.Khan A, Nikhil V, Pandey A, Chaturvedi P. Effectiveness of polyhexamethylene biguanide, chlorhexidine, and calcium hydroxide intracanal medicament against intraradicular mature polymicrobial biofilm: A microbiological study. J Conserv Dent 2022;25:536-40.

46.Chandki R, Nikhil V, Kalyan SS. Comparative evaluation of substantivity of two biguanides 0.2% polyhexanide and 2% chlorhexidine on human dentin. J Conserv Dent 2020;23:46–50.