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Antibacterial Efficacy of Super-Oxidized Water on *Enterococcus faecalis* Biofilms in Root Canal

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Abstract

Background: The success of endodontic treatment depends on a few crucial factors. One of these factors is the complete chemomechanic preparation of root canal against various bacteria. In particular, the effect of resistant bacteria may cause intense pain with flare-up and formation of periapical lesions. Therefore, the strong effect of irrigants plays an important role in terms of the complete elimination of these bacteria to achieve long-term successful treatment.
Objectives: The aim of this study was to investigate the antibacterial effects of super-oxidized water (SPO) in root canals infected with *Enterococcus faecalis* biofilms.
Methods: One hundred twenty single-root, premolar teeth were selected. Initially, the teeth were prepared and then disinfected. *E. faecalis* were inoculated and kept at 37°C for 24 hours in the root canals. The re-inoculation procedure was repeated on the first, fourth, seventh, and tenth days. The infected root canals were divided into one negative (saline) and one positive (sodium hypochlorite) control group and four experimental groups (super-oxidized water: 1, 2, 3, or 5 minutes) (n = 20). Paper points were placed in the root canals to control and evaluate the biofilm formation. Biofilms were counted on blood agar plates, and data was evaluated and statistically analyzed using one-way ANOVA and Tukey's test.
Results: Although sodium hypochlorite (NaOCl) showed no statistically significant difference when compared with three and five minutes of SPO irrigation ($P > 0.05$), NaOCl showed statistically significant differences among all other groups ($P < 0.05$).
Conclusions: Super-oxidized water indicated a remarkable and similar bactericidal effect to that of traditional NaOCl against *E. faecalis* biofilms. In terms of successful endodontic treatment approaches, super-oxidized water may be used as an effective irrigation solution in clinics.

Keywords: Super-Oxidized Water, Disinfection, Biofilm, Bacteria

1. Background

In endodontic literature, the importance of chemomechanic preparation has long been emphasized. In particular, the irrigation procedure has been a crucial step in endodontic treatments. More recently, various irrigants have been produced. Sodium hypochlorite is the most commonly used irrigant for non-surgical endodontic procedures, despite its limitations. Its allergenic potential, corrosive potential, and toxicity to periapical tissues are the main problems associated with sodium hypochlorite (1, 2).

Enterococcus faecalis is often isolated from infected teeth presenting with persistent disease (3). *Enterococci* are the leading resistant bacteria in different endodontic treatments (4, 5). This type of bacteria can penetrate into dentinal tubules (6), resist against high pH values (7), exhibit virulence factors (8), and form biofilms. A biofilm may be created when in vivo conditions are simulated (9). Moreover, biofilms can show strong antimicrobial tolerance through four mechanisms: the physical barrier properties of the extracellular polysaccharide matrix (10), slow growth of bacterial cells residing within a biofilm (11), deep locations of cells that stay alive under decreased oxygen tension (12), and highly resistant phenotypic states (13).

Electrochemically activated (ECA) water is one of the developed alternatives to. It shows potential as an efficient root canal irrigant. In the past 25 years, super-oxidized solutions have been shown to be potent antimicrobial agents and disinfectants through oxidative damage (14). Electrolyzed water contains a mixture of inorganic oxidants, such as hypochlorous acid (HClO), hypochlorous acidic ion (ClO^-), chlorine (Cl_2), hydroxide (OH), and ozone (O_3), which are effective for inactivating a variety of microorganisms responsible for endodontic infections (15). These

oxidants may provide bactericidal and sporicidal properties to the solutions. Moreover, they have been used safely for human tissues (16). Anolyte solution has been termed super-oxidized water (SPO) (17). The authors also noted that ECA resulted in more numerous open dentin tubules throughout the whole length of canals according to NaOCl.

Recently, new disinfectant agents have been produced for endodontic treatments. One of these disinfectants is SPO, which includes the highly reactive superoxide ion O_2^-, which is a common form of oxygen that is created when molecular oxygen gains a single electron. Superoxide radicals can attack susceptible biological targets, including lipids, proteins, and nucleic acids (18). One of the other advantages of SPO is its lower level of toxicity when compared to NaOCl (19) and hydrogen peroxide (20). Moreover, SPO has been investigated as an irrigation solution in root canals (16, 21) and recommended as a disinfectant for dental unit water lines and endoscopes (17).

2. Objectives

The aim of the present study was to evaluate and compare the antibacterial efficacy of SPO on *E. faecalis* biofilms in human root canals at different irrigation times.

3. Methods

3.1. Sample Preparation

Informed consent was obtained from the patients before the study, and the study was approved by the local ethics committee on human research of Cumhuriyet University (ethics committee code: 09/02/2013). One hundred twenty-six human mandibular premolar teeth with a single canal were used for the study.

After the residues were removed, following the extraction, teeth were kept in a 0.9% saline solution at 4°C. Sterile diamond discs (HORICO, Hopf, Ringleb & Co., GmbH & Cie, Berlin, Germany) were used to remove the coronal portions of the teeth, below the level of the cemento-enamel junction, under water cooling. The working length was detected as 14 - 16mm for each sample. The root canals were prepared using ProTaper Next (Dentsply, Tulsa Endodontics, Oklahoma, USA) Ni-Ti instruments with crown-down technique with an electric motor (Sendoline, Perfect Endo, Australia). PN instruments were used with a gentle in-and-out brushing motion at a speed of 300rpm, with light apical pressure. For optimum usage, the torque control device was set at 2N cm-1. All instruments were used to full working length.

The following instrumentation sequence was used: X1 (size 17, 0.4 taper), X2 (size 26, 0.6 taper), X3 (size 30, 0.7 taper), and X4 (size 40, 0.6 taper) instruments at the WL. One ml of 5.25% NaOCl solution was used after each instrumentation in the root canals.

Finally, the smear layer was removed with 17% EDTA and 5.25% NaOCl for five minutes. Bottles were sterilized in an autoclave for 20 minutes each at 121°C. Next, three layers of nail polish (L'Oreal Jet-Set Diamond, Paris, France) were applied to the whole external root surface of each tooth. The teeth were embedded in rubber caps. Finally, the rubber caps were sterilized by EtO (Melag, Euroklav 23V-S, Germany). These caps were then placed in bottles.

3.2. Biofilm Formation

Enterococcus faecalis (ATCC 29212) strains were cultured in blood agar (Brain-heart infusion agar, Acumedia Manufactures, Inc., Lansing, Michigan, USA). The incubation procedure was performed at 37°C for 24 hours. Before each experiment, 0.5 McFarland turbidity was set with a KristalSpecTM device. Then, the strains were subcultured in a Trypticase soy broth (Detroit, Michigan, USA) and incubated aerobically at 37°C for 24 hours. The turbidity of the *E. faecalis* cultures was adjusted to No. 0.5 McFarland standard. The value of 10μl of the bacterial suspension (final concentration of about 1.5×10^8) was transferred to the mechanically expanded lumen of the root canals, using a sterile micropipette (Blaubrand, Wertheim, Germany), except for ten canals, which were used as negative controls. They were then kept at 37°C for 24 hours. The orifices of the root canals were sealed with temporary filling material (3M ESPE, Dental Products, USA). All samples were stored at 37°C for ten days in a humid atmosphere, and the reinoculation procedure was repeated every 72 hours with fresh cultures on the first, fourth, seventh, and tenth days.

3.3. Scanning Electron Microscopy Analysis (SEM)

Scanning electron microscopy analysis was performed to identify the biofilm formation at the end of the tenth day (Figure 1). The SEM images of the remaining bacteria obtained from the root canals after super-oxidized water irrigation of Groups 3 and 5 are shown in Figures 2 and 3. For SEM preparation, the root canals were immersed in a fixative solution containing 4% buffered paraformaldehyde for 24 hours. The root canals were dehydrated in ascending degrees of ethanol series, following their separation with a diamond disc (HORICO, Hopf, Ringleb & Co., GmbH & Cie, Berlin, Germany) longitudinally. The X20.000 magnification with a scanning electron microscope (Jeol JSM 6400, Noran Instruments, Tokyo, Japan) was preferred for specimen examination. Digital images were taken at the center of the coronal (9 mm from apex), middle (6mm from apex), and apical (3 mm from apex) thirds of each canal in both root segments.

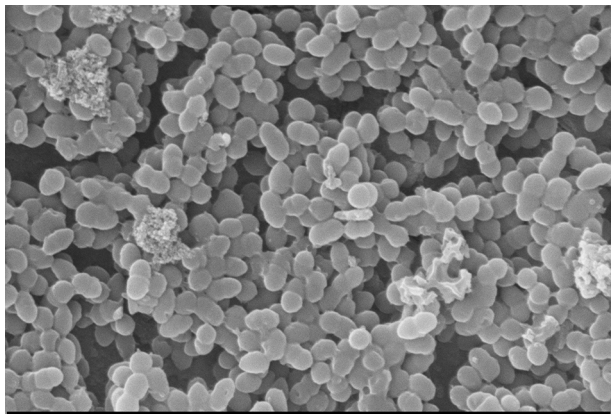

Figure 1. Stereomicroscopic Image of Developed *E. faecalis* Biofilm in a Root Canal

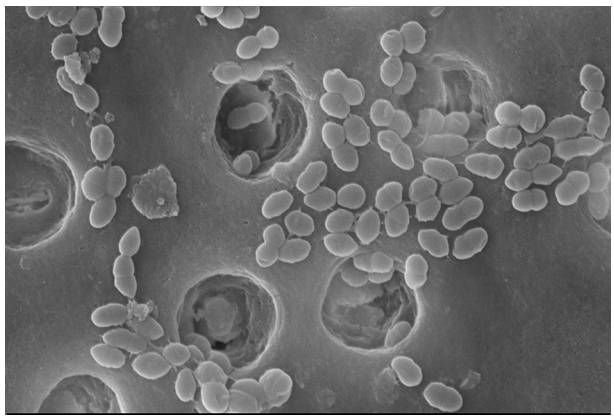

Figure 2. Stereomicroscopic Image of Remaining Bacteria in Group 2

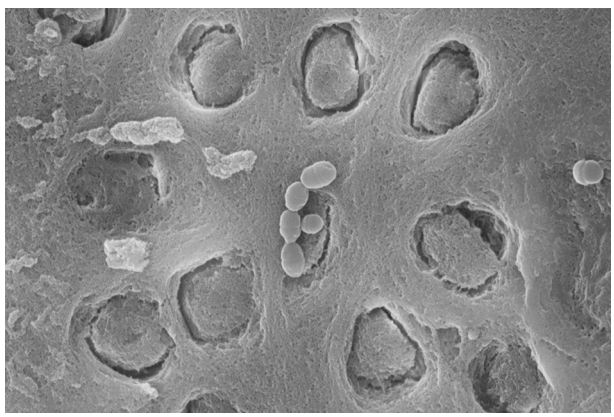

Figure 3. Stereomicroscopic Image of Remaining Bacteria in Group 3

3.4. Experimental Groups

Twenty teeth with biofilm processed canals were chosen for each group and randomly divided into six groups, as follows.

Group 1: Root canal irrigation was performed with 0.9% saline solution (negative control) with a 10mL/min flow rate for 2 minutes.

Group 2: Root canal irrigation was performed with SPO (Medilox; O-M Medical Dental Textile, Ankara, Turkey) that consists of a mixture of oxidizing substances, including hypochlorous acid at a concentration of 50 - 80mg/L, with a pH of 5.5 and a redox potential > 850 - 1000mV. The irrigation flow rate was 10mL/min for 1 minute.

Group 3: Root canal irrigation was performed with SPO with a 10mL/min flow rate for 2 minutes.

Group 4: Root canal irrigation was performed with SPO with a 10mL/min flow rate for 3 minutes.

Group 5: Root canal irrigation was performed with SPO with a 10mL/min flow rate for 5 minutes.

Group 6: Root canal irrigation was performed with 5.25% NaOCl (positive control) with a 10mL/min flow rate for 2 minutes.

3.5. Bacterial Evaluation

Bacterial cultures were taken with paper points (Dentsply, Maillefer, USA) that were inserted into the root canals, before and after root canal irrigation, to examine the bacteriological status. For standardization, specimens under 1.5×10^8 CFU/mL were excluded. Finally, a total of 120 teeth were evaluated after root canal irrigation, and CFU counts of the breeding colonies of microorganisms were calculated on blood agar plates.

3.6. Statistical Analysis

Variation data were analyzed using the SPSS statistical software program (version 22.0, SPSS Inc., Chicago, Illinois, USA). The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Tukey's post hoc test to examine pairwise differences at a significance level of 0.05.

4. Results

The comparisons and mean values of bacterial counting obtained before and after SPO irrigation in root canals are shown in Table 1. When the antibacterial effects of all disinfection agents were compared with each other, the following results were obtained. There were statistically significant differences between Group 1 (negative control) and all groups ($P < 0.05$).

Although Group 6 (positive control) showed no statistically significant difference when compared with Groups 4 and 5 ($P > 0.05$), Group 6 showed a statistically significant difference compared to all other groups ($P < 0.05$).

Table 1. The Comparisons and Mean Values of Bacterial Counting Obtained Before and After SPO Irrigation in Root Canals[a,b]

Groups, n = 20	Before Irrigation (Sx), CFU mL^{-1}	After Irrigation (Sx ± SD), CFU mL^{-1}
Group 1, saline (negative control)	1.5×10^8	1500000 ± 0
Group 2[A], super-oxidized water 1 min	1.5×10^8	4400 ± 1960
Group 3[A], super-oxidized water 2 mins	1.5×10^8	805 ± 123
Group 4[B,C], super-oxidized water 3 mins	1.5×10^8	39.5 ± 19.6
Group 5[B,D], super-oxidized water 5 mins	1.5×10^8	7.50 ± 5.96
Group 6[C,D], NaOCl (positive control)	1.5×10^8	0 ± 0

[a] CFU = colony-forming units; by the one-way ANOVA, F = 115.425; P = 0.000 (P < 0.05).
[b] Values with the same capital superscript letter are statistically no different at P > 0.05 by Tukey's test.

Although there was no statistically significant difference between Groups 2 and 3 (P < 0.05), both groups showed statistically significant differences when compared with other experimental groups (P > 0.05). Moreover, there was no statistically significant difference between Groups 4 and 5. Stereomicroscopic images obtained from the root canals in Groups 3 and 5 are shown in Figures 2 and 3. The SEM images of Group 3 indicated that some bacteria became intertwined with dentin tubules (Figure 2). The SEM images of Group 5 demonstrated a low number of bacteria (Figure 3). Moreover, the orifices of the dentinal tubules were observed more clearly, and the surfaces of the intertubular dentin showed roughness.

5. Discussion

Many researchers in endodontics have investigated the effects of various disinfectants on *E. faecalis* (22-24). Most of this research was performed under in vitro conditions. *E. faecalis* still poses a challenge from a clinical point of view, as it is a resistant species that is one of the major causes of endodontic treatment failure. Appropriate endodontic irrigation during root canal preparation is therefore necessary to adequately debride the canal system.

The actual aim of the present study was to examine the importance of bacterial biofilm during root canal irrigation procedures. The predominating species in post-treatment infection is *E. Faecalis*, which is the most resistant to intracanal medications and invades dentinal tubules as biofilm forms (25). The biofilm of *E. faecalis* may have a significant effect on the rate and extent of attachment by microorganisms and may be created under conditions that simulate conditions in vivo (9). Moreover, a biofilm can show strong antimicrobial tolerance, with physical barrier properties of the extracellular polysaccharide matrix (4).

Estrela et al. (26) put forth three aspects that are necessary for biofilm formation: the bacterial colonization structure, the biological indicator, and time. Biofilm formation was obtained in the present study. Finally, development and maturation of the bacterial biofilm was observed by SEM examination. Thus, the biofilm form of bacteria can be helpful in facilitating examination of the antimicrobial potential of endodontic materials.

The aim of irrigation by chemical solutions should be to eliminate both the smear layer formed during instrumentation and the biofilm, which is difficult to remove when compared with bacteria in a planktonic state. Furthermore, the irrigating solutions must indicate strong antimicrobial properties to remove the exopolymeric substance and to guarantee disruption of the biofilm (27).

Radcliffe et al. (22) examined the antibacterial efficacy of 5.25% NaOCl against *E. faecalis* at different times. The best result was obtained after 2 minutes' exposure. The result of regression analysis demonstrated the significant interaction between time and concentration. Alves et al. (23) investigated the antibiofilm and antibacterial effects of 2.5% NaOCl on *E. faecalis* biofilms, and most of the bacteria was eliminated in root canals. In another study using 5% NaOCl, Subbiya et al. (24) found consistent results with former studies, namely 100% biofilm reduction. In the present study, NaOCl irrigation showed insufficient bacterial elimination at 1 minute; therefore, 2 minutes' irrigation time was preferred. As a result, complete elimination of *E. faecalis* biofilm was achieved with 5.25% NaOCl for 2 minutes. This outcome demonstrated parallels with the aforementioned studies (22-24).

The present study purposed to evaluate the antimicrobial activity of SPO against *E. faecalis* biofilm in human root canals ex vivo. The antimicrobial activity of SPO was previously examined against bacteria, mycobacteria, viruses, fungi, and spores (17, 19, 28, 29). The microbicidal activity of SPO, when using membrane filters, was found to be highly active against several microorganisms on endoscope after 2 minutes (17-19). It was found to be nontoxic for biological tissues (19). Moreover, Middleton et al. (29) investigated the effect of SPO on the disinfection of bronchoscopes. They determined that this solution may be an effective alternative disinfectant against bacteria.

Rossi-Fedele et al. (21) investigated the antimicrobial activity of NaOCl and SPO as irrigating solutions on *E. fae-*

calis in bovine root canals for 3 minutes. In the present study, SPO was used at 3 and 5 minutes in human root canals infected by *E. faecalis* biofilms. The present study indicated a high antimicrobial effect of SPO after 3 and 5 minutes' contact time in root canals. SPO also showed a similar antibacterial effect with 5.25% NaOCl in the present study. Despite some variables, such as irrigation period, experimental samples, and types of microorganisms, the results of the present study are consistent with the above-mentioned studies. These results show a directly proportional relationship between the antibacterial effect of super-oxidized water and exposure time. In light of this in vitro study, SPO showed antimicrobial properties against *E. faecalis* biofilm, and its prolonged use in root canal was seen to enhance its activity.

Irrigation plays a key role in successful endodontic treatment. Detailed understanding of the mode of action of various solutions is important for optimal irrigation. Under the circumstances of the present study, SPO exhibited antimicrobial activity on *E. faecalis* biofilm in root canals with a 10 mL/min flow rate for 3 minutes. SPO is effective in terms of reduction of *E. faecalis*, and it has potential to be an efficient root canal irrigant. Moreover, SPO is an effective agent for eliminating the debris and smear layer (3) and is nontoxic in the apical third of the canal. More investigation is needed to examine irrigants in terms of toxicity, tissue dissolving ability, and strong antibacterial efficacy. None of the available irrigants has all of the characteristics of an ideal irrigant. Further research is warranted to determine an ideal material and/or technique to completely clean infected root canals.

5.1. Conclusion

Within the limitations of this in vitro study, it can be concluded that, as a new product, super-oxidized water had a highly antibacterial effect against *E. faecalis* biofilms in root canals. Moreover, super-oxidized water indicated a remarkable and similar bactericidal effect to that of traditional NaOCl against *E. faecalis* biofilms.

Acknowledgments

The authors do not have any conflicts of interest related to this study.

Footnote

Authors' Contribution: Recai Zan drafted the manuscript, study concept, and design and served as study supervisor. Tayfun Alacam contributed to critical revision of the manuscript for important intellectual content. Ihsan Hubbezoglu carried out statistical analysis and the analysis and interpretation of data. Tutku Tunc and Zeynep Sumer contributed to the acquisition of data. Oguzhan Alici and Tutku Tunc provided administrative, technical, and material support.

References

1. Zehnder M. Root canal irrigants. *J Endod.* 2006;**32**(5):389–98. doi: 10.1016/j.joen.2005.09.014. [PubMed: 16631834].
2. Harrison JW, Svec TA, Baumgartner JC. Analysis of clinical toxicity of endodontic irrigants. *J Endod.* 1978;**4**(1):6–11. doi: 10.1016/S0099-2399(78)80241-6. [PubMed: 277627].
3. Rocas IN, Siqueira JF, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. *J Endod.* 2004;**30**(5):315–20. doi: 10.1097/00004770-200405000-00004. [PubMed: 15107642].
4. Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2001;**91**(5):579–86. doi: 10.1067/moe.2001.113587. [PubMed: 11346739].
5. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998;**85**(1):86–93. [PubMed: 9474621].
6. Sedgley CM, Lennan SL, Appelbe OK. Survival of Enterococcus faecalis in root canals ex vivo. *Int Endod J.* 2005;**38**(10):735–42. doi: 10.1111/j.1365-2591.2005.01009.x. [PubMed: 16164688].
7. Portenier I, Waltimo TMT, Haapasalo M. Enterococcus faecalis- the root canal survivor and 'star' in post-treatment disease. *Endod Top.* 2003;**6**(1):135–59. doi: 10.1111/j.1601-1546.2003.00040.x.
8. Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, et al. Virulence, phenotype and genotype characteristics of endodontic Enterococcus spp. *Oral Microbiol Immunol.* 2005;**20**(1):10–9. doi: 10.1111/j.1399-302X.2004.00180.x. [PubMed: 15612939].
9. Donlan RM. Biofilms and device-associated infections. *Emerg Infect Dis.* 2001;**7**(2):277–81. doi: 10.3201/eid0702.700277. [PubMed: 11294723].
10. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. *Clin Microbiol Rev.* 1994;**7**(4):462–78. [PubMed: 7834601].
11. Elsner HA, Sobottka I, Mack D, Claussen M, Laufs R, Wirth R. Virulence factors of Enterococcus faecalis and Enterococcus faecium blood culture isolates. *Eur J Clin Microbiol Infect Dis.* 2000;**19**(1):39–42. [PubMed: 10706178].
12. ten Cate JM. Biofilms, a new approach to the microbiology of dental plaque. *Odontology.* 2006;**94**(1):1–9. doi: 10.1007/s10266-006-0063-3. [PubMed: 16998612].
13. Chavez de Paz LE. Redefining the persistent infection in root canals: possible role of biofilm communities. *J Endod.* 2007;**33**(6):652–62. doi: 10.1016/j.joen.2006.11.004. [PubMed: 17509401].
14. Huang YR, Hung YC, Hsu SY, Huang YW, Hwang DF. Application of electrolyzed water in the food industry. *Food Control.* 2008;**19**(4):329–45. doi: 10.1016/j.foodcont.2007.08.012.
15. Chittoria RK, Yootla M, Sampatrao LM, Raman SV. The role of super oxidized solution in the management of diabetic foot ulcer: our experience. *Nepal Med Coll J.* 2007;**9**(2):125–8. [PubMed: 17899964].
16. Solovyeva AM, Dummer PM. Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study. *Int Endod J.* 2000;**33**(6):494–504. [PubMed: 11307252].
17. Selkon JB, Babb JR, Morris R. Evaluation of the antimicrobial activity of a new super-oxidized water, Sterilox, for the disinfection of endoscopes. *J Hosp Infect.* 1999;**41**(1):59–70. [PubMed: 9949966].
18. David WMJ, Fehrenbach , Emmons M. Mosby's dental dictionary. 2 ed. USA: Mosby Inc; 2008.

19. Shetty N, Srinivasan S, Holton J, Ridgway GL. Evaluation of microbicidal activity of a new disinfectant: Sterilox 2500 against Clostridium difficile spores, Helicobacter pylori, vancomycin resistant Enterococcus species, Candida albicans and several Mycobacterium species. *J Hosp Infect.* 1999;**41**(2):101–5. [PubMed: 10063471].
20. Gonzalez-Espinosa D, Perez-Romano L, Guzman-Soriano B, Arias E, Bongiovanni CM, Gutierrez AA. Effects of pH-neutral, superoxidised solution on human dermal fibroblasts in vitro. *Int Wound J.* 2007;**4**(3):241–50. doi: 10.1111/j.1742-481X.2007.00331.x. [PubMed: 17924880].
21. Rossi-Fedele G, Figueiredo JA, Steier L, Canullo L, Steier G, Roberts AP. Evaluation of the antimicrobial effect of super-oxidized water (Sterilox(R)) and sodium hypochlorite against Enterococcus faecalis in a bovine root canal model. *J Appl Oral Sci.* 2010;**18**(5):498–502. [PubMed: 21085808].
22. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, et al. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. *Int Endod J.* 2004;**37**(7):438–46. doi: 10.1111/j.1365-2591.2004.00752.x. [PubMed: 15189432].
23. Alves FR, Neves MA, Silva MG, Rocas IN, Siqueira JF. Antibiofilm and antibacterial activities of farnesol and xylitol as potential endodontic irrigants. *Braz Dent J.* 2013;**24**(3):224–9. doi: 10.1590/0103-6440201302187. [PubMed: 23969910].
24. Subbiya A, Mahalakshmi K, Pushpangadan S, Padmavathy K, Vivekanandan P, Sukumaran VG. Antibacterial efficacy of Mangifera indica L. kernel and Ocimum sanctum L. leaves against Enterococcus faecalis dentinal biofilm. *J Conserv Dent.* 2013;**16**(5):454–7. doi: 10.4103/0972-0707.117507. [PubMed: 24082577].
25. Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J.* 1997;**30**(5):297–306. [PubMed: 9477818].
26. Estrela C, Sydney GB, Figueiredo JA, Estrela CR. Antibacterial efficacy of intracanal medicaments on bacterial biofilm: a critical review. *J Appl Oral Sci.* 2009;**17**(1):1–7. [PubMed: 19148398].
27. Chen L, Wen YM. The role of bacterial biofilm in persistent infections and control strategies. *Int J Oral Sci.* 2011;**3**(2):66–73. doi: 10.4248/IJOS11022. [PubMed: 21485310].
28. Tanaka H, Hirakata Y, Kaku M, Yoshida R, Takemura H, Mizukane R, et al. Antimicrobial activity of superoxidized water. *J Hosp Infect.* 1996;**34**(1):43–9. [PubMed: 8880549].
29. Middleton AM, Chadwick MV, Sanderson JL, Gaya H. Comparison of a solution of super-oxidized water (Sterilox) with glutaraldehyde for the disinfection of bronchoscopes, contaminated. *J Hosp Infect.* 2000;**45**(4):278–82. doi: 10.1053/jhin.2000.0772. [PubMed: 10973744].