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 acid ion (ClO−), chlorine (Cl2), hydroxide (OH), and ozone (O3), which are effective for inactivating a variety of microorganisms responsible for endodontic infections (15). These
oxidants may provide bactericidal and sporidical 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 \( \text{O}_2^- \), 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 \( E. \text{faecalis} \) 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, 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 (6 mm from apex), and apical (3 mm from apex) thirds of each canal in both root segments.

3.2. Biofilm Formation

\( Enterococcus \text{faecalis} \) (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 \( E. \text{faecalis} \) cultures was adjusted to No. 0.5 McFarland standard. The value of 10\(\mu\)l of the bacterial suspension (final concentration of about 1.5 \( \times 10^8 \)) 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 re-inoculation 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 (6 mm from apex), and apical (3 mm from apex) thirds of each canal in both root segments.
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 $1.5 \times 10^8$ 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 ($P < 0.05$).
Effects of various disinfectants on intertubular dentin showed roughness. Tubules were observed more clearly, and the surfaces of the 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 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).

Estrella 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. 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).

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).

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content. Ihsan Hubbezoglu carried out statistical analysis revision of the manuscript for important intellectual study supervisor. Tayfun Alacam contributed to critical

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.

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