OZONLU SU, BORİK ASİT, SÜPER OKSİDE SU VE KLORHEKSİDİN GLUKONAT İRRİGASYON SOLÜSYONLARININ KULLANIMI İLE KÖK KANAL BİYOFİLMİNİN APİKAL EKSTRÜZYONU

APICAL EXTRUSION OF INTRACANAL BIOFILM USING AQUEOUS OZONE, BORIC ACID, SUPER-OXIDIZED WATER AND CHLORHEXIDINE GLUCONATE IRRIGATION SOLUTIONS

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Özet

Endodontik tedavide kullanılan çeşitli irrigasyon solüsyonlarının, E. faecalis ile enfecte edilen kök kanallarının preparasyonu sırasında apikalden taşan bakteri miktarları üzerine etkilerinin değerlendirilmesi.

Seksen tek kök tek kanal mandibular premolar insan dişi kullanıldı. Başlangıçta giriş kavitesi hazırlandı ve kök kanallarının dezenfeksiyon işlemleri yapıldı. Daha sonra, kök kanalları ProTaper Next Nikel-titanyum (NiTi) rotary eğeleriyle prepare edildi. Enterococcus faecalis kök kanallarına inoküle edildi ve re-inokülasyon prosedürü birinci, dördüncü, yedinci ve onuncu günlerde yenilendi. İçerisinde E. faecalis biofilmi elde edilen örnekler rastgele dört gruba ayrıldı. Her grup ayrı ayrı ve sırasıyla Ozonlu su, borik asit, süper okside su (SPO) ve klorheksidin glukonat irrigasyon solüsyonları ile irrige edildi. Şekillendirme sonrasında apikal foramenden taşan bakteriler şişelerde toplandı. Şişede kalan bakteri miktarı beyin kalp infüzyon agar'a inkübe edildi ve 24 saat saklandı. Her bir örnekteki bakteri sayısı belirlendi. Elde edilen veriler tek yönlü varyans analizi (ANOVA) ve Tukey post-hoc testleri kullanılarak analiz edildi.

Taşan bakteri sayım sonuçlarına gore, borik asit en yüksek miktarda bakteri taşırırken, SPO en az miktarda bakteri taşmasına neden olmuştur. Tüm gruplar arasında yapılan ikili karşılaştırmaların sonuçlarına göre, tüm gruplar arasında istatistiksel olarak anlamlı farklar vardır ($p<0.05$).

Tüm irigasyon solüsyonları apikalden bakteri taşması ile ilişkili bulunmuştur. Ozonlu su ve SPO irrigasyon solüsyonlarının kök kanal tedavisi sırasında apikalden taşan bakteri açısından yapılan değerlendirmelerde güvenli irriganlar olarak tercih edilebileceği görülmüştür.

Anahtar Kelimeler: Apikal ekstrüzyon, irriganlar, biofilm.

Abstract

The evaluation of various irrigation solutions effect on amount of bacteria that extruded during preparation of root canal infected with E. faecalis.

Eighty the extracted single-root single-canal human mandibular premolar teeth were used. Initially, endodontic access cavities were prepared and the disinfection procedures of root canals were performed. Then, the root canals were instrumented with ProTaper Next Nickel titanium (NiTi) rotary files. Enterococcus faecalis were inoculated into the root canals and then the re-inoculation procedure was repeated on the first, fourth, seventh and tenth days. The samples in which E. faecalis biofilm was obtained, were randomly divided into four groups. Each group separately and respectively was irrigated with Aqueous ozone, Boric acid, Super-oxidized water (SPO) and Chlorhexidine gluconate. Intracanal bacteria extruded from the apical foramen after instrumentation were collected into vials. The amount of remaining bacteria in the vial were incubated in brain heart infusion agar and stored for 24 h. The number of bacteria was determined for each sample. The data obtained were analysed using the one-way ANOVA analysis of variance and Tukey tests.

As a result of extruded bacterial count, while boric acid produced the more amount of bacterial extrusion, SPO caused the least extrusion. According to the results of pairwise comparisons performed among all groups, there were statistically significant differences among all groups ($p < 0.05$).

All irrigation solutions were associated with apical bacterial extrusion. Aqueous ozone and SPO can be preferable as a safer irrigants in terms of intacanal bacterial extrusion that occurred during root canal treatment.

Key words: Apical extrusion, irrigants, biofilm.

Introduction

Root canal system is a complex system consisting of root canal dentine tubules, accessory canals, ramifications, apical delta and the transverse anastomosis. This complex systems should be cleaned as well as possible, prepared and three-dimensional be filled tightly. The cleaning and shaping the root canal provide the removing of infected dentin, necrotic or pulpal waste products, microorganisms and shaping with maintaining the original curvature of the canal. These important steps can be performed not only with

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biomechanic preparation but also with chemical agents. Consequently, the chemomechanical preparation is the most effective approach for ideal root canal treatment. Besides of all these, undesirable situations may occur during endodontic treatment that caused serious complications. One of the most common encountered mistakes was extrusion of debris or bacteria during chemomechanical preparation (1). The various irrigants have been used to minimize or eliminate this problem. In the light of this information, we aimed to evaluate and compare the efficacy of different irrigation solutions during root canal preparation.

Enterococcus faecalis is one of the highest-resistant bacteria against intracanal medicaments. E. faecalis frequently isolated from recurrent root canal treatments and plays a major role in the etiology of primary endodontic infections and persistent infections (2, 3). Additionally, E. faecalis is commonly observed in root canal treatment failures and it is able to survive in infected root canal either a single organism or a dominant component of the flora (4).

In recent times, aqueous ozone gains popularity as a effective irrigant for endodontic treatments. Various kinds of microorganisms may be eliminated with using ozone that exhibit strong oxidizing ability (5). Some of important features of ozone can be listed as follows: bactericidal, antiviral, and antifungal effects (2, 6). On the other hand, the low concentration of aqueous ozone was found insufficient against pathogenic microorganisms in dental plaque (2), root canals (7), and acrylic resin plates (8). Furthermore, the ozone gas level can not remain stable in aqueous ozone. Therefore, it should be used as soon as possible after being mixed. It is very difficult to keep this mixture at the same concentration for a long time (9).

Boric acid and its salts, borates, have been used in medicine as a bactericide, a fungicide, and an antiseptic as a wettable powder, liquid (applied as a spray or aerosol), emulsifiable concentrate, granule or dusts. Boric acid is especially effective when used as part of an ongoing integrated pest management (IPM) program that incorporates sanitation, cultural, mechanical, and biological practices (10). Moreover, it has been researched as an irrigation solution for periodontal therapy in patients with chronic periodontitis (11) and has been recommended systemic boric acid for osteoblastic activity (12).

Super-oxidized water (SPO) includes the highly reactive superoxide ion O_2^-. It 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 (13). It has been researched as an irrigation solution in root canals (14, 15) and has been recommended as a disinfectant for endoscopes (16), dental unit water lines (17) and dental impression materials (18).

Chlorhexidine gluconate (CHX), that is a common used irrigant in endodontic treatments. CHX may binds to hydroxyapatite and soft tissues, changing their electrical field to compete with bacterial binding. It has lower toxicity than sodium hypochlorite (NaOCl) but lacks tissue dissolving property. Moreover, antimicrobial effect of irrigant combinations within dentinal tubules has been suggested in endodontics (19). The antimicrobial effect of CHX is mediated by several mechanisms. It binds electrostatically to negatively charged sites on bacteria. By attaching to the bacterial cytoplasmic membrane, CHX causes the osmotic balance to be lost, resulting in leakage of intracellular material.

In light of this information, the aim of this study was to evaluate the amount of apically extruded biofilm after root canal irrigation with Aqueous Ozone, Boric Acid, SPO and CHX.

Materials and Methods

Selection and Preparation of Teeth

This study was approved by the Local Ethics Committee on Human Research of Cumhuriyet University (2014-10-21). The 80 extracted human single-rooted mandibular premolar teeth were used for this study. Criteria for tooth selection included a single root canal, no visible root caries, fractures, or cracks, no signs of internal or external resorption or calcification, a completely formed apex, and a curvature < 5° according to Schneider (20). The teeth were cleaned of debris and soft tissue remnants and were stored in physiological saline solution at +4 °C until required. Periapical radiographs (Schick Tech. Inc., Long Island City, NY, USA) were taken in the buccolingual and mesiodistal directions to select only teeth with

oval shaped root canals – long/short cross section diameter ratio of ≥2.5, at 5 mm from the apex (21).

Endodontic access cavities were prepared using diamond (Endo Access Bur; Dentsply Maillefer, Ballaigues, Switzerland) with a high-speed hand piece under water cooling. The pulp chambers were accessed, and any missing coronal tooth structure was replaced with acid-etched composite resin (Charisma; Heraeus Kulzer, Dormagen, Germany) to create a reservoir for contamination of the root canals with a suspension of E. faecalis.

Canals that were patent to greater than International Standards Organization (ISO) size 15 were discarded (22) and eithty teeth were finally selected. To ensure standardization and obtain a reference point, the both cusp edge of each tooth was flattened using a high-speed bur and the length of all teeth was standardized to 19 mm. Working length determination in all teeth was achieved using an Endomaster (EMS, SA, Switzerland) endodontic handpiece with the electronic apex locator mode switched on. The lip clip was attached to the needle. K-files were attached to the file holder cord and placed into the root canals and the tip of the file was transmitted to measure the length of each canal until it became visible in the apical foramen. It was then withdrawn 1 mm from the measured length.

Test Apparatus

A previously described method was used (9). The vials with rubber cap were adjusted for use by using a heated instrument to create a hole through the in centre. A hole was created on each cap and a 25-G needle alongside the cap to equalize the air pressure inside and outside the tubes. Then, each cap with the tooth and the needle was attached to its Eppendorf tube, and the tubes were fitted into vials. The entire apparatus was handled only by the vial. In no case was the Eppendorf tube touched with fingers. All vials were covered with aluminum leaf to prevent the operator from viewing irrigant extrusion during the irrigation phase.

One operator, using aseptic techniques, carried out the canal preparation and sampling procedures on each specimen under a Class I laminar airflow cabinet to prevent airborne bacterial contamination. The root canals were shaped with ProTaper (Dentsply, Tulsa Endodontics, OK, USA) rotary instruments using the crown-down method with an the electric motor (Denta Port DP-ZX, J. Morita MFG, CORP, Kyoto, Japan). Firstly, the coronal third of the roots were expanded with SX files. The median third of roots were then reached with S1 and S2 files. The F1, F2, and F3 files were applied, respectively, to shape the apical third of the canals. The canals were irrigated with 1 ml of 5.25% NaOCl solution after the variation of each file.

The roots were irrigated with 17% EDTA, 5.25% NaOCl, and distilled water for 5 min each to remove the smear layer, which was formed during the root canal preparation and then dried with paper point. All teeth were coded and then randomly assigned to 4 groups of 20 specimens each.

The entire model system was sterilized in ethylene oxide gas for a 12-h cycle using the anprolene and 74 °C gas sterilizer (Andersen Products Inc., Haw River, NC, USA).

Contamination with E. faecalis Biofilm

E. faecalis (ATCC 29212) strains were cultured on blood agar (Brain-heart infusion agar, Acumedia Manufactures, Inc., Lansing, Michigan, USA) and were incubated at 37°C for 24 h. Prior to the each experiment, 0.5 McFarland turbidity was set with a kristalspecTM device. Then was subcultured on Trypticase soy broth (Detroit, Michigan, USA) and incubated aerobically at 37°C for 24 h. The turbidity of E. faecalis culture was adjusted to No. 0.5 Mc Farland Standard. The value of 10 µl of bacterial suspension (final concentration of about 1.5 X 10^8) were transferred to the mechanically expanded lumen of the root canal using a sterile micropipette except 10 canals which prefered as negative control and then kept at 37°C for 24 h. The entrance of root canals were sealed with temporary filling material (Cavit; 3M ESPE, USA). All samples were stored at 37°C for 10 days in a huminity atmosphere and the reinoculation procedure was repeated every 72h with fresh culture at first, fourth, seventh and tenth days. Scanning electron microscopy (SEM) micrograph of the biofilm was examined at 5,000x manification, is shown in Fig. 1.

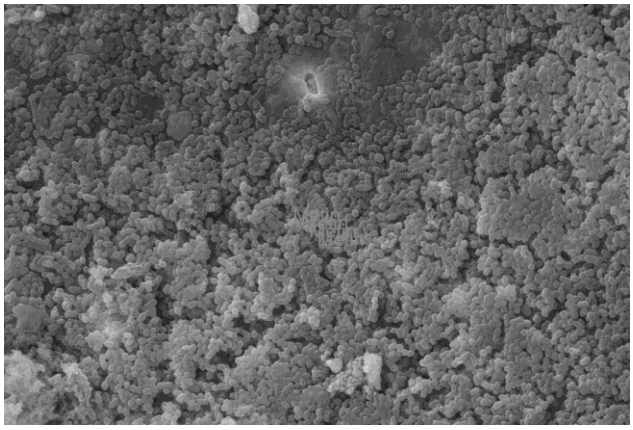

Figure 1. Scanning electron microscopy (SEM) micrograph of the biofilm.

Experimental Groups and Instrumentation Procedures

The irrigant was delivered by disposible plastic syringes with a 27-gauge stainless steel needle that had been placed passively down the canal, up to 3 mm from the apical foramen without binding. The irrigation sequences used were:

Group 1, Aqueous ozone group. Aqueous ozone was obtained with a custom-made ozone generator (TeknO3zone, Izmir, Turkey) from TeknO_3zone company. The ozone density of the distilled water was shown by the digital indicator (Fig. 2a) on the generator. The concentration of aqueous ozone was measured with the help of the probe that was in the reaction tank (Fig. 2b) connected to the generator. Power was controlled automatically by means of the automatic balancing system. Root canals infected by E. faecalis biofilm were irrigated for a duration of five min with 4 ppm aqueous ozone (TeknO_3zone, Izmir, Turkey).

Figure 2a. Digital indicator on the generator; **2b**. Reaktor tank of generator

Group 2, Boric acid group. Root canals infected by E. faecalis biofilm were irrigated with %6 Boric acid prepared in combination with distilled water at a 10 mL/min flow rate for 1 min.

Group 3, CHX group. Root canals infected by E. faecalis biofilm were irrigated with 2% CHX. The irrigation flow rate was 10 mL/min for 1 min.

Group 4, SPO group. Root canals infected by E. faecalis biofilm were irrigated with SPO (Medilox; O-M Medical Dental Textile, Ankara, Turkey) (Fig. 3) that consists of a mixture of oxidizing substances including hypochlorous acid (HOCl) at a concentration of 50-80 mg/L, with a pH of 5.5 and a redox potential > 850- 1000 mV. The irrigation flow rate was 10 mL/min for 1 min.

Figure 3. Super-oxidized water

Evaluation of Apically Extruded Bacteria

Evaluation was done by a second examiner who was blinded to group assignment. Paper points were placed in the eppendorf tubes before and after irrigation to control and evaluate the biofilms' formation. Biofilm counting ensured standardization; examples with colony forming units (CFUs) values under 1.5 X 10^8 CFU/ml were excluded. After irrigation, CFU counts of the breeding colonies of microorganisms were performed in blood agar plates. Then, the CFUs were calculated.

Statistical analysis

The variation data for the irrigation solutions were analyzed using SPSS statistical software (Version 14.0, SPSS Inc., Chicago, USA). The data were subjected to statistical analysis among the six different groups using one-way ANOVA. Tukey's test was applied when significant differences appeared, in order to examine pairwise differences at a significance level of 0.05.

Results

The mean and standard deviations of debris extruded apically in each group is shown in Table 1.

Groups	Mean (SD) (CFU mL^{-1})	Minimum	Maximum
Group 1 *Aqueous ozone*	76.00 (8.05)	60.00	90.00
Group 2 *Boric acid*	172.00 (23.97)	140.00	220.00
Group 3 *Super-oxidized water*	43.50 (7.63) [a]	30.00	50.00
Group 4 *Chlorhexidine gluconate*	137.00 (21.30) [a]	100.00	180.00

By the one way ANOVA, F= 234.356; P= 0.000 (P<0.05).
Groups with the same letter (a) was not significantly different at p>0.05 by Tukey's Test

Table 1. Mean **(**Standard Deviation) together with their statistical comparisons, minimum and maximum values were obtained from all groups.

The results of the one-way ANOVA test indicated that boric acid group extruded significantly more biofilm than all other groups (p<0.05). Besides that, super-oxidized water extruded significantly least amount of biofilm (p<0.05). The result of pairwise comparisons indicated that; There were statistically significant differences among all groups (p<0.05).

Discussion

The successful endodontic treatment depends on various factors. Specially, the complete elimination of debris and microorganisms play a crucial role in this respect. Because, the remaining or extruded intracanal waste products are mostly resulting in inflammatory reactions such as postoperative pain and swelling following the overinstrumentation of root canal. In this context, the preferred irrigation solution may be effective on the amount of extruded bacteria. In recent times, various irrigation solutions have examined in terms of different subjects by researchers for root canal systems. To our knowledge, no previous study has compared the effect of various irrigants on biofilm extrusion to the apical. In present study, we investigated the effect of aqueous ozone, boric acid, SPO and CHX on bacterial extrusion. In this study, straight single-rooted mandibular premolar teeth were prefered to prevent possible complications, such as WL loss or non-standardized preparation and irrigation in the curved root canals. Some investigators suggested the use of a barrier material to simulate the apical resistance (23,24). However, barrier materials, such as agar or foam, may absorb some irrigation solution and debris. Therefore, no attempt was made to simulate the presence of periapical tissues. An in vivo model may give different results because the periapical tissues, which serve as a natural barrier, may inhibit debris extrusion (25). Therefore, it must be emphasized that the results of this study should not be directly extrapolated to the clinical situation. On the other hand, E. faecalis biofilm was prefered to provide exactly the human infected intracanal conditions. For instance, most biofilm cells have higher levels of secondary metabolites, waste products, and secreted factors, as well as lower nutrient and oxygen limitations than planktonic bacteria (26, 27). Moreover, biofilms are much more metabolically active than planktonic cells in the stationary phase (28). Consequently, the biofilms are difficult to treat and destroy in root canals so that they may simulate the conditions in vivo. For these reasons, we used biofilms like those used in the recent studies (29, 30).

Aqueous ozone is one of the more investigated solution by endodontics. Although there is not enough study about ozone, its significant antimicrobial effect was emphasized by few researchers (5,8,31-33). Especially, engaged in research related to this issue in human root canals indicated that aqueous ozone caused considerable decrease in the amount of bacteria in root canals. For instance, Hems et al. (31) was applied aqueous ozone for 240 sec and determined a significant reduction of bacteria. In another study, eradication effect of aqueous ozone was examined on E. faecalis and C. albicans. Resultly, the ability of aqueous ozone related with bacterial elimination was

demonstrated in infected root canals (8, 33). The current research that planned considering the results of the these study indicated similar results as aforementioned studies in terms of bacterial elimination after aqueous ozone irrigation. However there is no research conducted yet about extrusion of intracanal biofilm following the aqueous ozone irrigation. The findings of the present study showed that although the amount of extruded biofilm was higher than SPO, it extruded less biofilm compared with CHX and Boric acid. Therefore, in our opinion, although aqueous ozone is a new irrigant can be recommended agent, there is a need for more research about this irrigant.

Bor is recently gaining popularity material that can indicate antimicrobial properties against the gram-negative bacterium, the gram-positive bacterium and the fungi in the field of health (34). However, only a single study has investigated the relation between boric acid and dental treatment. Saglam et al. (11) examined the efficacy of boric acid irrigation on chronic periodontitis patients. Boric acid ensured the reductions in deep pockets, plaque index and gingival index scores. Based on these results, we investigated the extruded intracanal biofilm after boric acid irrigation. However, it was not achieve the desired level of healthy results in terms of extrusion of bacteria. Therefore, boric acid cannot be safely recommended as a root canal irrigation solution.

SPO has been searched against bacteria, mycobacteria, viruses, fungi, and spores (16,35,36) on various materials and surfaces in medical literature. In line of these studies, this solution was seen to be an alternative disinfectant against various microorganisms. Especially, the antibacterial effect of SPO was demonstrated against cultured planktonic cells of cariogenic and periodontopathic bacteria on dental equipment (37). Moreover, Rossi-Fedele et al. (14) investigated the antimicrobial ability of SPO irrigation on E. faecalis in bovine root canals for 3 min. Both of studies indicated strong antimicrobial effects of SPO against E. faecalis. Unlike these studies, in the present study, SPO was tested in the first time in infected human root canal in terms of extrusion. The present study concluded that SPO caused the least amount of biofilm extrusion thus, it might be also preferable as safer for endodontic treatments.

CHX is one of the other conventional irrigant that indicates a certain level of antibacterial activity antibacterial property in endodontic treatments. Antibacterial activity of 2% CHX was demonstrated in root canals by Shailaja et al. (38) Recently, Goztas et al. (39) was used the CHX in primary root canals and exhibited antimicrobial effect. Besides these studies, in the present study, CHX was evaluated in terms of extruded intracanal biofilm during root canal irrigation. Resultly, CHX caused high amount of bacterial extrusion. It extruded more bacteria than all experimental irrigants accept boric acid. For this reason, CHX can not advisable as a useful irrigant during chemomechanic preparation.

Conclusions

Under the limitations of this study, it can be concluded that aqueous ozone and SPO extruded less biofilm compared to other irrigants. In our opinion, the amount of bacterial extrusion related with antibacterial ability of used irrigation solution type in root canals. There may be an inversely proportional relationship between bactericidal activity of irrigation solutions and the extruded bacteria during endodontic treatments. Therefore, the clinicians should be preferred non-toxic, highly effective antibacterial irrigants in order to minimize the extruded bacteria to the surrounding tissues. Whereby postoperative complication may be prevented and the success of endodontic treatment can be increased in the long term.

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