

Antibacterial effect of urushiol on *E. faecalis* as a root canal irrigant

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Objectives: The purpose of this study was to compare the antibacterial activity of urushiol against *Enterococcus faecalis* (*E. faecalis*) to that of NaOCl. **Materials and Methods:** The canals of thirty two single rooted human teeth were instrumented with Ni-Ti files (ProTaper Next X1, X2, X3, Dentsply). A pure culture of *E. faecalis* ATCC 19433 was prepared in sterile brain heart infusion (BHI) broth. The teeth were submerged in the suspension of *E. faecalis* and were incubated at 37°C for 7 days to allow biofilm formation. The teeth were randomly divided into three experimental groups according to the irrigant used, and a negative control group where no irrigant was used ($n = 8$). Group 1 used physiologic normal saline, group 2 used 6% NaOCl, and group 3 used 10 wt% urushiol solution. After canal irrigation, each sample was collected by the sequential placement of 2 sterile paper points (ProTaper NEXT paper points, size X3, Dentsply). Ten-fold serial dilutions on each vials, and 100 μ L were cultured on a BHI agar plate for 8 hours, and colony forming unit (CFU) analysis was done. The data were statistically analyzed using Kruskal-Wallis and Mann-whitney U tests. **Results:** Saline group exhibited no difference in the CFU counts with control group, while NaOCl and urushiol groups showed significantly less CFU counts than saline and control groups ($p < 0.05$). **Conclusions:** The result of this study suggests 10% urushiol and 6% NaOCl solution had powerful antibacterial activity against *E. faecalis* when they were used as root canal irrigants. (*Restor Dent Endod* 2017;42(1):54-59)

Key words: Antibacterial effect; *Enterococcus faecalis*; Root canal irrigant; Sodium hypochlorite; Urushiol

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Introduction

Bacteria usually prefer biofilm forms because they protect bacteria from existing physical forces and chemical attack. It is also indicated that the resistance of bacteria in biofilm is 2 to 1,000 times higher than that of the planktonic forms.¹ Furthermore, biofilms can rebuild themselves after being partially affected.² It is well known that microbial biofilm in the infected root canal system should be eliminated to achieve successful result with endodontic treatment,³ since pulpal and periapical diseases as well as failure of endodontic therapy are caused by these microbes.

Enterococcus faecalis (*E. faecalis*), a facultative anaerobic gram-positive coccus, is one of the most resistant microorganisms among the root canal microflora.⁴ It is known as the most commonly identified species in the failed root canals from earlier endodontic treatment,⁵ and also present in 24 - 74% of asymptomatic and persistent endodontic

infections.⁶

Mechanical instrumentation only removes approximately 50% of the bacteria in the canal.⁷ Therefore, chemomechanical means using various instrumentation techniques, irrigants, and intracanal medications have been used to exterminate bacteria from an infected canal. However, it is unlikely to remove microorganisms completely because of the complex anatomy of the canal system such as fins, isthmuses, lateral canals, apical deltas, etc.⁸ Therefore, persistence of intracanal bacteria is believed to be one of the most common causes of endodontic treatment failure.⁹

Root canal irrigation plays an important role in endodontic treatment. It has two goals; the physical one is to make the irrigant flow throughout the entire root canal system to wash out all debris, and the chemical one is to destroy biofilms and endotoxins and to dissolve tissue remnants and smear layer on canal walls.¹⁰

Sodium hypochlorite (NaOCl) is the main endodontic irrigant used due to its ability to dissolve organic tissues and its excellent antibacterial properties against most microorganisms.¹¹ Furthermore, NaOCl solutions are economical, easily obtainable, and have a good shelf life.¹² However, NaOCl has several disadvantages, including unpleasant smell and taste, high toxicity if extruded beyond the apex and its inability to kill all bacteria present in the root canal system.¹³ In addition, it may alter the dentin structure and leave residues that may interfere with pulp regeneration procedures or weaken adhesive bonding to dentin.¹⁴ Therefore, the efforts to search for more efficient and safer root canal irrigants should be continued.

Urushiol, a natural extract from the sap of the lacquer tree, consists of a catechol with an n-C15 or n-C17 alkyl side chain, and its antibacterial activity depends on the unsaturation of the alkyl chain.¹⁵ Furthermore, powder-type urushiol exhibits not only significant antimicrobial activity against gram-positive and gram-negative microorganisms but also excellent antioxidant activity.¹⁶ Recently, 0.01% urushiol solution was advocated as a cavity disinfectant due to its strong antibacterial capacity against *Streptococcus mutans* (*S. mutans*) like other well-known cavity disinfectants (2% chlorhexidine gluconate [CHX] and 6% NaOCl), and its preservation capacity of adhesive's bond strength to dentin.¹⁷ Additionally, it was revealed in our pilot study that *E. faecalis* was not detected after 30 minutes incubation in brain-heart infusion (BHI, Becton, Dickinson and Co., Franklin Lakes, NJ, USA) medium containing 0.01% urushiol.

The purpose of this study was to compare the antibacterial activity of urushiol against *E. faecalis* to that of NaOCl, a main root canal irrigant. The null hypothesis tested was that there is no difference between the two irrigants.

Materials and Methods

Non-carious, unrestored thirty two human teeth with single root canal were used throughout this study, which were extracted in the department of oral and maxillofacial surgery in Dankook university dental hospital. Permission by the institutional review board (IRB) of Dankook university dental hospital, Cheonan, Korea, was taken for collection and use of extracted teeth (DKUDH IRB 2015-09-003). All the teeth were cleaned to remove superficial debris, calculus, and other contaminants, and were stored in 6% NaOCl solution (RC CLEANER, Ilchung Dental Co. Ltd., Seoul, Korea) for 30 minutes. The crown and part of root portion were removed to standardize the length of the remaining tooth to 13 mm.

The root canals were instrumented at the apical foramen with K files up to size 15. Then, rotary Ni-Ti files (ProTaper Next X1, X2, X3, Dentsply, York, PA, USA) were used to standardize the size of the apical constriction. Two milliliters of 6% NaOCl was used between each instrument during the cleaning and shaping procedure. The smear layer was removed by copious irrigation with 17% ethylene diamine tetra-acetic acid (EDTA) solution (2 minutes, 1 mL) and NaOCl (1 mL) using 30 gauge syringe in an up and down motion. Finally, the samples were rinsed with distilled water for 4 minutes in an ultrasonic bath (Sankei Giken Industry Co. Ltd., Tokyo, Japan) and stored for 24 hours in sterilized distilled water to extract the remnant of NaOCl solution.

A pure culture of the test strain, *E. faecalis* ATCC 19433 (Koram Biotech Corp., Seoul, Korea) was prepared in sterile BHI broth (Becton, Dickinson and Co.). Cells of *E. faecalis* were grown as a suspension and incubated at 37°C for 24 hours. The teeth were submerged in the suspension of the *E. faecalis* and were incubated at 37°C for 7 days to allow biofilm formation. BHI broth was added every 2 days for the growth of microorganisms. After 7 days of contamination, microbe loading level was confirmed with colony forming unit (CFU) per millilitre (9.2×10^6 CFU/mL).

The teeth were randomly divided into three experimental groups with 8 teeth in each group according to 4 mL of irrigant used, and a negative control group with 8 teeth where no irrigant was used. The flow rate of irrigants were 3 - 3.5 mL/min. Group 1 used physiologic normal saline (Daihan normal saline, Daihan Pharm Co., Ltd., Ansan, Korea). Group 2 used 6% NaOCl (RC CLEANER, Ilchung Dental). Group 3 used 10 wt% urushiol solution (Table 1). Urushiol was extracted in a way as follows: the mixture (1 gm) of urushiol (Ikkake-Urushi, Watanabe-Shoten, Tokyo, Japan) was diluted with 300 mL methanol (Duksan Chem. Co., Seoul, Korea) and washed with 300 mL toluene (Duksan Chem. Co.) three times. The organic layer was evaporated in a vacuum to give the crude product, which was purified by column chromatography (GC-Mass, JMS-600W, JEOL, Tokyo,

Table 1. Groups classified according to the canal irrigants

Group	Irrigation procedure	Manufacturer
Control	No irrigation	
Saline	0.9% normal saline, 4 mL	Daihan normal saline, Daihan Pharm Co., Ansan, Korea
NaOCl	6% NaOCl, 4 mL	RC CLEANER, Ilchung dental, Seoul, Korea
Urushiol	10% urushiol, 4 mL	Ikkake-Urushi, Watanabe-Shoten, Tokyo, Japan

Japan) using ethyl acetate. Canals were irrigated with 4 mL of each irrigant using a 30 gauge needle (Septodont, Lancaster, PA, USA) for approximately 30 seconds without any further instrumentation. The needle was initially placed in to 1 mm short of the working length, and the irrigation was done with in and out motion.

The final irrigation of the canals was done with 2 mL of saline in the same way, and excess fluid was removed by drying with gentle air stream. Each sample was collected by sequential placement of 2 sterile paper points (ProTaper NEXT paper points, size X3, Dentsply). Each paper point remained in the canal for 1 minute. To avoid contamination, all the procedures were performed in a laminar flow hood. The paper points were transferred into a vial containing 1 mL of sterile distilled water, vortexing for 2 minutes. Ten-fold serial dilutions on each vials, and 100 μ L were cultured on a BHI agar plate for 8 hours, and CFU analysis was done. The data were statistically analyzed using Kruskal-Wallis and Mann-whitney U tests. The significance level was set to $p < 0.05$. All statistical tests were conducted using IBM SPSS Statistics ver. 15.0 (IBM SPSS Corp., Amarak, NY, USA).

Results

Table 2 shows the mean and standard deviation values in the CFU of *E. faecalis*. The Kruskal-Wallis test showed significant differences among the groups ($p < 0.05$). NaOCl and urushiol groups showed significantly less CFU

than saline and control groups ($p < 0.05$). There was no statistical difference between the NaOCl and urushiol groups. Saline group exhibited no difference in the CFU with control group, either.

Discussion

E. faecalis, a target microorganism in this study, is a normal inhabitant of the oral cavity. Its prevalence is believed to increase in patients receiving initial endodontic treatment and retreatment when compared to those with no endodontic problem.¹⁸ The prevalence of *E. faecalis* is low in primary endodontic infections (4 - 40%)¹⁹ and high in persistent infections (24 - 77%).²⁰⁻²²

E. faecalis has the ability to survive in various harsh circumstances including hyperosmotic conditions, at temperatures ranging from 10 to 60°C and at a pH of over 9.6.²³ It was also reported *E. faecalis* can survive long-term entombment without additional nutrients and keep viability *in vitro* for 12 months.²⁴ There are several ways for *E. faecalis* to survive within the canal system. It possesses collagen-binding proteins, which help it to bind to the dentin.²⁵ It is small enough to easily penetrate and lives within dentinal tubules.²⁶ In addition, it is able to form the biofilm that helps the bacteria to become 1,000 times more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing organisms.²⁷

Mechanical instrumentation techniques including rotary instruments produce a 1 to 2 μ m thick smear layer, which

Table 2. Colony forming units per milliliter (CFU/mL) collected from the canals which were irrigated with irrigants and cultured on a BHI agar plate

Group	No. of specimen	Mean	1 st Quartile	Median	3 rd Quartile
Control	8	97,190 ^a	34,260	47,000	152,000
Saline	8	95,875 ^a	39,000	76,000	168,000
NaOCl	8	0.0 ^b	0	0	0
Urushiol	8	0.38 ^b	0	0.38	1.88

BHI, brain-heart infusion.

Same superscripts indicate that there was no statistically significant difference.

seals the remaining bacteria in dentinal tubules after root canal preparation. This smear layer prevents irrigants from penetrating into the irregularities of the root canal system and the dentinal tubules.²⁸ In order to eliminate already formed smear layer in this study, copious irrigation was done with 17% EDTA solution (2 minutes, 1 mL) and NaOCl (1 mL) using a 30 gauge syringe. Additionally, the canal system was not mechanically re-instrumented after *E. faecalis* inoculation in this study because the exclusive purpose of this study was to determine the antimicrobial effectiveness of the irrigants following a standardized irrigation protocol.

Dentin and organic materials within root canals as well as smear layer can affect the antibacterial effect of the irrigants.²⁹ It was also noted that dentin powder markedly reduces or at least delays antimicrobial effect of NaOCl.³⁰ Therefore, antibacterial irrigants used for canal disinfection should penetrate or remove the smear layer to kill the bacteria in infected dentin.

Regardless of irrigation solution used, the endodontic microorganisms are reduced by the mechanical action of the irrigation.³¹ However, it was reported that sterile saline irrigation with conventional needle did not show any antibacterial effect, although a little improvement was obtained when it was used with piezoelectric ultrasonic device.³² Another study through observation of no dead bacteria after canal irrigations demonstrated normal saline had no antibacterial effect against *E. faecalis*.³³ These results are consistent with this study, which showed no difference in CFU from that of control group.

NaOCl is the well-known principal endodontic irrigant because of its excellent antibacterial property and its ability to dissolve organic tissue.¹¹ Chlorine affects a broad range of microbes including viruses and fungi, and oxygen kills anaerobic bacteria. Additionally, dissolution of necrotic pulp tissue and organic debris can be achieved with the proteolytic effect of free chlorine.³⁴ However, the use of NaOCl has various inherent disadvantages, such as unpleasant smell and taste, high toxicity, extreme corrosiveness to metals, etc.³⁵ Furthermore, it was also shown that its clinical performance is inferior to its effects *in vitro*, and about 40 - 60% of the root canals irrigated with NaOCl still keep bacteria in the main canal.³⁶ Canal irrigation with 6% NaOCl or 10% urushiol in this study resulted in the zero or extremely few CFU numbers, showing more potent antibacterial effect against *E. faecalis* than sterile saline irrigation. Thus, the hypothesis, no difference between the two irrigants (6% NaOCl and 10% urushiol), was accepted.

Because this is the first study evaluating urushiol as a canal irrigant, it is not clear what makes urushiol antibacterial against *E. faecalis*. However, it may be possible to presume that urushiol is able to disrupt the bacterial cell membrane,³⁷ and unsaturation of the alkyl

chain controls this trait of antibacterial characteristic.¹⁵ It was reported that urushiol rapidly promoted bleb formation and lysis of *Helicobacter pylori* and it had antibacterial effects by acting on the cell membrane, not on the bacterial ribosome, leaving the bacterium unable to synthesize proteins essential for its growth.³⁷

Although NaOCl and urushiol showed very potent antibacterial effects at the time of post canal irrigation in this study, persistent bacteria residing deeply in the dentinal tubules might recover and redevelop a mature biofilm in the dentin. It is well-known that NaOCl effect is short lasting and insufficient to eradicate *E. faecalis*, thus bacteria still survived after the treatments.³⁸

This study has a limitation of using only a single species. Usually one or just a few species were recovered in cases of teeth with persistent lesion, and there was a consensus of a high manifestation of *Enterococci* and *Streptococci*.³⁹ The multispecies biofilm model might be closely similar to the *in vivo* biofilm⁴⁰ and allow standardized comparison of the efficacy of the antibacterial irrigants. In addition, antimicrobial activity is not the exclusive requirement of an endodontic irrigant. It should be also lubricating, nontoxic, minimally destructive to tooth structure, provide dissolution of organic and inorganic materials, and be relatively expedient and easy to use.⁴¹ Although NaOCl and urushiol demonstrated their satisfactory antibacterial activity on *E. faecalis* in this study, more work remains to be done to identify another characteristics of urushiol solution, such as toxicity, structural damage, etc, which are the requirements for the endodontic irrigation solution.

Conclusions

Within the limitations of this *in vitro* study, its result suggest 6% NaOCl and 10% urushiol solution had powerful antibacterial activity against *E. faecalis* when they were used as root canal irrigants.

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