# The efficacy of plasma-treated water as a root canal irrigant

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**Purpose:** In recent years, attention has been drawn to disinfection techniques by plasma irradiation on human bodies, however, the efficacy is not sufficient in the root canal. Therefore, the authors generated plasma-treated water (PTW) to concentrate the active species, and investigated the efficacy of PTW as a root canal irrigant.
**Materials and Methods:** PTW was produced using plasma prepared from helium containing nitrogen. Three tested microorganisms, *Enterococcus faecalis*, *Candida albicans,* and *Candida glabrata*, were selected, and their growth with PTW was evaluated to determine minimum inhibitory concentration (MIC). Infected root canal models were created using 76 extracted human anterior teeth whose root canals were prepared and inoculated with one of the bacterial/fungal suspensions and cultured. A bacterial/fungal test of the root canals was performed following PTW treatment. The effect of PTW was evaluated in an *in situ* model of infected root canal with *E. faecalis* of 12 bilateral mandibular first molars of six 4-week-old male Wistar rats.
**Results:** PTW had a sterilizing effect against all tested microorganisms significantly. The MIC of PTW was a dilution ratio of 0.125 against *E. faecalis* and a dilution ratio of 0.25 against *C. albicans* and *C. glabrata*. No growth of microorganisms were observed in any of PTW experimental groups, that means the significant efficacy of PTW in all test-infected root canal models *in vitro* and *in situ* of the rat.
**Conclusion:** PTW showed a sterilizing effect against *E. faecalis, C. albicans,* and *C. glabrata,* even in root canals without obvious effect on oral mucosal tissue.

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**Key Words:** *Candida*, *Enterococcus faecalis*, plasma disinfection, root canal irrigation

## Introduction

The mechanical cleaning is essential for root canal treatment. Traditionally, sodium hypochlorite has often been used for chemical cleaning [1,2]. However, while sodium hypochlorite has a strong sterilization action, it also strongly irritates tissue and is associated with risk of injury caused by extravasation of sodium hypochlorite to the periapical tissue, leakage of sodium hypochlorite into the oral mucosa, and dispersion of sodium hypochlorite to skin and clothes [3,4]. To perform effective and safe treatment, a new root canal irrigant that is both less harmful to living organisms and has sufficient sterilizing activity needs to be developed.

In recent years, attention has been drawn to plasma sterilization techniques for human body. Because various active oxygen and nitrogen species can be produced from plasma generated, various types of plasma sterilization is being reported [5-7]. Atmospheric pressure plasma in a state of thermal equilibrium can reach a very high temperature, but it can be transformed to a state of non-equilibrium by subjecting it to pulse discharge, which generates low-temperature atmospheric pressure plasma (LTAPP) that can avoid a thermal load. This LTAPP is capable of irradiating living organisms in addition to sterilizing medical equipment. It is therefore expected to be applied to treatments such as the promotion of burn healing or hemostasis, in addition to skin disinfection [8]. When applying LTAPP to living organisms, the plasma also needs to be effective against bacteria present in fluids because of the large amount of target bacteria present in damp environments. However, direct plasma irradiation of bacterial cells present in fluids is difficult, and the sterilizing effect is limited. Ikawa et al. [9] therefore developed a "reduced-pH method" where the sterilizing effect against bacteria in fluid is markedly enhanced by keeping the pH level of the fluid itself below 4.8. Research into the application of this technique is

already progressing even in the field of dentistry [10]. Caries-infected dentin is reported to reduce cariogenic bacteria in dentin to a level below the detection limit [11]. The sterilization mechanism of direct plasma irradiation is thought to result from HOO· generated by acid dissociation equilibrium of $O_2^-$·; however, there is concern about the effect on the living organism in order for the simultaneously irradiated OH·, $^1O_2$, and O to bring about a nonspecific chemical reaction.

The authors are continuing to conduct various studies with the objective of clinically applying LTAPP in the field of dentistry and have reported that it is possible to produce plasma-treated water (PTW) with bactericidal activity through active species by performing plasma irradiation of distilled water (DW) [12]. The properties of PTW are similar to those of direct plasma irradiation, and PTW has strong bactericidal activity when the "reduced-pH method" is applied. Meanwhile, the half-life of PTW activity is relatively short, and the impact on living organisms is considered very low. The half-life depends on the temperature; the half-life is several seconds at 37°C, several minutes at room temperature, several hours at 0°C. However, when PTW is frozen at −80°C, it can be semimaintained permanently [12]. When PTW is produced, it can be prolonged by cooling it in ice cold water, which also allows the key active species to be maintained. PTW therefore has both extremely strong bactericidal activity and a very small impact on living organisms, which makes the application of PTW to dental treatment promising.

The purpose of this study was to investigate the efficacy of PTW when used as a root canal irrigant in extracted human teeth and molars of living rat.

## Materials and Methods

### Generation of PTW

PTW was generated in the same way as research by Tasaki et al [13]. Helium containing several percent nitrogen was used as the plasma generating gas. DW was poured into a quartz discharge tube (length: 1,000 mm, inner diameter: 8 mm) to which electrodes were attached, and the plasma generating gas was simultaneously controlled by mass flow controllers (Model 8500, Kofloc, Kyoto, Japan). Using a high-voltage pulse (20 kV, 10 kHz, 5-ns pulse width) generated by power supply (High Voltage Pulse Generator FPG 20-10NKN5; FID GmbH, Burbach, Germany), atmospheric pressure glow plasma was generated through dielectric barrier discharge to irradiate DW. To ensure continuous generation of PTW and as long an exposure time as possible, the discharge tube was placed at a slight angle. The equipment was totally cooled with ice cold water in an effort to avoid losing PTW activity within the processing time. The generated PTW was immediately cooled with liquid nitrogen and stored at −80°C. Upon initiation of the experiment, the cryopreserved PTW was thawed with keeping almost 0°C while being stirred, after which 200 mM citrate buffer (pH 3.5) was added to adjust the pH before PTW could be used.

### Tested microorganisms and culture

The tested microorganisms were *Enterococcus faecalis* (ATCC19433), a bacterium, and *Candida albicans* (ATCC18804) and *Candida glabrata* (ATCC90030), two fungi. Cryopreserved *E. faecalis* suspension (500 μL) was poured into 3 mL of brain heart infusion broth (BHI; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and precultured for 24 hours at 37°C under aerobic conditions, after which the concentration was adjusted to $10^7$-$10^8$ CFU/mL. Cryopreserved *C. albicans* and *C. glabrata* suspensions were each transferred by one inoculation loop to 10 mL of tryptic soy broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with

5% dextrose (TSBD) for 24 hours preculturing at 30˚C under aerobic conditions, after which the concentrations were adjusted to $10^7$-$10^8$ CFU/mL.

**Measurement of minimum inhibitory concentration (MIC)**

Tested microorganism suspensions were distributed in 10 µL portions to a 96-well microtiter plate, to which 10 µL of 200 mM citrate buffer (pH 3.5) and 80 µL of PTW were added. The samples were then kept for one minute at room temperature, after which 100 µL of BHI were injected into the *E. faecalis* samples, and 100 µL of TSBD were injected into the *C. albicans* and *C. glabrata* samples. The *E. faecalis* samples and *C. albicans* and *C. glabrata* samples were subsequently subjected to 48 hours of aerobic culture at 37˚C and 30˚C, respectively. Evaluation was done by observation of the presence or absence of microorganism growth and culture solution turbidity under room light. The tested PTW was observed at five different concentrations in a two-fold serial dilution series. In the control groups, 90 µL of DW were added to samples, and four samples each were measured under each condition.

**Creation of infected root canal models *in vitro***

The Ethical Review Board of Tsurumi University School of Dental Medicine (No. 865) gave its approval for this study to be conducted. The tested teeth were 76 safely extracted human anterior teeth that had been stored in saline. The crowns were resected at a position 10 mm from the root apex, and the root canals were prepared using hand files (Zipperer, München, Germany) up to size #60. During preparation, the root canals were also irrigated frequently with 3% sodium hypochlorite. The root canals were immersed in DW in a 1.5 mL polypropylene microtube, and sterilized with an autoclave. The root canals were then dried with a sterile paper point, and #60 paper point impregnated with 10 µL of each suspension was inserted into the root canals randomly divided into three groups. The *E. faecalis* group (n = 10) underwent aerobic culture within microtubes for 24 hours at 37˚C, while the *C. albicans* (n = 16) and *C. glabrata* (n = 26) groups underwent aerobic culture within microtubes for 24 hours at 30˚C.

**Observation of the efficacy of PTW in infected root canal models**

The infected models were stored before the experiment at 37˚C, and the experiment was subsequently conducted at room temperature. The paper points used for bacterial/fungal seeding were removed from the root canal models, after which a syringe mounted with a 27G needle was used to irrigate the models with 1 mL of PTW to which 100 µL of 200 mM citrate buffer (pH 3.5) had been added. The needle was inserted until it was near the root apex, after which the model was carefully irrigated while extracting bubbles. The root canals were left filled with PTW for one minute, and PTW was removed with a paper point. A bacterial/fungal test was then conducted by using liquid medium. In other words, the #60 paper point was inserted into the root canal and impregnated with 20 µL of DW and then injected into a liquid medium after one minute. The *C. glabrata* group of models (n = 10) underwent one minute of prewashing with 1 mL of 200 mM citrate buffer (pH 3.5) before irrigating with PTW, in addition to the procedure used in the aforementioned experimental group (n = 16). The control groups were formed of a group irrigated with DW (n = 4) and a group of root canal models irrigated with citrate buffer (n = 4). The models underwent 48 hours culturing under aerobic conditions, after which the culture solution turbidity was observed under room light.

**Observation of the efficacy of PTW *in situ* models**

The Animal Experiment Board of Tsurumi University School of Dental Medicine (No. 28A032) gave its approval for this study to be conducted. The tested teeth were 12 bilateral mandibular first molars of six

4-week-old male Wistar rats. After sedating the rats using ether, a triple anesthesia mix of 1.0 mg/mL medetomidine hydrochloride (Domitor; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), 5.0 mg/mL midazolam (Dormicum; Astellas Pharma Inc., Tokyo, Japan), and 5.0 mg/mL butorphanol tartrate (Betorphal; Meiji Seika Pharma, Co., Ltd., Tokyo, Japan) was administered intraperitoneally at 0.1 mL/10 g [14]. The rats' jaws were held open with a mouth-opening device, and the access cavity was expanded with a round bur (0.8 mm in diameter), after which the root canal was prepared using hand files (Zipperer, München, Germany) up to size #15. Then, each root canal was injected with 10 µL of *E. faecalis* suspension using a micropipette, and this suspension was made to fill the entire root canal by moving the file in a vertical up-and-down motion.

In each rat, one side was randomly assigned to the experimental group, while the opposite side was assigned to the control group. The root canal was then irrigated with 1 mL of PTW or DW. After drying with a paper point, bacterial test was conducted within the root canal by a simple culture method. In addition, changes in oral mucosal tissue with which PTW came into contact were observed using a magnifying glass.

**Statistical analysis**

The efficacy of PTW in an infected root canal model and *in situ* model was statistically investigated between conditions using chi-squared tests ($p < 0.05$).

# Results

**Measurement of MIC**

PTW had a sterilizing effect against all tested microorganisms (Table 1). The MIC of PTW was a dilution ratio of 0.125 against *E. faecalis* and a dilution ratio of 0.25 against *C. albicans* and *C. glabrata*. Bacterial/fungal growth was seen in all control groups.

**Table 1** Minimum inhibitory concentration (MIC)

| Tested microorganisms | Dilution rate of PTW |
|---|---|
| *E. faecalis* | 0.125 |
| *C. albicans* | 0.25 |
| *C. glabrata* | 0.25 |

**The efficacy of PTW in infected root canal models**

The results are presented in Table 2. No growth of residual microorganisms was seen in any of the infected root canal models in the *E. faecalis* and *C. albicans* groups, even without prewashing. In the *C. glabrata* group, growth of residual microorganisms was seen in two samples in which prewashing with citrate buffer was not performed; however, no growth was seen in any of the groups in which prewashing was performed. Growth was seen in all control groups. The results of the statistical analysis showed significant efficacy of PTW in all test-infected root canal models.

**Table 2** Success rate of sterilization in the models of infected root canal *(%)

| Tested microorganisms | without pre-wash | | with pre-wash | |
|---|---|---|---|---|
| | Control (DW) | PTW | Control (Citric acid buffer) | PTW |
| *E. faecalis* | 0 (n = 4) | 100 (n = 10) | 0 (n = 4) | Not done |
| *C. albicans* | 0 (n = 4) | 100 (n = 16) | 0 (n = 4) | Not done |
| *C. glabrata* | 0 (n = 4) | 87.5 (n = 16) | 0 (n = 4) | 100 (n = 10) |

*The rate of root canals which did not detect the microorganisms

**The efficacy of PTW *in situ* models**

No growth of residual microorganisms was seen in any of the experimental groups of rat molars (Table 3). Meanwhile, bacterial/fungal growth was seen in all control groups. The results of this statistical analysis showed significant efficacy of root canal irrigation with PTW in the experimental groups compared with the control groups. In addition, no changes were seen in oral mucosal tissue that came into contact with PTW that overflowed after root canal irrigation.

**Table 3** Success rate of sterilization in the models of Wistar rats *(%)

| Tested microorganisms | Control (DW) | PTW without pre-wash | with pre-wash |
|---|---|---|---|
| *E. faecalis* | 0 (n = 6) | 100 (n = 6) | Not done |

*The rate of root canals which did not detect the microorganisms

## Discussion

The application of LTAPP to living organisms for skin disinfection and other purposes is being studied due to the hypoallergenic nature of its sterilizing effect [6,7]. The sterilizing effect on intraoral microorganisms and application to the root canal of LTAPP are also being reported in the field of dentistry [15-18]. However, these applied studies involve direct irradiation with LTAPP, which makes practical application of LTAPP difficult for reasons including a required long irradiation time and the accompanying generation of ultraviolet light, heat, and harmful radicals. Meanwhile, Usui et al. [11] report achieving a dramatic sterilizing effect against bacteria in carious dentin in a short period of time by using direct plasma irradiation and the "reduced-pH method," thereby demonstrating the potential of plasma for clinical application. Prior to the present study, we therefore evaluated the *in vitro* sterilizing effect of direct plasma irradiation in *E. faecalis* infected root canals for the purpose of applying plasma to root canal disinfection. When we directly irradiated infected root canal models filled with citrate buffer with plasma in the direction of the tooth axis, we did not see a sufficient sterilizing effect against *E. faecalis* within the root canals. This is likely because convection of active species is unlikely to occur within root canals with a complex microstructure. In the application of plasma sterilization to root canal disinfection, clinical application time, reliable bactericidal activity, and access to microstructures are all necessary. To solve these problems, in the present study, the efficacy of plasma in root canal disinfection was investigated by using PTW generated through the application of LTAPP as a root canal irrigant.

Three tested microorganisms were chosen: *E. faecalis*, *C. albicans,* and *C. glabrata*. *E. faecalis* is a bacterium with low drug susceptibility among intraoral microorganisms. It is frequently detected in infected root canals with refractory apical periodontitis and is reported to be involved in the acute transformation of apical periodontitis [19]. To date, *E. faecalis* has often been used in studies of the sterilizing effect of plasma irradiation [17,18]. *C. albicans* is frequently detected within the oral cavity and is resistant to various agents for root canal therapy including calcium hydroxide [20]. It has also been detected in infected root canals with refractory apical periodontitis with persistent percussion pain. *C. glabrata* is the next most frequently detected fungus in the oral cavity, and both fungi are typical oral fungi.

MIC measurements were performed to confirm the bactericidal activity of PTW against the tested microorganisms and if this bactericidal activity is concentration-dependent. The results showed that PTW had a sterilizing effect against all tested microorganisms (Table 1), and that this effect occurred at different

concentrations between *E. faecalis* and *Candidas*. The reason for this difference is likely the larger number of *C. albicans* and *C. glabrata* cells than *E. faecalis*, which require a larger amount of active species to achieve sterilization. Yamazaki et al. also reported seeing greater resistance of *C. albicans* than *E. faecalis* in sterilization by direct plasma irradiation [10]. Furthermore, superoxide dismutase is a known factor behind the decomposition of active oxygen, and its production volumes and types are greater in eukaryotes than in prokaryotes, which likely explains the higher MIC of *C. albicans* and *C. glabrata* as compared with *E. faecalis*.

In creating the infected root canal models, the root canals were prepared up to size #60 in order to form root canals that were as uniform as possible. Irrigation with PTW alone had an effect below the detection limit in all *E. faecalis* and *C. albicans* groups (Table 2). The detection limit was set as the minimum concentration at which growth was detectable. Pre-measured detection limit concentration was 4.9 CFU/10 µL for *E. faecalis*, 0.2 CFU/10 µL for *C. albicans*, and 0.6 CFU/10 µL for *C. glabrata*. Fungal growth was seen in two samples in the *C. glabrata* group that were only irrigated with PTW. PTW shows strong bactericidal activity under reduced pH conditions, but its sterilizing effect can diminish when the pH rises as a result of the decalcification action of citrate buffer on root canal dentin. Thus, when irrigating with PTW was performed after one minute of prewashing with citrate buffer, there was an effect below the detection limit in all samples, even in the *C. glabrata* group. *C. glabrata* is reported to have a lower susceptibility to azole antifungals than *C. albicans*; however, the detailed mechanism remains unclear [21]. The difference in susceptibility to PTW may also be attributable to a similar mechanism.

The purpose of the *in situ* study using rat molars was to confirm the effects of PTW on microstructures in experimental systems closer to clinical reality. The tested microorganism used was *E. faecalis*, and sufficient bacterial seeding was achieved by moving the root canal preparation instrument vertically up and down. An effect of irrigation with PTW below the detection limit was seen in all root canals, and no effect was seen in the oral mucosal tissue that came into contact with PTW during the experiment. It suggests that the residual activity of PTW disappeared immediately.

In current clinical practice, chemical root canal cleaning requires removal of objects including hard tissue shards resulting from root canal preparation, smear layers, and residual pulp tissue. Sodium hypochlorite is typically used to dissolve soft tissue, while ethylenediaminetetraacetic acid (EDTA) is typically used to dissolve hard tissue. Citrate buffer is added to PTW to adjust the pH. Citric acid has a chelating action and mineral solubility, leading to reports of studies in which citrate buffer is applied to root canals to remove smear layers [22]. Due to the potential additional effect of citrate buffer, irrigating with EDTA or similar substances was not performed in preparation of infected root canal models or rat molar root canals in the present study when observing the efficacy of PTW as a root canal irrigant. Reduction in efficacy of PTW in the *C. glabrata* group (Table 2) was thought to be attributable to the rise in pH that occurs as a result of the decalcification action of citric acid. Thus, to achieve a reliable effect, prewashing with citrate buffer or irrigating with a sufficient amount of PTW is needed.

Sufficient caution also needs to be paid to the effect of PTW on healthy dentin and periodontal tissue during root canal irrigation. Sodium hypochlorite, which is frequently used in clinical practice, is also reported to have an effect on dentin and periodontal tissue, and reports of accidents resulting from extravasation of sodium hypochlorite outside of the apical foramen are not uncommon [4,23-25]. On the other hand, PTW has a strong bactericidal activity and a short half-life of the active species that it contains, which suggests that it is a very safe

root canal irrigant with low residual activity. This property of PTW, wherein it detoxifies within a short period of time, which has not been present in conventional disinfectants, gives PTW a strong advantage as a disinfectant that can be applied to living organisms.

PTW used in the present study was confirmed to both have a sterilizing effect on microorganisms in the root canal after about one minute of action and the ability to reach root canal microstructures. PTW can also be cryopreserved at −80°C, which was additionally confirmed to have no impact on its sterilizing effect during use after thawing [12]. Studies and the scope of clinical application are likely to expand, because PTW combines both long-term storability and convenience when moved. A future study is needed to investigate the specific effects of PTW on hard and soft tissues and to confirm the biological safety of PTW for practical application.

In conclusion, PTW generated using LTAPP exhibited a sterilizing effect against *E. faecalis, C. albicans,* and *C. glabrata*, which are sometimes detected in apical periodontitis. PTW also showed a sterilizing effect in molars of rat. Moreover, the lack of any observed effect on oral mucosal tissue suggested that PTW is an effective root canal irrigant.

# References

1. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983; 55: 307-12.
2. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, et al. Antimicrobial activity of varying concentration of sodium hypochlorite on the endodontic microorganisms Actinomyces israeli, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J 2004; 37: 438-46.
3. Bowden JR, Ethunandan M, Brennan PA. Life-threatening airway obstruction secondary to hypochlorite extrusion during root canal treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101: 402-4.
4. Kleier DJ, Averbach RE, Mehdipour O. The sodium hypochlorite accident: experience of diplomates of the American Board of Endodontics. J Endod 2008; 34: 1346-50.
5. Heinlin J, Morfill G, Landthaler M, Stolz W, Isbary G, Zimmermann JL, et al. Plasma medicine: possible applications in dermatology. J Dtsch Dermatol Ges 2010; 8: 968-76.
6. Daeschlein G, Scholz S, Ahmed R, von Woedtke T, Haase H, Niggemeier M, et al. Skin decontamination by low-temperature atmospheric pressure plasma jet and dielectric barrier discharge plasma. J Hosp Infect 2012; 81: 177-83.
7. Takai E, Hirano A, Shiraki K. Chemical modification of amino acids by atmospheric-pressure cold plasma in aqueous solution. J Phys D Appl Phys 2014; 47: 1-15.
8. Rad ZS, Davani FA. Non-thermal atmospheric pressure dielectric barrier discharge plasma source construction and investigation on the effect of grid on wound healing application. Clin Plasma Med 2016; 4: 56-64.
9. Ikawa S, Kitano K, Hamaguchi S. Effects of pH on bacterial inactivation in aqueous solutions due to low-temperature atmospheric pressure plasma application. Plasma Process Polym 2010; 7: 33-42.
10. Yamazaki H, Ohshima T, Tsubota Y, Yamaguchi H, Jayawardena JA. Microbicidal activities of low atmospheric pressure plasma jet on oral pathogens. Dent Mater J 2011; 30: 384-91.
11. Usui E, Ohshima T, Yamazaki H, Ikawa S, Kitano K, Maeda N, et al. Plasma sterilization of caries-infected dentin model with reduced-pH method. Jpn J Conserv Dent 2015; 58: 101-8.
12. Ikawa S, Tani A, Nakashima Y, Kitano K. Physicochemical properties of bactericidal plasma-treated water. J Phys D Appl Phys 2016; 49: 401-25.
13. Tasaki T, Ohshima T, Usui E, Ikawa S, Kitano K, Maeda N, et al. Plasma treated water reduced cariogenic bacteria in infected dentin model using Streptococcus mutans, Dent Mater J 2017; in press 10.4012/dmj.2016-358.
14. Kawai S, Takagi Y, Kaneko S, Kurosawa T. Effect of types of mixed anesthetic agents alternate to ketamine in mice. Exp Anim 2011; 60: 481-7.
15. Goree J, Liu B, Drake D, Stoffels E. Killing of S. mutans bacteria using a plasma needle at atmospheric pressure. IEEE Trans Plasma Sci 2006; 34: 1317-24.
16. Rupf S, Lehmann A, Hannig M, Schäfer B, Schubert A, Feldmann U, et al. Killing of adherent oral microbes by a non-thermal atmospheric plasma jet. J Med Microbiol 2010; 59: 206-12.
17. Du T, Shi Q, Shen Y, Cao Y, Ma J, Lu X, et al. Effect of modified nonequilibrium plasma with chlorhexidine digluconate against endodontic biofilms in vitro. J Endod 2013; 39: 1438-43.
18. Li Y, Sun K, Ye G, Liang Y, Pan H, Wang G, et al. Evaluation of cold plasma treatment and safety in disinfecting 3-week root canal Enterococcus faecalis biofilm in vitro. J Endod 2015; 41: 1325-30.
19. Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod 1990; 16:

        580-8.
20. Velera MC, Rego JM, Jorg AOC. Effect of sodium hypochlorite and five intracanal medications on Candida albicans in root canals. J Endod 2001; 27: 401-3.
21. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Bijie H, Dzierzanowska D, et al. Results from the ARTEMIS DISK global antifungal surveillance study, 1997-2005: an 8.5-year analysis of susceptibilities of Candida species and other yeast species to fluconazoleand voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol 2007; 45: 1735-45.
22. Baumgartner JC, Brown CM, Mader CL, Peters DD, Shulman JD. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. J Endod 1984; 10: 525-31.
23. Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int Endod J 2001; 34: 120-32.
24. Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine −mechanical, chemical and structural evaluation. Int Endod J 2007; 40: 786-93.
25. Ghisi AC, Kopper PM, Baldasso FE, Stürmer CP, Rossi-Fedele G, Steier L, et al. Effect of superoxidized water and sodium hypochlorite, associated or not with EDTA, on organic and inorganic components of bovine root dentin. J Endod 2015; 41: 925-30.

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