Entrococcus faecalis Elimination in Root Canals Using Silver Nanoparticles, Photodynamic Therapy, Diode Laser, or Laser-activated Nanoparticles: An *In Vitro* Study

Farzaneh Afkhami, DDS, MSc,[*,†] *Saba Akbari, DDS,*[‡] *and Nasim Chiniforush, DDS, PhD*[§]

Abstract

Introduction: The aim of this study was to compare the efficacy of silver nanoparticles (AgNPs), an 810-nm diode laser (DL), conventional photodynamic therapy (PDT) with the use of indocyanine green (ICG) photosensitizer, and modified PDT with the use of AgNPs for the disinfection of root canals inoculated with *Enterococcus faecalis*. **Methods:** The root canals of 65 extracted human single-rooted teeth were prepared, and *E. faecalis* was incubated in the root canals for 4 weeks. The teeth were then randomly divided into the following 4 experimental groups: the DL group: 810-nm DL irradiation (1 W, 4 times for 10 seconds), the AN group: 5 minutes of irrigation with 5 mL AgNPs (100 ppm), the ICG/DL group: conventional PDT with ICG (1 mg/mL)/810-nm DL (200 mW, 30 seconds), and the AN/ICG/DL group: modified PDT with AgNPs/ICG/810-nm DL (200 mW, 30 seconds). There was also a control group, which consisted of 5 minutes of irrigation with 5 mL 2.5% sodium hypochlorite ($n = 9$). Samples were obtained from dentin chips before and after the interventions. A reduction in colony count was assessed by counting the colony-forming units. **Results:** Significant reductions were noted in *E. faecalis* colony counts in all groups ($P < .05$). The greatest reduction in colony count (99.12%) was noted in the AN/ICG/DL group (AgNPs/ICG/810-nm diode laser); however, the differences in this respect between the AN/ICG/DL group and the DL (97.41%), AN (94.42%), and control groups (94.61%) were not significant ($P > .05$). **Conclusions:** PDT with ICG, an 810-nm diode laser, and AgNPs has the potential to be used as an adjunct for disinfection of the root canal system. *(J Endod 2016;■:1–4)*

Key Words

Biofilm, disinfection, *Enterococcus faecalis*, indocyanine green, laser, nanoparticles, root canal

Significance

Photodynamic therapy with indocyanine green photosensitizer, an 810-nm diode laser, and silver nanoparticle suspension has high potential to be used as an adjunct for disinfection of the root canals inoculated with *Enterococcus faecalis*.

Mechanical debridement and cleaning of the root canal system (RCS), the use of irrigants, and microbial control and filling of the RCS are the main factors influencing the outcome of endodontic treatments (1). The complex anatomy of the RCS (ie, the presence of isthmuses, accessory canals, and dentinal tubules) enables the survival of bacteria after the completion of conventional cleaning of the RCS (2). Instrumentation alone cannot completely eliminate the bacteria from the RCS. Thus, chemical agents and different disinfection techniques are used for further decreasing the intracanal bacterial count (3). Mechanical root canal cleaning alone is capable of decreasing the bacterial count by 100- to 1000-folds; however, it cannot completely eliminate the bacteria from the RCS (4). *Enterococcus faecalis* is a gram-positive anaerobic cocci responsible for most cases of endodontic treatment failures (5). A previous study showed that *E. faecalis* was isolated from 18% of the primary endodontic infections and 67% of cases of endodontic failures (4). *E. faecalis* triggers the body immune response and impairs the function of lymphocytes (5). It is capable of surviving in inadequate nutritional conditions and can stay viable as a single microorganism (6). It can penetrate into the dentinal tubules and is capable of biofilm formation (7). Bacterial biofilm is highly resistant to conventional irrigants because of formation of the extracellular polymeric matrix by this microorganism (8). Moreover, bacterial biofilm protects the bacteria from the immune system and provides nutrients; resultantly, it increases the resistance of microorganisms (9).

Various irrigants at variable concentrations such as sodium hypochlorite (NaOCl) and chlorhexidine are used as an adjunct to conventional mechanical root canal cleaning methods (10). However, intracanal irrigants are only effective when in contact with the surface, and they cannot penetrate deep into the surfaces because of anatomic barriers (3). NaOCl can penetrate into the dentinal tubules by 130 μm, whereas bacteria can penetrate into the dentinal tubules by 1000 μm (11). Silver nanoparticles (AgNPs) can also be used for disinfection because of their optimal antimicrobial properties. They are effective against many microorganisms including *E. faecalis* (12). Nanoparticles have polycationic/polyanionic properties with a high surface area and positive charge density, which enhance their antibacterial activity (7, 13). High-power lasers are among

From the *Department of Endodontics, School of Dentistry; †Dental Research Center, Dentistry Research Institute; ‡School of Dentistry, International Campus; and §Laser Research Center of Dentistry, Dentistry Research Institute; Tehran University of Medical Sciences, Tehran, Iran.

Address requests for reprints to Dr Farzaneh Afkhami, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, International Campus, Mahan Ave, Khaniabadno Blv, Tehran, Iran. E-mail address: f-afkhami@tums.ac.ir or farzaneh_afkhami@yahoo.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.08.029

the newly introduced techniques for root canal disinfection (14). Evidence shows that diode lasers (DLs) can decrease the intracanal bacterial count and penetrate 500 μm into the surface. Moreover, application of a DL in conjunction with irrigants has shown more favorable results. A DL has the advantage of penetrating into the anatomic irregularities of the RCS. A study showed that an 810-nm DL obstructed the dentinal tubules and decreased the *Escherichia coli* and *E. faecalis* bacterial counts (9).

Photodynamic therapy (PDT) is an adjunct method for the inactivation of bacteria (8). In PDT, a reaction occurs between light and a photosensitizer in the presence of oxygen. This reaction generates reactive oxygen species and causes oxidative damage to the respective cells (5). PDT has the advantage of specifically targeting the microorganisms without affecting the host (8). There is a possibility that this method improves dentin strength via increasing the cross-links in collagen fibers (15). Indocyanine green (ICG) has gained special attention as a new photosensitizer. ICG has low host tissue toxicity, high absorption in the near-infrared (IR) spectrum, and fungal and bacterial elimination efficacy and causes powerful photosensitized cellular damage. It is generally believed that ICG affects the target cell mainly through a photothermal effect rather than a photochemical effect and performs its bactericidal effect through oxidative stress (16, 17). ICG can be beneficial because of its peak absorption at 810 nm, which coincides with the commercially available dental DLs. Moreover, this wavelength has more penetration depth than red lasers used for toluidine blue and methylene blue, which is favorable in the elimination of bacteria in root canal therapy (17, 18).

This *in vitro* study aimed to compare the efficacy of an 810-nm DL, PDT with ICG photosensitizer in conjunction with an 810-nm DL, AgNPs, and modified PDT with AgNPs for decreasing the *E. faecalis* count in the RCS. The study hypothesis was that the modified PDT with the use of AgNPs would have greater efficacy than conventional PDT for the elimination of *E. faecalis*.

Materials and Methods

Preparation of Teeth

This study was conducted on 65 single-canal freshly extracted human teeth. The teeth were radiographically confirmed to have a single canal. Roots were cut at a 14-mm distance from the apex using a diamond disc. The working length was considered 1 mm short of the apical foramen (13 mm). Root canals were instrumented using ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) up to F4 (master apical file) along with the application of RC-Prep (Premier, Norristown, PA). In between the use of rotary files, canals were rinsed with 2.5% NaOCl (Barf, Paksan Co, Tehran, Iran) using a 1-mL insulin syringe and 30-G needle (Soha Co, Tehran, Iran). After completion of instrumentation, the canals were rinsed with 1 mL 17% EDTA, 5 mL normal saline, and 1 mL NaOCl, respectively, each for 3 minutes using a 30-G insulin syringe to remove the smear layer. Eventually, all canals were rinsed with 5 mL saline solution. The apical foramen was then sealed with self-cure glass ionomer (GC Co, Tokyo, Japan), and the root surfaces were covered with 2 layers of a nail varnish. The teeth were then transferred into 2-mL microtubes and autoclaved at 121°C for 15 minutes.

Bacterial Inoculation of Root Canals

E. faecalis standard strains (American Type Culture Collection 29212) were cultured in 5 mL brain-heart infusion (BHI) broth (33 g/1 L; Merck, Darmstadt, Germany) and incubated at 37°C for 24 hours. Next, 100 μL sterile BHI was added to sterile teeth in microtubes, and 30 μL of the culture medium containing approximately 10^9 *E. faecalis* bacteria was also added into the canals. The specimens were then incubated at 37°C for 4 weeks to form biofilm, and, every 2 days, 30 μL of the suspension (BHI containing *E. faecalis*) was aspirated from the canal and replaced by fresh sterile BHI medium under sterile conditions.

Experimental Procedures

The specimens were randomly divided into 4 experimental groups ($n = 14$) and a control group ($n = 9$) as follows:

1. Group DL (810-nm DL): the teeth were irradiated with an 810-nm DL (ARC Laser, Nürnberg, Deutschland) (1 W, CW). A DL with a 200-μm diameter bur tip was used 1 mm short of the apex and moved from the apex toward the coronal part in a rotary motion for 10 seconds. This process was repeated 4 times with 15-second intervals.
2. Group AN (AgNPs/100 ppm): root canals were rinsed with 5 mL AgNP suspension (Nanopoosheshfelez, Tehran, Iran) at a 100-ppm concentration (the average particle size was 30 nm), and the solution remained in the root canal for 5 minutes and then was aspirated by a 30-G insulin syringe.
3. Group ICG/DL (ICG/810-nm DL): root canals were filled with ICG (Pulsion Medical Systems AG, Munich, Germany) at the concentration of 1 mg/mL. After 5 minutes, the canals were irradiated with an 810-nm DL (200 mW, CW) for 30 seconds with a bulb-shaped tip.
4. Group AN/ICG/DL (AgNPs/ICG/diode): a suspension of 100 ppm AgNP suspension and ICG with a 1:40 ratio was injected into the root canals using a 30-G insulin syringe and irradiated with an 810-nm DL as in group ICG/DL.
5. Group N (2.5% NaOCl): root canals were rinsed conventionally with 5 mL 2.5% NaOCl, and the solution remained in the root canals for 5 minutes. Next, 1 mL 5% sodium thiosulfate was injected into the root canals by a 30-G insulin syringe to neutralize NaOCl and remained in the root canals for 30 seconds.

Microbiological Procedures

Bacterial samples were obtained of each specimen at baseline and after each intervention protocol.

Primary Sampling. A baseline microbial sample of the root canal was taken just before canal disinfection. For primary sampling, BHI was aspirated from inside the root canals using a 12-mm, 30-G syringe. Then, the root canals were filled with sterile saline solution using a 30-G syringe, and dentin was scraped from inside the canals using a #40 Hedstrom file (Mani Inc, Tochigi, Japan). A #40 sterile paper point (Gapadent Co, Hamburg, Germany) was placed inside the canals for 60 seconds and was then transferred into a sterile microtube containing 1 mL saline solution and vortexed for 20 seconds. A serial 10-fold dilution up to $1:10^2$ was prepared. Finally, 100 μL of each dilution was spread onto blood agar plates (30 g/1 L distilled water in 50 mL sheep blood) and incubated at 37°C for 24 hours. The number of colony-forming units was counted afterward and transformed into actual counts according to the dilution factor.

Final Sampling. To standardize all groups, root canals were irrigated with 5 mL sterile saline, which remained in the root canals for 30 seconds, and then the canals were dried using paper points. Samples were obtained from inside the canals using Mtwo rotary files (35/0.4%; Dentsply, Munchen, Germany) at the working length operating at 300 rpm and 1.2-Ncm torque as recommended by the manufacturer for 1 minute. Under sterile conditions, files were transferred into sterile microtubes containing 1 mL of the saline solution and vortexed for 20 seconds. Bacterial culture was prepared as described for primary sampling.