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Photodynamic Therapy for Root Canals Infected with *Enterococcus faecalis*

Michelle Bernardes Fonseca, D.D.S., M.Sc.,[1] Paulo Odair Tessare Júnior, D.D.S., M.Sc.,[1]
Raul Capp Pallota, D.D.S., M.D.Sc., Ph.D.,[2] Hely Ferreira Filho, Ph.D.,[3]
Odilon Victor Porto Denardin, Ph.D.,[4] Abrão Rapoport, Ph.D.,[5] Rogério Aparecido Dedivitis, Ph.D.,[6]
João Francisco Veronezi, M.Sc.,[7] Walter João Genovese, D.D.S., M.D.Sc., Ph.D.,[8]
and Ana Lucia Franco Ricardo, D.D.S., M.D.Sc.[9]

Abstract

Objective: The aim of this study was to investigate the effects of photodynamic therapy (PDT) on endodontic pathogens by evaluating the decrease in numbers of *Enterococcus faecalis* colonies in the canals of extracted human teeth.
Background Data: Failure in endodontics is usually related to inadequate cleaning and disinfection of the root canal system. This is due to the establishment of microorganisms in areas where the instruments and chemical agents used during root canal preparation cannot eliminate them. PDT is a complementary therapeutic method that could be used to eliminate these remaining bacteria. PDT is a process in which radiation acts on a dye that is applied to the target organism, resulting in bacterial death.
Materials and Methods: Forty-six uniradicular teeth had their canals contaminated with bacteria and were incubated for 48 h at 35°C. After that, the teeth were divided into a control group (CG) and a test group (TG). The 23 CG teeth did not undergo any intervention, whereas in the TG the teeth received a solution of 0.0125% toluidine blue for 5 min followed by irradiation using a 50-mW diode laser (Ga-Al-As) at a wavelength of 660 nm. Bacterial samples were taken before and after irradiation. In each of the samples, the number of colony-forming units (CFU) was counted.
Results: The mean decrease in CFU was 99.9% in the TG, whereas in the CG an increase of 2.6% was observed.
Conclusion: PDT was effective as a bactericidal agent in *Enterococcus faecalis*–contaminated root canals.

Introduction

The success of endodontic therapy has dramatically increased with the development and adoption of new technologies. In spite of this, most failures are related to inadequate cleaning and disinfection of the root canal system.[1,2] These remaining bacteria can become established and proliferate in the root canal, inside the dentin, or in the periapical area.[3] These bacteria may also invade the system when there is impairment of the blood supply and also after previous infection by caries or periodontal problems.[4] One of the aims of endodontic therapy is the decontamination of the root canal system. However, microorganisms can establish themselves in areas where the instruments and chemical agents used during root canal preparation cannot eliminate them, such as in the intercanal isthmus and accessory canals, as well as in the recesses and diverticula of instrumented main canals.

Currently, anaerobic bacteria are isolated in 60–90% of the cases of symptomatic teeth. One of the most common bacteria associated with endodontic failures is *Enterococcus faecalis*,[5] which is capable of surviving without nutrients for long periods of time. Additionally, this microorganism may infect the length of the dentinal tubules,[6] thus establishing the need for augmentation of the decontamination with drugs that increase the antibacterial action and

[1]Hospital Heliópolis, São Paulo, [2]University of São Paulo School of Dentistry, São Paulo [3]Laboratory of Clinical Pathology, Hospital Heliópolis, São Paulo, [4]Federal University of São Paulo, São Paulo, [5]Department of Surgery, University of São Paulo Medical School, São Paulo, [6]Department of Otorhinolaryngology–Head and Neck Surgery, Federal University of São Paulo, São Paulo, [7]Head of Administration, Hospital CEMA, São Paulo, [8]Professor of Semiology, University of São Paulo Dentistry School, São Paulo, [9]São Leopoldo Mandic University, São Paulo, Brazil.

allow closer contact between the decontaminating agent and the medium.

A new alternative method for disinfecting root canals is the use of photodynamic therapy (PDT).[7] PDT is based on associating photosensitizing agents with light within the visible spectrum. Initially described for promoting death of cancer cells, PDT has recently also been used in dentistry to kill microorganisms.[7] The laser radiation is used to activate a dye that is applied to the target. Sensitization of the target occurs in two distinct ways: by means of a redox system that promotes a cytotoxic response and generates free radicals and cell death after the interaction with the medium; or through energy release that transforms the molecular oxygen into singlet oxygen, which is cytotoxic for microorganisms.[7]

There is limited knowledge about the behavior of PDT in the interior of root canals. Thus this study had the objective of evaluating the *in vitro* action of laser light associated with a photosensitizing dye on *E. faecalis*, with the aim of assessing the use of PDT as an adjuvant to endodontic treatment for root canal decontamination.

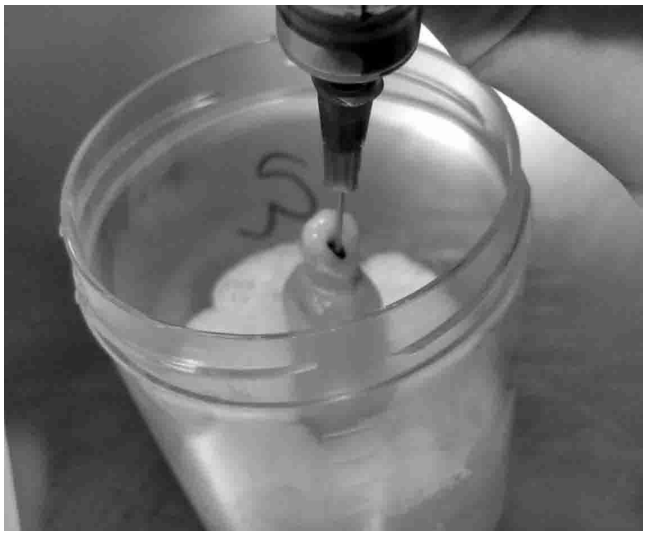

FIG. 2. Radicular canal receiving 0.0125% toluidine blue.

Materials and Methods

Forty-six uniradicular human teeth with an average length of 21 mm were used in this investigation. Access cavities were made using round #2 or #3 diamond burs (Microdent Materiais Odontológicos, Ribeirão Preto, Brazil). The canals were then explored using a size 10K file (Dentsply Maillefer®, Ballaiges, Switzerland). The working length was determined by subtracting 1 mm from the length of the file at the moment the tip of the instrument was seen at the apical foramen.

The teeth were instrumented using the crown-down technique in accordance with Machado[8] until reaching a size 50K file. Throughout instrumentation, irrigation using 3 mL of 0.5% sodium hypochlorite was performed between the files. At the end of root canal preparation, all the teeth were irrigated with 1.8 mL of 17% EDTA (Formula & Ação Farmácia Magistral, São Paulo, Brazil) followed by 10 mL of 0.5% sodium hypochlorite. The canals were dried with paper points (Dentsply Maillefer). The roots were then coated with thermally activated acrylic resin in order to protect them from external contamination.

To check the accuracy of the sterilization procedure, five uniradicular teeth were wrapped in surgical paper (Baumer®, Mogimirim, Brazil) and autoclaved at 121°C for 15 min (Sterilizer Dabi Atlante®, Ribeirão Preto, Brazil). Subsequently, their canals received a sterile brain heart infusion (BHI) solution. After 24 h, a size 50 sterile paper point was inserted into the canal for 20 sec; this paper point was then placed in a Petri dish with bile azide agar. After the incubation period, bacterial growth was not observed.

FIG. 1. The wet chambers developed by the author for use in this study.

The 46 teeth were then divided into two groups of 23: the control group (CG) and the test group (TG). The creation of a control group that did not receive treatment with PDT served to check whether the contaminated teeth would be influenced by the medium that they were in. In such an event, colony growth might undergo some modification over a period of time, in this case the duration of PDT application to the test group. All the root canals were filled with 10 μL of ATCC 29212 *Enterococcus faecalis* suspension BHI medium, using an automatic pipette provided by the Microbiology Laboratory of Hospital Heliópolis. The teeth were placed in semi-covered wet chambers (Fig. 1) and incubated at 35°C for 48 h, so that the microorganisms could penetrate the dentinal tubules.[9] The CG received no PDT treatment and the teeth were incubated for 48 h. Meanwhile, in the TG the canals were filled with an aqueous solution of 0.0125% toluidine blue that was kept inside the root canals for 5 min (Fig. 2). The canals were then washed with 9% sterile saline solution.

In the present study, a multi-functional gallium-aluminum-arsenide (Ga-Al-As) diode laser/LED unit (Eccofibras, São Paulo, Brazil) was used. The LED mode remained deactivated throughout the experiment.

The laser had a wavelength of 660 nm and an output power of 50 mW, and the emission of light was continuous. The laser had an acrylic optical fiber with a diameter of 600 μm that had been subjected to autoclaving at 121°C for 20 min for disinfection. This fiber optic provided an energy fluence of 400 J/cm^2, such that the energy deposited was 6.4 J, according to the "PDT function" of the apparatus. The irradiation method involved helicoidal traction movements of the fiber, from apical to cervical, lasting 320 sec (Fig. 3).

Bacterial samples were taken before and after the therapy from the test group and before and after 48 h of incubation from the control group. A size 50 paper point was inserted into the canal for 20 sec in accordance with the procedure described by Hartroth et al.[6] (Fig. 4) and then placed in 10 mL of sterile saline solution. The tubes were agitated in a

FIG. 3. Optical fiber with a diameter of 600 μm positioned inside the radicular canal.

Vortex® (Eletrolab, São Paulo, Brazil) device for 30 sec to disaggregate the microbial pellets. From each suspension, an aliquot of 0.1 mL was transferred to a test tube containing 0.9 mL of saline solution, and the method of successive dilutions for counting the colony-forming units (CFU) was then followed. Three dilutions (1:10, 1:100, and 1:1000) were carried out in bile azide agar medium. The plates were then incubated for 15 h at 35°C. The number of CFU/mL was determined by visual observation of the number of bacterial colonies that developed in the culture medium, using the 1:100 dilution.

Pos Combo Type 21 panels (Dade Behring®, Deerfield, IL) for gram-positive bacteria were used. These are commercial microdilution plates containing 26 substrates and 23 dehydrated antimicrobial agents that are used for identifying gram-positive cocci at species level and for determining their susceptibility to antimicrobial agents.

Identification of the bacteria was based on detecting changes in pH, use of substrates, and bacterial growth in the presence of antimicrobial agents after 16–44 h of incubation at 35°C, carried out using the automated MicroScan WalkAway-96 scanner (Dade Behring).

Confirmation of the identification of *E. faecalis* was also performed. After the incubation period, samples of the colonies from the agar plate were taken and identified by means of the MicroScan WalkAway identification equipment (MicroScan Dried Gram-Positive®).

The distribution of the study variables in relation to the study groups is presented in Table 1, as associations with simple descriptive functions. Because of the characteristics of the variables (the initial number of *E. faecalis* colonies and changes in the numbers of CFU following the procedures), non-parametric tests were used to investigate associations from before and after the procedures within the PDT and control groups separately (Wilcoxon test), and for comparing the numbers seen before and after the procedures between the groups (Mann-Whitney test). Statistical significance was set at $p < 0.05$.

Results

Table 1 shows the number of CFU before the procedure and 48 h post-PDT, and in the control group. The median calculated for the test group was 840,000 CFU in the pre-PDT samples and 700 CFUs in the post-PDT samples. This signifies a reduction of 99.9% after the use of PDT. Also, the mean calculated for the control group was 1,800,000 CFU at the first collection, and 1,950,000 CFU at the second collection, thus showing an increase of 2.6%. Thirteen of the 23 samples increased their CFU values.

Discussion

Instrumentation of infected root canals does not assure complete removal or inactivation of microorganisms. Because of anatomical variations and the bacterial characteristics of contamination, the use of chemical antibacterial agents (such as sodium hypochlorite, chlorhexidine, and calcium

FIG. 4. A size 50 absorbent paper cone introduced into the radicular canal.

Table 1. Distribution of the Study Variables in Relation to the Study Groups

Group	Mean initial count (CFU/mL)	Mean final count (CFU/mL)	Change in the number of viable cells (%)
Test group (23 teeth)	840,000	700	−99.9
Control group (23 teeth)	1,800,000	1,950,000	+2.6

Mann-Whitney Test: pre-PDT × pre-control ($p = 0.07$); post-PDT × post-control ($p < 0.001$).

hydroxide) is required to facilitate adequate decontamination of the root canal system. Sodium hypochlorite can be used as an irrigating agent during root canal preparation; reductions of up to 93.25% of the bacteria tested in the present study (*E. faecalis*) have been obtained.[10] It can also be used as an intracanal medication between sessions. However, no substance currently used has been able to kill all the remaining bacteria.[6,9,11] Persistence of bacteria or their byproducts inside the root canal system may compromise the prognosis for endodontically treated teeth.[11] Thus, the present study on the use of photodynamic therapy as a method for achieving decontamination of the root canal system was put forward.

In this investigation, the smear layer was removed to enable penetration of both the bacteria and the photosensitive dye.[12] The presence of the smear layer on the canal walls blocks the penetration of the drugs into the dentinal tubules.[9,13]

E. faecalis is a facultative anaerobic bacterium. It is one of the most common bacteria in persistent endodontic infections, refractory infected lesions,[8,14] and periapical biofilms.[4,13] This bacterial species is able to survive for long periods without nutrients. It invades dentinal tubules,[15] which provide this bacterium protection against the usual irrigating agents. This makes it difficult to eliminate *E. faecalis*, even in *in vitro* studies.[4,7,16,17] In the present investigation, after instrumentation and irrigation, the 46 root canals were filled with a suspension of *E. faecalis* and incubated at 35°C for 48 h, in accordance with the standards of the National Committee for Clinical Laboratory Standards (NCCLS). The incubation period provided enough time for the microorganisms to penetrate the dentinal tubules.[6]

Following this, PDT was applied inside the root canals. The radiation sources for PDT are low-power lasers that supply radiation at the appropriate fluence and wavelength for the different photosensitizing agents. This therapy may act as a bactericidal agent in endodontic treatment, but for this to be achieved, a system that can maximize its action needs to be used. In the present study, an efficient optical system was used with an optical fiber (600 μm in diameter) that was capable of directing the radiation. The root canals were instrumented up to a 50K file, which allowed the beam to get close to the root apex and the periapical region as well. In thin roots and curved canals, it is not possible to instrument up to a size 50K file; however, it is known that most of the microorganisms are in the cervical area of the canal,[18] and that the laser light is able to reach the apical third of the canal or even outside the canal in the periapical area by diffusion.

There are laser devices on the market that cover the entire visible and infrared spectrum. These devices might act on a large number of photosensitizing agents. The Ga-Al-As laser was used in this investigation. This device is a low-power laser that emits red light of wavelength 660 nm, and in the present study it was adjusted to a power of 50 mW, which has yielded good results in the literature.[19]

Except for black-pigmented species such as *Porphyromonas* and *Prevotella*,[18] most oral bacteria are unable to directly absorb laser light at the red portion of the spectrum. In these cases, administration of a photosensitizing agent to facilitate the therapy is necessary. The choice of toluidine blue as the photosensitizing agent was made because of its capacity to absorb light at wavelengths from 620–660 nm,[20] such as that from the red laser we used. Thus, it can be seen that it is important not only to choose the proper laser or dye separately, but also to choose the appropriate laser-dye combination. Although results of PDT studies have varied with regard to the laser used and the concentrations and types of dye, this therapeutic method has shown excellent results, as shown in the study by Garcëz et al.,[10] in which a reduction in *E. faecalis* of 99.2% was achieved using a blue photosensitizing agent in association with laser light of 685 nm.

Various light and drug parameters must be further explored in order to define the appropriate dosages for eliminating microorganisms in the root canal.[21]

Further studies should be carried out to investigate the clinical use of the proposed protocol, as well as the effects of photodynamic therapy on periodontal and periapical tissue. Also, dentin staining should be avoided by using low-concentration dyes. Because of the good results obtained from antibacterial evaluations, this should encourage further investigations of PDT, as well as the development of new devices that may decrease the existing limitations.

Conclusion

Our results demonstrate that photodynamic therapy is viable as a bactericidal agent in a tooth model contaminated with *Enterococcus faecalis*, but it did not totally eradicate the contaminating bacteria.

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Address reprint requests to:
Michelle Bernardes Fonseca, D.D.S., M.Sc.
Rua Apucarana 326 sala 92
03311-000 São Paulo SP, Brazil

E-mail: essencialsaude@yahoo.com.br