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The antimicrobial effectiveness of photodynamic therapy used as an addition to the conventional endodontic re-treatment: A clinical study

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KEYWORDS Endodontic re-treatment; Laser; Photodynamic therapy; Root canal infection	Summary <i>Background:</i> The purpose of the study was to evaluate the efficacy of antimicrobial photody- namic therapy (aPDT) used as an adjunct to the endodontic re-treatment in the eradication of microorganisms from previously filled root canals. <i>Methods:</i> The study sample consisted of 21 randomly selected patients with root filled and infected root canal system with chronic apical periodontitis on incisors or canines, who have had previously endodontic treatment. Microbiological samples from the root canals were collected after accessing the canal, following the endodontic re-treatment and after the aPDT procedure. During instrumentation, the root canals were irrigated with 2.5% sodium hypochlorite (NaOCl), and the final irrigation protocol included 17% ethylenediaminetetraacetic acid followed by NaOCl. Root canals were filled with a phenothiazinium chloride and irradiated with a diode laser ($\lambda = 660$ nm, 100 mW) for 1 min. Microbiological samples from the root canals were cultivated on selective plates, and the identification was done by micromorphology, macromorphology and different API strips as well as bacterial counts (colony forming units). <i>Results:</i> Fourteen bacteria species were isolated from the root canals initially, with a mean value of 4.57 species per canal. Although endodontic re-treatment alone produced a significant reduction in the number of bacteria species ($p < 0.001$), the combination of endodontic treat-
	reduction in the number of bacteria species ($p < 0.001$), the combination of endodontic treat- ment and aPDT was statistically more effective ($p < 0.001$). No bacteria were cultivated from the main root canals of 11 teeth.

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Conclusion: The results indicated that the aPDT used as an adjunct to the conventional endodontic therapy achieved a significant further reduction of intracanal microbial load. © 2014 Elsevier B.V. All rights reserved.

Introduction

The outcome of the endodontic treatment depends significantly on the elimination of bacterial population from the root canal, or maximum reduction in bacterial count to the levels compatible with the periradicular tissue healing [1]. Accepted treatment procedures to eliminate the intracanal infection include mechanical instrumentation. irrigation with different disinfectant agents, and application of interappointment dressing based on the calcium hydroxide [2]. Although this conventional technique is able to reduce the number of bacteria in the root canal, complete disinfection of the whole root canal system is difficult to achieve [3-5]. The reasons are the inability of instruments and irrigants to decontaminate irregular areas of the root canal system, especially the apical part of the canal, which can serve as a reservoir for the microorganisms [6-8]. As the persistence of microorganisms is one of the main causes of failed endodontic treatment outcome [9], additional antimicrobial treatment strategies have been recommended in order to eliminate and neutralize the remaining microorganisms and toxins [2].

Antimicrobial photodynamic therapy (aPDT) is an antimicrobial strategy which is based on the chemical interaction of a nontoxic photosensitive agent and a low power laser of particular wavelength. The excited photosensitizer reacts with the molecular oxygen from the environment in order to produce highly reactive oxygen species (ROS), which cause damage to the membrane and intracellular molecules including the proteins and the nucleic acids [10]. The photosensitizers with strong cationic charge bind and penetrate rapidly in the bacterial cells, without affecting the host cells viability [11]. Many in vitro [12,13] and ex vivo [14,15] studies using the aPDT have shown that this approach has the potential to maximize the root canal disinfection. Yet, there are still some controversies regarding the effect of the aPDT on the multibacterial biofilms in the root canals [16,17]. In addition, there are still a few clinical studies evaluating the efficacy of the aPDT used at the end of the conventional endodontic treatment [18-20].

The aim of this clinical study was to evaluate whether there was any additional antimicrobial effect of the aPDT, used at the end of the conventional one-session endodontic re-treatment, against the polymicrobial intracanal infection in cases of the persistent periapical periodontitis.

Materials and methods

Selection of the patients

A clinical study was conducted at the Department of Endodontics and Restorative Dentistry, School of Dental

Medicine, University of Zagreb between April 2012 and November 2012. The study protocol was approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb. Standards of the Helsinki Declaration were followed [21].

The study sample consisted of 21 patients (9 men and 12 women), aged between 20 and 45 years, who were randomly selected from a group of patients who had been referred to the Department of Endodontics and Restorative Dentistry, School of Dental Medicine (Zagreb, Croatia) for root canal re-treatment. A periapical radiograph was taken, with the long cone paralleling technique (Gendex, KaVo, Biberach, Germany), for each case to determine the presence of periapical radiolucency, that was at least $3 \text{ mm} \times 3 \text{ mm}$ in diameter, the canal morphology, and its length. The diagnosis in all cases was root-filled teeth and infected root canal system with chronic apical periodontits on incisors or canines. All of them had undergone endodontic therapy more than 2 years earlier. There was no overfilled case. All teeth were coronally restored, without evident direct exposure of the root canal filling material to the oral cavity at the time of the clinical examination. Selected teeth had no gingival recession, nor periodontal pockets greater than 4mm deep, measured by a graduated periodontal probe model 0703L-1 Williams (ASA Dental, Bozzano, Italy). The selected patients did not use antibiotics for at least four weeks before the initial treatment. Excluded from the study were patients with systemic diseases such as diabetes mellitus, chronic infections, rheumatoid arthritis or any other disease that could compromise the immune system, patients on immunosuppressive therapy, pregnant and nursing women. Also, patients with teeth, which were difficult to isolate adequately, were not included in the study. All selected patients signed an informed consent before undergoing the research procedure, agreeing to their participation in the study. The whole clinical protocol was carried out by the same practitioner. Microbiological samples were collected by another practitioner.

Endodontic procedures

Twenty-one single canal endodontically treated teeth with chronic apical peridodontitis were re-treated followed by the aPDT. Microbiological samples from the root canals were collected immediately after the accessing the canal, following endodontic re-treatment (standard therapy) and after the aPDT procedure (experimental therapy).

Following rubber dam isolation (Dental Dam, Coltene Whaledent, NY, USA), the access to the pulp chamber was performed with a sterile high speed diamond drill number 016 (Komet, Rock Hill, SC, USA) under water coolant. The surrounding area was cleansed with 3% hydrogen peroxide

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