Acta Odontologica Scandinavica. 2014; 72: 673–680

informa healthcare

ORIGINAL ARTICLE

In vitro activity of photoactivated disinfection using a diode laser in infected root canals

ULRICH SCHIFFNER[1]*, GEORG CACHOVAN[1]*, JOCHEN BASTIAN[1], ANTON SCULEAN[2] & SIGRUN EICK[2]

[1]*Center for Oral and Dental Medicine, Department of Restorative and Preventive Dentistry, University Center Hamburg-Eppendorf, Hamburg, Germany, and* [2]*Department of Periodontology, Laboratory of Oral Microbiology, Dental School, University of Bern, Bern, Switzerland*

Abstract

Objective. To investigate the lethal activity of photoactivated disinfection (PAD) on *Enterococcus faecalis* (ATCC 29212) and mixed populations of aerobic or anaerobic bacteria in infected root canals using a diode laser after the application of a photosensitizer (PS). ***Materials and methods.*** First, the bactericidal activity of a low power diode laser (200 mW) against *E. faecalis* ATCC 29212 pre-treated with a PS (toluidine blue) for 2 min were examined after different irradiation times (30 s, 60 s and 90 s). The bactericidal activity in the presence of human serum or human serum albumin (HSA) was also examined. Second, root canals were infected with *E. faecalis* or with mixed aerobic or anaerobic microbial populations for 3 days and then irrigated with 1.5% sodium hypochlorite and exposed to PAD for 60 s. ***Results.*** Photosensitization followed by laser irradiation for 60 s was sufficient to kill *E. faecalis.* Bacteria suspended in human serum (25% v/v) were totally eradicated after 30 s of irradiation. The addition of HSA (25 mg/ml or 50 mg/ml) to bacterial suspensions increased the antimicrobial efficacy of PAD after an irradiation time of 30 s, but no longer. The bactericidal effect of sodium hypochlorite was only enhanced by PAD during the early stages of treatment. PAD did not enhance the activity of sodium hypochlorite against a mixture of anaerobic bacteria. ***Conclusions.*** The bactericidal activity of PAD appears to be enhanced by serum proteins *in vitro,* but is limited to bacteria present within the root canal.

Key Words: *photoactivated disinfection, diode laser, Enterococcus faecalis, root canal infection, human serum albumin*

Introduction

Disinfection of the root canal system is crucial in endodontics. Mechanical instruments alone are not effective at reducing bacterial counts in the infected root canal due to the complex anatomy of the root [1,2]. Failed endodontic treatment often leaves bacteria within the root canal system. Photoactivated disinfection (PAD) is a novel method that is used to treat endodontic inflammation and other inflammatory diseases, such as periodontitis [3,4]. Photodynamic therapy (PDT) was originally developed as a therapy for pre-malignant diseases, tumors and other oral lesions [5]; however, PAD is increasingly being used in the field of dentistry [6]. PAD is effective at reducing bleeding on probing in patients undergoing periodontal maintenance [7]. It is also as efficient method as the local delivery of antibiotics in cases of early peri-implantitis [8]. One clinical study, focusing on endodontic treatment, showed that PAD eliminates bacteria from root canals [9].

PAD is based on the principle that a photoactivated substance (a photosensitizer; PS) is bound to a target cell (pathogenic bacteria in this case) and then activated by irradiation with light, at a specific wavelength; the resulting free radicals, such as singlet oxygen species, destroy the pathogenic bacteria [6,10]. PAD uses a non-toxic dye (a PS), which is activated by a visible light source. Methylene blue, toluidine blue and acridine orange are all potent PSs [11]. Both the dentine tubule fluid [12] and the gingival crevicular fluid contain serum, the main

Correspondence: Professor Dr Ulrich Schiffner, DMD, PhD, Center for Oral and Dental Medicine, Department of Restorative and Preventive Dentistry, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany. Tel: +49 40 7410 54000. Fax: +49 40 7410 55168. E-mail: schiffner@uke.de
*Both authors have made an equal contribution to the manuscript.

(Received 26 February 2013; accepted 21 January 2014)
ISSN 0001-6357 print/ISSN 1502-3850 online © 2014 Informa Healthcare
DOI: 10.3109/00016357.2014.898087

protein constituent of which is human serum albumin (HSA) [13]. The concentration of HSA in human plasma is between 3.5–5% (and up to 4% in inflammatory exudates) [14], where it exists in two states: mercaptoalbumin (reduced state) and non-mercaptoalbumin (oxidized state) [15]. The infected root canal system contains different tissues, bacteria and inflammatory exudates; the latter contains numerous proteins, including albumin [16]. A previous study shows that leakages along root canal fillings enabled bovine serum albumin (BSA) to penetrate into the root canal [17]. Indeed, several studies have used BSA to investigate the effects of albumin. BSA reduces the antimicrobial activity of chlorhexidine [16,18] and sodium hypochlorite [14] and appears to affect the efficacy of more modern antibacterial treatments, including PAD [11]. To the best of our knowledge, there are no reports examining the possible effects of HSA on the bactericidal properties of PAD.

Enterococcus faecalis is one of the most important micro-organisms responsible for failed root canal treatments [19,20]. However, using nucleic acid based techniques it has been shown that the microbiota causing treatment failures are more complex, often being a mixture of aerobes and anaerobes [21]. Therefore, the aims of this study were: (i) to examine the *in vitro* antibacterial activity of PAD against *E. faecalis* ATCC 29212 in the presence of serum or HSA; and (ii) to examine the ability of PAD, in combination with different dental irrigants, to disinfect root canals infected with *E. faecalis* or defined mixed cultures of aerobic or anaerobic bacteria.

Materials and methods

Specimen preparation

A total of 125 extracted human single-rooted permanent teeth, with neither an open apical foramen nor a distinct root curvature, were selected for the study. The crowns were removed with a diamond saw at a defined root length of 15 mm from the apex. Root canals were prepared in a standard manner using the Mtwo NiTi rotary system (VDW, Munich, Germany) according to the manufacturer's protocol. The single-length technique was employed, and the instruments (0.04 taper, sizes #10 to #40) were used to the full working length of the root canal. After sterilization at 121°C for 5 min, the prepared roots were coated with the self-curing resin, Paladur® (Heraeus, Hanau, Germany), to prevent any penetration of bacteria or diffusion of substrates through the dentine. Fifty prepared roots were used for the short-term experiments, whereas 75 prepared roots were used in the experiments lasting up to 4 days after application of the disinfectant (long-term experiments).

Microorganisms

Enterococcus faecalis ATCC 29212 was maintained by sub-cultivation on Columbia agar (BioMerieux, Cambridge, UK) containing 8% sheep blood. The bacteria were suspended in 0.9% v/v NaCl and the concentration adjusted to yield an optical density of 0.1 at a wavelength of 640 nm (Bio-Rad SmartSpec 3000™, Bio-Rad Laboratories, Philadelphia, PA).

In addition to monocultures of *E. faecalis* ATCC 29212, the root canals were inoculated with a mixed culture of aerobic bacteria mixture (*E. faecalis* WK16-1 and *Shewanella putrefaciens* WK16-2) or a mixed culture of anaerobic bacteria (*Actinomyces naeslundii* WK2-1, *Bifidobacterium adolescentis* WK2-2, *Peptostreptococcus* sp. WK2-3, and *Eggerthella lenta* WK2-4). Both mixtures were isolated from failed, infected human root canals. The bacteria were suspended in nutrient broth (brain heart infusion broth [Oxoid, Basingstoke, UK] for the aerobes and Schaedler broth [Oxoid] for the anaerobes) and inoculated into the root canals. The roots were then incubated for 3 days. The nutrient broth was changed daily.

Laser

A low power diode laser (PACT 200-Laser®; Cumdente Ltd, Tübingen, Germany) with a power output of 200 mW was used for irradiation. The light emitted from the laser had a wavelength of 632–644 nm, i.e. visible red light. For all experiments, laser light was applied to the root canals using 'PACT Light Guide Endo' tips. After loading the root canals with the photosensitizer, the tips were placed into the root canals until slight contact with the canal walls occurred, which was at a depth of ~5 mm.

Photosensitizer

PACT-200® solution (Cumdente Ltd, Tübingen, Germany), which contains toluidine blue as the PS, was used according to the manufacturer's instructions. For PAD, the PS was applied 2 min before exposure to the laser light.

Photoactivated disinfection of Enterococcus faecalis

E. faecalis samples were suspended in 0.9% v/v sodium chloride (NaCl), adjusted to OD_{640nm} = 0.1 and diluted 1:100 with 0.9% v/v NaCl (equivalent to a concentration of 5×10^6 bacteria/ml). Aliquots (10 µl) of each sample were placed in Eppendorf tubes and 10 µl of PS was added to half of them (10 µl of 0.9% v/v NaCl were added to the remaining tubes, which were used as 'laser only' controls). The tubes were then divided into eight groups: control, PS only, laser irradiation only (30 s, 60 s and 90 s) and PAD (PS plus laser irradiation for 30 s, 60 s or 90 s). After the respective treatments, 80 µl of 0.9% NaCl were added to each tube; 25 µl was then removed, serially 10-fold diluted and plated on Columbia agar plates containing 8% sheep blood. After incubating the plates for 24 h at 37°C, the number of colony forming units (cfu) was counted and expressed as Log_{10} cfu.

Next, bacterial suspensions were adjusted to OD_{640nm} = 0.2 and mixed (1:1 v/v) with inactivated human serum (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) or 0.9% NaCl. These mixtures were then mixed (1:1 v/v) with either PS or 0.9% NaCl (control tubes with and without serum, respectively). Again, the tubes were divided into eight groups: control groups (with or without serum at a final concentration 25%, v/v) and PAD groups (30 s, 60 s or 90 s, each with or without serum). After PAD, the samples were examined as described in the previous section. Finally, HSA (25 mg/ml or 50 mg/ml final concentration) was added to the samples instead of serum. The tubes were then split into 12 groups: no HSA, 25 mg/ml or 50 mg/ml HSA, each with its appropriate controls, and PAD (30 s, 60 s or 90 s and 0 s). After PAD, the tubes were processed as described in the previous section. All experiments were made in independent duplicates.

Photoactivated disinfection of infected root canals

For the short-term experiments, 50 roots were infected with *E. faecalis* ATCC 29212 and then divided into five groups (n = 10 per group). One group was left untreated (control). The roots in the other four groups were irrigated with 5 ml of 0.9% NaCl to mimic the mechanical effects of root canal irrigation. One group was not treated further (NaCl alone), one group was exposed to PAD (PS plus irradiation) for 60 s, one group was treated with the PS alone and one group was irradiated for 60 s alone.

Seventy-five roots were used for the long-term experiments (25 per experiment). After infection and incubation, the teeth were irrigated with 2.5 ml of 0.9% NaCl. Thereafter, the 25 roots used for each experiment were split into three groups: one group was used as a control (no further treatment; n = 5), one group was irrigated with 2.5 ml of 1.5% sodium hypochlorite solution (NaOCl; n = 10) and one group was irrigated with 2.5 ml of 1.5% sodium hypochlorite solution followed by PAD for 60 s (n = 10).

Microbial samples were taken after 5 min of treatment by inserting paper points (ISO 40) into the root canals for 30 s. Microbial samples obtained from the teeth used in the short-term experiments were serially 10-fold diluted and 25 µl aliquots were plated on agar plates and incubated at 37°C. Two days later, the number of cfu was counted. Samples were obtained from each tooth after 5 min of treatment (d0; baseline) and nutrient broth was added to the root canals. Microbial samples were then taken from the root canals after a further 2 (d2) or 4 (d4) days of incubation to examine if, after an initial suppression, the bacteria were able to re-grow. The samples were plated on tryptic soy agar plates (Oxoid) to cultivate *E. faecalis* ATCC 29212 or the mixture of aerobic bacteria. To culture the mixture of anaerobic bacteria, samples were plated on Schaedler agar. For the long-term experiments, mainly focusing on a total eradication of bacteria as an outcome, the number of cfu was recorded semi-quantitatively.

Statistical analysis

For the short-time experiments, the number of cfu was counted, expressed as log_{10} cfu, and tested for normal distribution using the Kolmogorov Smirnov test. One-way ANOVA (with the *post hoc* LSD modification) was used to examine differences between groups. Non-parametric tests (the Kruskal-Wallis test and the Mann-Whitney test) were used to compare treatment outcomes between groups. The Wilcoxon test was used to compare the results at follow-up with those at baseline between the respective groups. All statistical analyses were performed using SPSS version 21.0 (SPSS Statistics, IBM Corporation, New York, NY). The level of significance was set to $p < 0.05$.

Results

Effect of photoactivated disinfection on Enterococcus faecalis ATCC 29212 in suspension

E. faecalis ATCC 29212 in suspension was completely eliminated by PAD for 60 s or 90 s, whereas PAD for 30 s resulted in a 2 log_{10} cfu reduction in the number of cfu. The experiments conducted in the presence/absence of serum showed that maximum killing was achieved by PAD in the presence of serum. No viable bacteria were recovered from serum-containing samples, regardless of the irradiation time. However, a reduction depending on the irradiation time was observed in the samples without serum. The results of the experiments designed to examine the effects of HSA were dependent on the irradiation time. In the absence of HSA, killing rates improved as the irradiation time increased. In contrast, >99.9% of the bacteria were killed after 30 s of irradiation in the presence of HSA (either 25 mg/ml or 50 mg/ml) (Figure 1).

Photoactivated disinfection of Enterococcus faecalis ATCC 29212 in root canals

Irrigation of infected root canals with 5 ml of 0.9% NaCl solution decreased the cfu count by

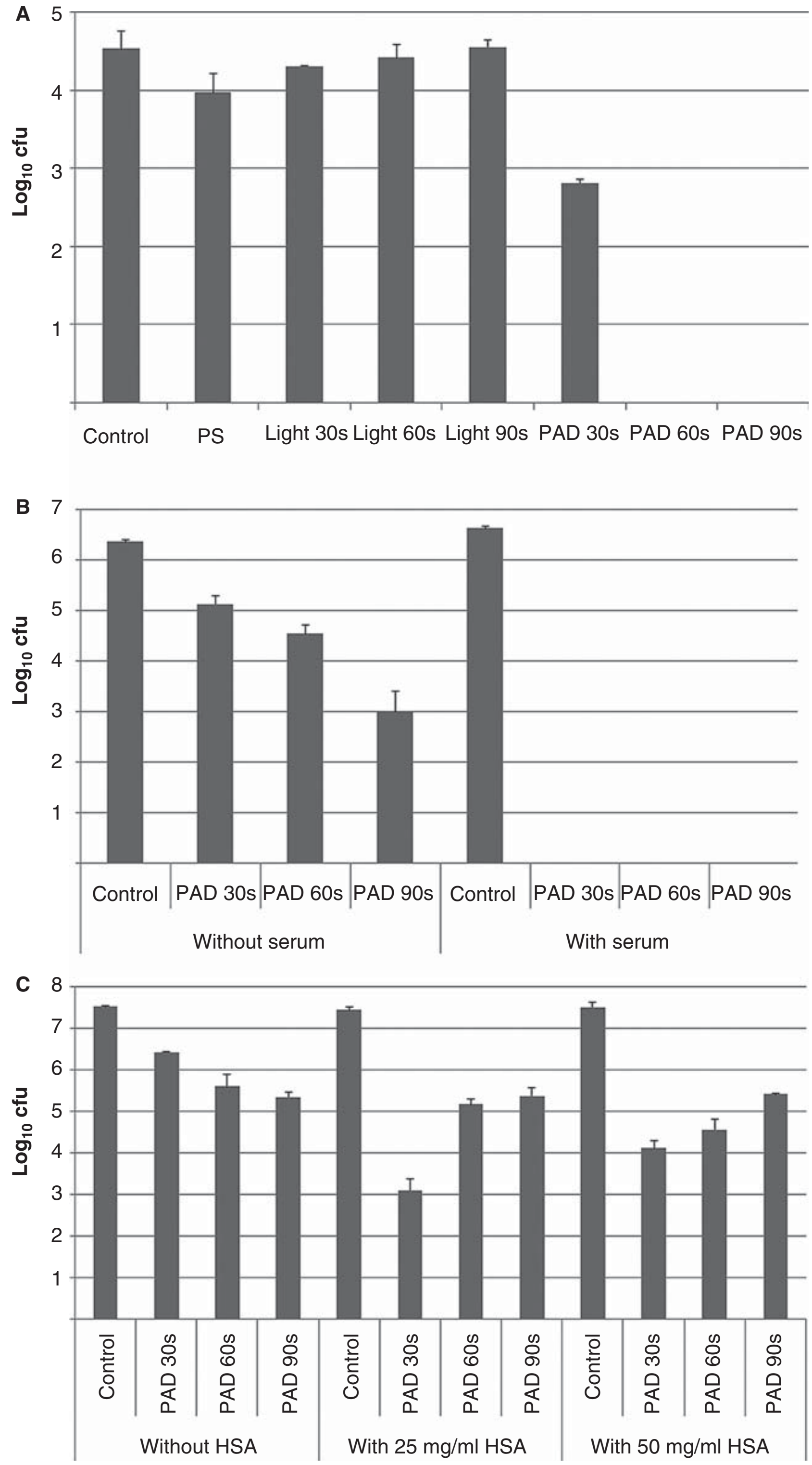

Figure 1. Number of *Enterococcus faecalis* ATCC 29212 colony forming units (cfu)/ml after treatment in suspension. Number of cfu derived from suspensions of *Enterococcus faecalis* ATCC 29212 after (A) treatment with a photosensitizer (PS) alone, treatment with laser irradiation for 30 s, 60 s or 90 s or after photoactivated disinfection (PAD; PS + laser) for 30 s, 60 s or 90 s. (B) Number of cfu after treatment with PAD (30 s, 60 s or 90 s) in the presence or absence of 25% inactivated human serum. (C) Number of cfu after treatment with PAD (30 s, 60 s or 90 s) in the presence or absence of human serum albumin (HSA; 25 mg/ml or 50 mg/ml). The number of cfu is expressed as the $\log_{10}$ cfu.

1.14 ± 0.23 $\log_{10}$ ($p < 0.001$). Irrigation plus laser irradiation and PS alone did not significantly reduce the cfu count further. Irrigation plus PAD resulted in a reduced cfu count (reduced by 0.72 ± 0.23 $\log_{10}$ cfu) compared with irrigation alone ($p = 0.033$). None of the treatments resulted in the complete eradication of bacteria (Figure 2).

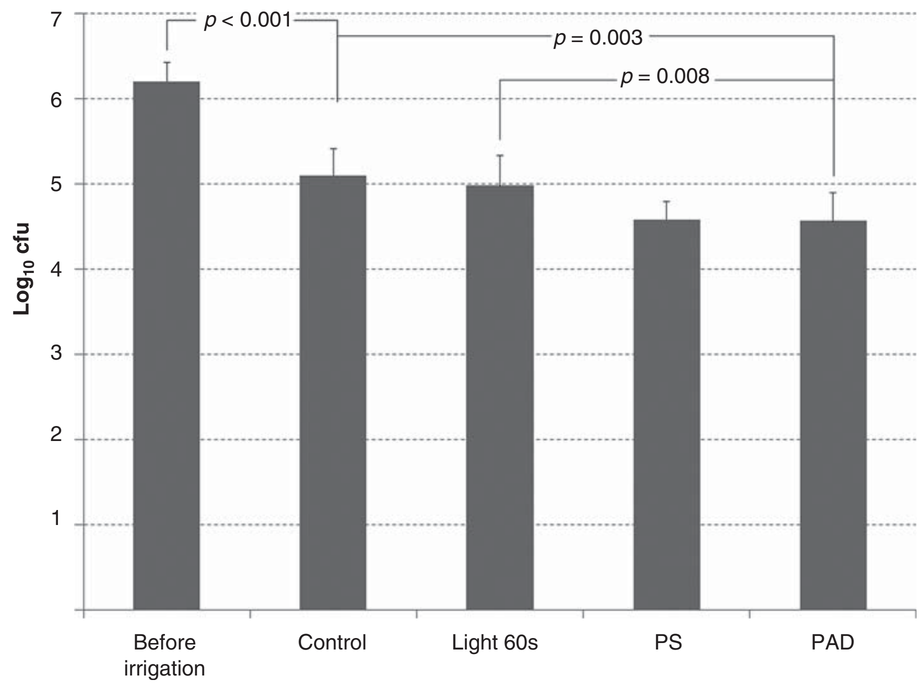

Figure 2. Number of *Enterococcus faecalis* ATCC 29212 colony forming units (cfu) derived from infected root canals before irrigation, after irrigation with 5 ml of 0.9% NaCl solution (control), after irrigation with 5 ml of 0.9% NaCl solution plus laser irradiation (light, 60 s), after treatment with a photosensitizer (PS) alone and after photoactivated disinfection (PAD) alone. The number of cfu is expressed as $\log_{10}$ cfu.

Long-term results of photoactivated disinfection of root canals

The root canals were irrigated with NaOCl before treatment with PAD, which is the normal procedure during endodontic treatment. In roots infected with *E. faecalis* ATCC 29212, the bactericidal activity of PAD (as an adjunct to NaOCl) was only apparent at d0 ($p = 0.001$). Neither NaOCl alone or NaOCl plus PAD had a significant activity on the bacterial load at d2 or d4 (Figure 3A). However, the aerobic mixture comprising *E. faecalis* WK16-1 and *S. putrefaciens* WK16-2 was susceptible to irrigation by NaOCl ($p = 0.003$) and to treatment with NaOCl plus PAD ($p = 0.001$) at d0; although neither regimen was effective at later time points (Figure 3B). The mixture of anaerobic bacteria (four different Gram-positive species) was very susceptible to both treatment regimens; all bacteria were completely eradicated at d4 (Figure 3C).

Discussion

The elimination of bacteria and their by-products from infected root canals is vital if endodontic treatment is to be successful. Novel approaches to root canal disinfection include the application of high powered lasers and the use of PAD. Previous studies show that PAD significantly decreases the bacterial load in infected root canals [5,22,23].

In the present study, PAD after the application of a PS efficiently killed *E. faecalis* ATCC 29212 in suspension. In suspension cultures containing a moderate bacterial load of *E. faecalis* ATCC 29212, all of the pathogens were eradicated after an irradiation time of 60 s. This observation is in line with the effects reported by other studies that used other laser systems in conjunction with a PS [24,25]. As observed in the present study, PAD is more potent in the presence of human serum. The absorption of the PS (toluidine blue in this case) is affected by variations in the cell walls of Gram-positive and Gram-negative bacteria [26]. PAD may oxidize the proteins and fats present in human serum, resulting in the formation of free radicals [26], which penetrate and damage the bacterial cells. Also, a recent study identified an interaction between PAD and bacterial virulence factors, which results in a decrease in the activity of bacterial proteases [27].

The present study showed that, in the presence of HSA, an irradiation time of 30 s had a greater bactericidal activity against *E. faecalis* than irradiation times of 60 s or 90 s. Serum albumin is an important antioxidant; however, it can itself be oxidized [28]. It may be that the structure of the HSA molecule is altered upon exposure to laser light or to the activated PS; thus, the protein may disintegrate after exposure to longer pulses of irradiation (60 s or 90 s), thereby losing its antibacterial activity. Further studies are needed to clarify the effects of PAD in the presence of human serum and HSA.

The experiments involving *E. faecalis*-infected roots shed further light on the results of the

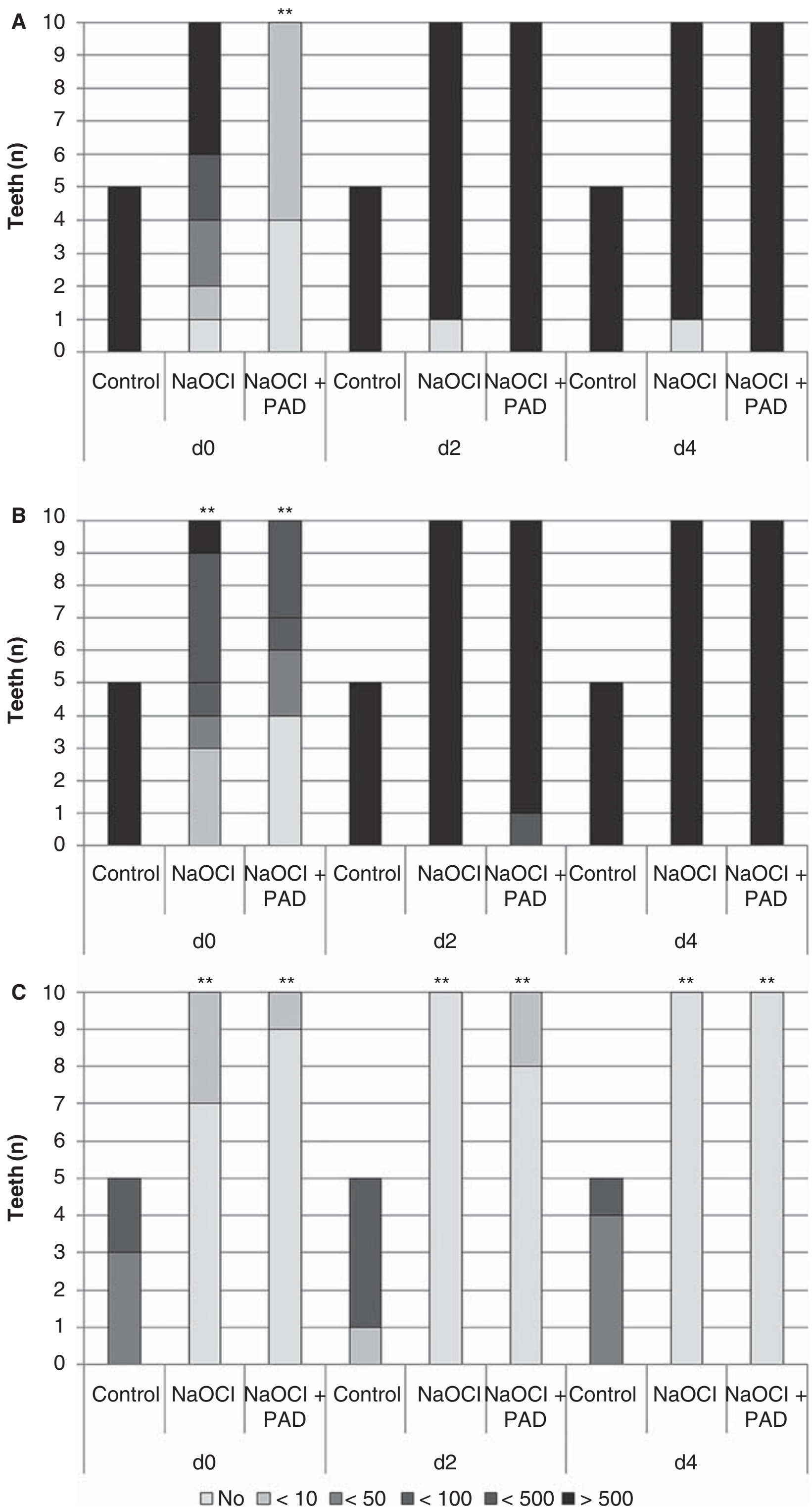

Figure 3. Number of teeth with no, <10, <50, <100, <500 and >500 colony forming units (cfu) derived from (A) *Enterococcus faecalis* ATCC 29212, (B) a mixed aerobic population and (C) an anaerobic population isolated from infected root canals after irrigation with 2.5 ml of 0.9% NaCl solution (control), irrigation with 2.5 ml of 1.5% sodium hypochlorite (NaOCl) and after irrigation with 2.5 ml of 1.5% sodium hypochlorite (NaOCl) plus photoactivated disinfection (NaOCl + PAD) up to 4 days (d4) after treatment.

experiments involving bacterial suspensions. Irrigation of the infected roots with saline solution, which mimics the mechanical characteristics of root canal irrigation, eliminated 92% of the bacteria. Although PAD killed 70% of the remaining bacteria, the total reduction was still only ~98%. None of the experiments resulted in 100% eradication. These results are in good agreement with those of Foschi et al. [23], who reported that PAD treatment of root canals *in vitro* destroyed 77.5% of *E. faecalis*.

In the 4-day experiments, the root canals were always irrigated with NaOCl before PAD. However, most studies examined chemical disinfection before PAD [29,30]. The findings of the present study confirm those of a previous study showing that the antibacterial action of NaOCl enhances the activity of PAD in the short-term [3]; however, we found that, in the longer-term, irrigation with NaOCl plus PAD was no better than irrigation with NaOCl alone. These results are in line with those of Souza et al. [29], who found that irrigation with NaOCl plus PAD did not result in improved outcomes.

NaOCl or NaOCl plus PAD had a long-lasting (up to 4 days) effect on the anaerobic mixture; however, roots infected with *E. faecalis* ATCC 29212 or the aerobic mixture always showed bacterial re-growth. Moreover, treatment with PAD plus NaOCl appeared to be no more effective than treatment with NaOCl alone. This may be due to the survival of *E. faecalis* within the deeper dentin layers. A previous study showed that bacteria may survive in these deep layers even after treatment with PAD plus chemical disinfection [31]. The surviving bacteria in the dentin tubuli can then re-colonize the root canals.

It is difficult to translate the findings of the present study to the clinic because little data is available from clinical trials evaluating the effects of photodynamic therapy. To date, the only clinical study focusing on PAD as an endodontic treatment shows that treatment with PAD plus different disinfecting irrigants, followed by filling with calcium hydroxide paste for 1 week, was able to completely eradicate bacteria from root canals [9]. Hence, further studies are needed to evaluate the clinical utility of PAD.

Conclusions

The present study shows that PAD plus irrigation with NaOCl acts bactericidal against anaerobic bacteria but does not completely eradicate aerobic bacteria from infected root canals, although there is some evidence that human serum might increase the activity of PAD.

Acknowledgments

This study was supported by the participating institutions. The laboratory work of Marianne Weibel and Regula Hirschi is highly appreciated. Furthermore, the authors are grateful to Cumdente Ltd (Tübingen, Germany) for providing the laser system. The authors are also very grateful to Thomas Spitzer for linguistic assistance when writing the article.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Spratt DA, Pratten J, Wilson M, Gulabivala K. An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. Int Endod J 2001;34:300–7.
[2] Pataky L, Ivanyi I, Grigar A, Fazekas A. Antimicrobial efficacy of various root canal preparation techniques: an in vitro comparative study. J Endod 2002;28:603–5.
[3] Rios A, He J, Glickman GN, Spears R, Schneiderman ED, Honeyman AL. Evaluation of photodynamic therapy using a light-emitting diode lamp against Enterococcus faecalis in extracted human teeth. J Endod 2011;37:856–9.
[4] Atieh MA. Photodynamic therapy as an adjunctive treatment for chronic periodontitis: a meta-analysis. Lasers Med Sci 2010;25:605–13.
[5] Gursoy H, Ozcakir-Tomruk C, Tanalp J, Yilmaz S. Photodynamic therapy in dentistry: a literature review. Clin Oral Investig 2013;17:1113–25.
[6] Konopka K, Goslinski T. Photodynamic therapy in dentistry. J Dent Res 2007;86:694–707.
[7] Chondros P, Nikolidakis D, Christodoulides N, Rossler R, Gutknecht N, Sculean A. Photodynamic therapy as adjunct to non-surgical periodontal treatment in patients on periodontal maintenance: a randomized controlled clinical trial. Lasers Med Sci 2009;24:681–8.
[8] Schar D, Ramseier CA, Eick S, Arweiler NB, Sculean A, Salvi GE. Anti-infective therapy of peri-implantitis with adjunctive local drug delivery or photodynamic therapy: six-month outcomes of a prospective randomized clinical trial. Clin Oral Implants Res 2013;24:104–10.
[9] Garcez AS, Nunez SC, Hamblim MR, Suzuki H, Ribeiro MS. Photodynamic therapy associated with conventional endodontic treatment in patients with antibiotic-resistant microflora: a preliminary report. J Endod 2010;36: 1463–6.
[10] Schneider M, Kirfel G, Berthold M, Frentzen M, Krause F, Braun A. The impact of antimicrobial photodynamic therapy in an artificial biofilm model. Lasers Med Sci 2012;27: 615–20.
[11] Shrestha A, Kishen A. The effect of tissue inhibitors on the antibacterial activity of chitosan nanoparticles and photodynamic therapy. J Endod 2012;38:1275–8.
[12] Knutsson G, Jontell M, Bergenholtz G. Determination of plasma proteins in dentinal fluid from cavities prepared in healthy young human teeth. Arch Oral Biol 1994;39:185–90.
[13] Tew JG, Marshall DR, Burmeister JA, Ranney RR. Relationship between gingival crevicular fluid and serum antibody titers in young adults with generalized and localized periodontitis. Infect Immun 1985;49:487–93.
[14] Pappen FG, Qian W, Aleksejuniene J, Leonardo Rde T, Leonardo MR, Haapasalo M. Inhibition of sodium hypochlorite antimicrobial activity in the presence of bovine serum albumin. J Endod 2010;36:268–71.
[15] Kawakami A, Kubota K, Yamada N, Tagami U, Takehana K, Sonaka I, et al. Identification and characterization of oxidized human serum albumin. A slight structural change impairs its ligand-binding and antioxidant functions. FEBS J 2006;273: 3346–57.
[16] Portenier I, Haapasalo H, Rye A, Waltimo T, Orstavik D, Haapasalo M. Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin. Int Endod J 2001;34:184–8.
[17] Malcic A, Jukic S, Brzovic V, Miletic I, Anic I. Leakage of bovine serum albumin in root canals obturated with super-EBA and IRM. J Endod 2006;32:368–71.
[18] Portenier I, Waltimo T, Orstavik D, Haapasalo M. Killing of Enterococcus faecalis by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentine powder or BSA. J Endod 2006;32:138–41.

[19] Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86–93.

[20] Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J 2001;34:429–34.

[21] Sakamoto M, Siqueira JF Jr, Rocas IN, Benno Y. Molecular analysis of the root canal microbiota associated with endodontic treatment failures. Oral Microbiol Immunol 2008;23: 275–81.

[22] Nunes MR, Mello I, Franco GC, de Medeiros JM, Dos Santos SS, Habitante SM, et al. Effectiveness of photodynamic therapy against Enterococcus faecalis, with and without the use of an intracanal optical fiber: an in vitro study. Photomed Laser Surg 2011;29:803–8.

[23] Foschi F, Fontana CR, Ruggiero K, Riahi R, Vera A, Doukas AG, et al. Photodynamic inactivation of Enterococcus faecalis in dental root canals in vitro. Lasers Surg Med 2007; 39:782–7.

[24] Kranz S, Guellmar A, Volpel A, Gitter B, Albrecht V, Sigusch BW. Photodynamic suppression of Enterococcus faecalis using the photosensitizer mTHPC. Lasers Surg Med 2011;43:241–8.

[25] Bergmans L, Moisiadis P, Huybrechts B, Van Meerbeek B, Quirynen M, Lambrechts P. Effect of photo-activated disinfection on endodontic pathogens ex vivo. Int Endod J 2008;41:227–39.

[26] Pinheiro SL, Schenka AA, Neto AA, de Souza CP, Rodriguez HM, Ribeiro MC. Photodynamic therapy in endodontic treatment of deciduous teeth. Lasers Med Sci 2009; 24:521–6.

[27] Braham P, Herron C, Street C, Darveau R. Antimicrobial photodynamic therapy may promote periodontal healing through multiple mechanisms. J Periodontol 2009;80:1790–8.

[28] Guedes S, Vitorino R, Domingues R, Amado F, Domingues P. Oxidation of bovine serum albumin: identification of oxidation products and structural modifications. Rapid Commun Mass Spectrom 2009;23:2307–15.

[29] Souza LC, Brito PR, de Oliveira JC, Alves FR, Moreira EJ, Sampaio-Filho HR, et al. Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/ irrigation procedures in promoting intracanal reduction of Enterococcus faecalis. J Endod 2010;36:292–6.

[30] Ng R, Singh F, Papamanou DA, Song X, Patel C, Holewa C, et al. Endodontic photodynamic therapy ex vivo. J Endod 2011;37:217–22.

[31] Cheng X, Guan S, Lu H, Zhao C, Chen X, Li N, et al. Evaluation of the bactericidal effect of Nd:YAG, Er:YAG, Er, Cr:YSGG laser radiation, and antimicrobial photodynamic therapy (aPDT) in experimentally infected root canals. Lasers Surg Med 2012;44:824–31.