doi:10.1111/j.1365-2591.2008.01532.x

Effectiveness of different laser systems to kill *Enterococcus faecalis* in aqueous suspension and in an infected tooth model

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Abstract

Meire MA, De Prijck K, Coenye T, Nelis HJ, De Moor RJG. Effectiveness of different laser systems to kill *Enterococcus faecalis* in aqueous suspension and in an infected tooth model. *International Endodontic Journal*, **42**, 351–359, 2009.

Aim To assess the antibacterial action of laser irradiation (Nd:YAG, KTP), photo activated disinfection (PAD) and 2.5% sodium hypochlorite (NaOCl) on *Enterococcus faecalis,* in an aqueous suspension and in an infected tooth model.

Methodology Root canals of 60 human teeth with single straight canals were prepared to apical size 50, autoclaved, inoculated with an *E. faecalis* suspension and incubated for 48 h. They were randomly allocated to four treatment and one control groups. After treatment, the root canals were sampled by flushing with physiological saline, and the number of surviving bacteria in each canal was determined by plate count and solid phase cytometry. The same experimental or control treatments were completed on aqueous suspensions of *E. faecalis,* and the number of surviving bacteria was determined in the same way.

Results In aqueous suspension, PAD and NaOCl resulted in a significant reduction in the number of *E. faecalis* cells ($P < 0.001$), whilst Nd:YAG or KTP had no effect. In the infected tooth model, only the PAD and NaOCl treated teeth yielded significantly different results relative to the untreated controls ($P < 0.001$).

Conclusions The laser systems as well as PAD were less effective than NaOCl in reducing *E. faecalis*, both in aqueous suspension and in the infected tooth model.

Keywords: disinfection, laser, photodynamic therapy, root canal.

Received 27 March 2008; accepted 2 December 2008

Introduction

The role of micro-organisms and their by-products in the pathogenesis of apical periodontitis has clearly been established (Kakehashi *et al.* 1965, Sundqvist *et al.* 1998). Likewise, disinfection of the root canal system has been recognized as an essential aspect of root canal treatment. Traditionally, this is accomplished by chemo-mechanical cleaning, a combination of (i) mechanical instrumentation; (ii) use of disinfecting solutions for irrigation of the root canal space; and (iii) placement of intracanal medication between appointments (Byström & Sundqvist 1981, 1983, 1985, Sjögren *et al.* 1991). However, despite meticulous chemo-mechanical cleaning, eradication of all micro-organisms from the root canal system is difficult (Sjögren *et al.* 1997, Nair *et al.* 2005). Micro-organisms have been shown to persist in the anatomical complexities of the root canal system and to be the cause of treatment failure (Lin *et al.* 1991, Sundqvist *et al.* 1998). Therefore, various laser systems have been examined as adjuncts to currently used disinfection methods in root canal treatment. The laser light is thought to be able to reach areas that are inaccessible

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with the traditional techniques (e.g. bacteria located deeply in fins, isthmuses, lateral canals and in the dentinal tubules). Indeed, it has been demonstrated that dentinal tubules effectively act as fibre optic channels, redirecting the light in multiple directions (Odor *et al.* 1996).

Lasers such as CO_2 (wavelength of 10 600 nm), Nd:YAG (neodymium-doped yttrium aluminium garnet) (1 064 nm), Er:YAG (erbium-doped yttrium aluminium garnet) (2 940 nm) and diode (810 or 980 nm) have been tested for disinfection of root canals. However, the reported success rate varies between studies. The Nd:YAG laser is probably the best documented laser for this application. Moritz *et al.* (1999) found a 99.16% reduction of bacterial numbers in inoculated root canals after Nd:YAG irradiation (Moritz *et al.* 1999). Folwaczny *et al.* (2002) and Piccolomini *et al.* (2002), in comparable experiments, also found the Nd:YAG laser to reduce the bacterial content, but they reported NaOCl to be more effective.

The KTP (potassium titanyl phosphate) laser, emitting at 532 nm, a new wavelength for dental applications, has been used primarily for tooth bleaching procedures (De Moor & Vanderstricht 2009). Recently, Schoop *et al.* (2006) reported on the antibacterial effectiveness of this laser. Using a dentine slice model, they observed a considerable reduction in the number of bacteria in most samples and concluded that the KTP laser was a suitable tool for root canal disinfection.

The bactericidal effects of these high-power lasers are the result of dose-dependent heat generation. The amount of heat delivered may have undesirable effects such as charring and cratering of dentine (Depraet *et al.* 2005) or possible thermal injury to the periodontal ligament, resulting in root resorption, ankylosis or periradicular necrosis (Bahcall *et al.* 1992). However, these disadvantages may be overcome by the use of a low-power laser to activate a dye (photosensitizer), which in turn exerts a lethal effect on bacteria (Wilson *et al.* 1992). This photo-activated disinfection (PAD) technique can be undertaken with a range of visible red and near infrared lasers. Systems using low power (100 mW) visible red semiconductor lasers in conjunction with tolonium chloride dye are now commercially available. It has been shown that the PAD technique kills several bacterial species commonly found in the oral cavity, even if located within biofilms (Dobson & Wilson 1992).

Experimental studies reporting on the antimicrobial effectiveness of various chemicals and techniques are numerous in endodontics. Most of them are based on comparison of bacterial counts before and after disinfection. For enumeration of viable bacteria, culture-based techniques are mostly used, such as the plate count method or examination of turbidity in a broth. At present, nonculture based approaches are becoming increasingly important (Malacrino *et al.* 2001). Amongst these, solid phase cytometry (SPC) offers the advantages of a low detection limit, high speed and the ability to detect viable nonculturable bacteria (D'Haese & Nelis 2002).

In the infected root canal, micro-organisms can be found within the canal lumen, attached to the root canal wall, and invading the dentinal tubuli (Nair 1987, Siqueira *et al.* 2002a). The bacteria sticking to the root canal wall (i.e. a bacterial biofilm, Costerton *et al.* 1999) are of particular interest, as it is known that a significant part of the root canal wall often remains untouched by instruments during conventional treatment (Peters *et al.* 2003). In these areas, the bacterial biofilm cannot be removed mechanically.

The aim of this study was to assess the antibacterial action of Nd:YAG and KTP laser irradiation, PAD and sodium hypochlorite (NaOCl) on *Enterococcus faecalis*. This study was carried out both *in vitro* and on the uninstrumented infected root canal walls of extracted teeth (*ex vivo*), by means of a culture-dependent and a culture-independent microbiological technique.

Materials and methods

Bacteria and culture conditions

A pure culture of *E. faecalis* ATCC 10541 was grown in brain heart infusion (BHI) broth and incubated overnight at 37 °C. Ten-fold serial dilutions were then prepared in physiological saline (PS, 0.9% (w/v) NaCl), to obtain working concentrations of 10^5 to 10^8 CFU mL^{-1}.

Preparation of teeth

A total of 60 extracted, single-rooted human teeth, stored in physiologic saline, were decoronated following an informed consent protocol approved by the Ethics Committee of the Ghent University Hospital (ref. B67020084251). The external root surface was cleaned with curettes to remove calculus and periodontal soft tissues. Root canal preparation commenced with Gates Glidden Burs 4-3-2 (Dentsply Maillefer, Ballaigues, Switzerland) in a crown-down mode. Working length was established by passing a size

10 K-file (Dentsply Maillefer) in the canal until visible at the apex and subtracting 1 mm. Instrumentation was continued until MAF 50 (taper 0.02) under irrigation with physiologic saline. The apical foramen was sealed by composite resin (Z250, 3M ESPE, Seefeld, Germany) and the root surface covered with bonding agent, to prevent bacterial leakage. Teeth were then placed in test tubes and sterilized by autoclaving (134 °C, 10 min).

Ex vivo experiments

Ten microlitre aliquots of a freshly prepared *E. faecalis* suspension were transferred into the root canals, resulting in approximately 10^3–10^4 cells per root canal. Sterile PS was added until the entire canal space was filled with fluid. The tooth specimens were then enclosed in sterile Eppendorf tubes and incubated in a humid atmosphere at 37 °C for 48 h. Any residual medium in the root canals after incubation was carefully removed with sterile paper points and the teeth were randomly allocated to the experimental or control groups. In the first group, teeth were treated with pulsed Nd:YAG laser (Fidelis, Hightechlaser, Herzele, Belgium) irradiation at 1064 nm (n = 10), using the following lasing parameters: power: 1.5 W; frequency: 10 Hz. The light was delivered through a 200 μm flexible fibre. The fibre tip was applied with a spiral movement from apical to coronal, for five times 5 s with 20 s intervals (total energy: 37.5 J).

In the second experimental group, teeth were treated with KTP laser (Smartlite KTP laser, Deka, Calenzano, Italy) irradiation at 532 nm (n = 10) in pulsed mode. The power was set at 1 W and the pulse frequency at 10 Hz, again applying a spiral movement from apical–coronal with the 200 μm fibre, for five times 5 s with 20 s intervals (total energy: 25 J). The actual output of both lasers was controlled with a power meter (Ophir Nova, Ophir Optronics Ltd, Jerusalem, Israel) before each experiment, and regularly checked during the experiments.

In the third group, teeth (n = 10) were subjected to PAD treatment. To this end, a photosensitizing agent (PAD solution: i.e. toluidine blue O (TBO) at a concentration of 12.7 mg mL^{-1}, pH 5) was added to fill the canal. After a 2 min pre-irradiation time; laser light (635 nm) generated by a diode laser (Denfotex, Inverkeithing, UK) was applied through an endodontic handpiece consisting of a flexible cylinder (15 mm length, 400 μm diameter), emitting 70% of the light radially as a cylinder uniformly along the length and 30% of the total light intensity at the tip. Power was set at 100 mW and irradiation time was 150 s (total energy: 15 J), according to the manufacturer's recommendations. The endotip was gently moved up and down the canal during irradiation.

In the fourth group (n = 10), canals were filled to the orifice with NaOCl solution (2.5% w/v). After 5 min, the NaOCl was removed from the canal with sterile paper points and fresh NaOCl solution was added. Five minutes later, this procedure was repeated, resulting in a total contact time of 15 min.

The fifth group (n = 20) served as a positive control; i.e. teeth received no treatment. Uninoculated negative control teeth (n = 3) were also included in the experiment.

Root canal sampling

In all groups, root canals were flushed with 990 μL sterile PS by means of a 3 mL syringe with a 27-gauge endodontic needle (Monoject, Sherwood Medical, St Louis, MO, USA) of which the apical 4 mm had been removed. This needle was gently moved up and down in the canal space whilst applying firm pressure on the syringe plunger. To this end, the teeth were held upside down, to collect the sampling liquid in a test tube, resulting in a volume of 1 mL. Following aspiration of the sampling liquid, flushing was repeated. A 1/10 dilution series of the collected bacterial suspension was prepared in sterile PS.

In vitro experiments

Sterile Eppendorf tubes (n = 24) were filled with 10 μL of a freshly prepared *E. faecalis* suspension (10^5–10^6 bacterial cells). The following treatment protocols were used. A first treatment consisted of pulsed Nd:YAG laser irradiation at 1064 nm (n = 6) with a power of 1.5 W and a frequency of 15 Hz. Using the 200 μm fibre, the suspension was uniformly irradiated by applying a scanning movement just above the liquid surface. This was done five times 5 s, with 20 s intervals. In the second group, KTP laser irradiation at 532 nm (n = 6) was used (1 W; 10 Hz). Using the 200 μm fibre, the aliquot was uniformly irradiated by applying a scanning movement just above the fluid surface. This was done five times 5 s, with 20 s intervals. For the PAD treatment (n = 6), 20 μL of PAD solution was added to each tube. This solution was filtered over a 0.22 μm membrane filter (Millipore, Bedford, MA, USA) prior to use. After a 2 min pre-irradiation time; the

bacterial suspension was uniformly irradiated for 150 s at 100 mW (according to the manufacturer's recommendations), holding the spherical (caries) tip in the centre of the liquid. In the NaOCl group (n = 6), 10 μL of NaOCl solution (2.5% v/v) was added to each tube. A contact time of 15 min was allowed.

Sterile PS was added to each tube to obtain a working volume of 1 mL, except for the NaOCl group, where 980 μL of a 5% sodium thiosulphate solution was added to inactivate the NaOCl. Prior experiments had proven this solution to effectively inactivate sodium hypochlorite whilst not influencing the enterococcal viability (data not shown). A 1/10 dilution series of the final bacterial suspension was prepared in sterile PS.

Solid phase cytometry (SPC)

In order to count the surviving micro-organisms using SPC, 100 μL of each bacterial suspension was filtered through a black polyester membrane filter (Cycloblack CB 04, 25 mm diameter, 0.4 μm pore size, Chemunex, Ivry-sur-Seine, France). Subsequently, these filters were incubated with a labelling solution (1% dilution of ChemChrome V6 in ChemSol B16, Chemunex) for 30 min at 30 °C. The fluorescent labelling of viable bacteria in SPC is based on the cleavage of a fluorescein type ester by intracellular esterases and retention of the free fluorescein in cells with an intact cytoplasmic membrane. Consequently, only viable bacteria with an intact membrane will be detected by this technique. After labelling, the membrane filter was scanned in the ChemScan® (Chemunex). This apparatus was equipped with an argon laser, emitting light of 488 nm with a (variable) power of 25 mW that scanned the 25-mm diameter membrane filter in 3 min. Two photomultiplier tubes with wavelength windows set for the green (500–530 nm) and amber (540–585 nm) regions of the emission spectrum of fluorescein detected the fluorescence light emitted by the labelled cells. The signals produced were processed by a PC using a series of software discriminants, to differentiate between valid signals (labelled bacteria) and background. Scan results were displayed as coloured spots on a membrane filter image. These spots were visually confirmed using epifluorescence microscopy.

Plate count

Aliquots of each (diluted) bacterial suspension were transferred to petridishes and mixed with molten (45 °C) brain heart infusion agar (BHIA). After solidification of the agar, the plates were incubated aerobically at 37 °C for at least 48 h. Finally, the number of colony forming units (CFUs) in the undiluted suspension was calculated.

Cell counts were logarithmically transformed to normalize the data prior to statistical comparison (one-way ANOVA, *post hoc* Scheffe test, level of significance set at 5%). The mean and standard deviation of each group were calculated.

Results

In vitro experiments

In Table 1 the reductions in the number of viable (SPC) and culturable *E. faecalis* cells after the various treatments are listed. The results represent the mean values calculated with reference to the nontreated control samples. No significant differences between SPC and plate count values were observed. Nd:YAG or KTP laser irradiation did not result in a reduction in the number of surviving *Enterococci*. PAD treatment using the filter-sterilized photosensitizer resulted in a significant reduction (2.7 log units), whilst contact with NaOCl killed all the cells present.

The use of the PAD photosensitizer, as provided by the manufacturer, in combination with low power laser

Table 1 Effect of Nd:YAG and KTP laser irradiation, photo activated disinfection and sodium hypochlorite on the survival of *Enterococcus faecalis in vitro*

	Before ± SD		After ± SD		Log reduction	
Treatment	SPC	Culture	SPC	Culture	SPC	Culture
Nd:YAG	5.20 ± 0.18	5.27 ± 0.12	5.45 ± 0.08	5.42 ± 0.14	−0.25	−0.15
KTP	5.20 ± 0.18	5.27 ± 0.12	5.37 ± 0.25	5.28 ± 0.28	−0.17	−0.01
PAD	6.13 ± 0.07	6.14 ± 0.07	3.47 ± 0.68	3.44 ± 0.49	2.66	2.69
NaOCl	5.13 ± 0.11	5.14 ± 0.09	0	0	5.13	5.14

Results are expressed as mean (logarithmic) before and after treatment figures (± SD) and as reductions in viable (SPC) and culturable (plate count) cells. n = 6 in each group.

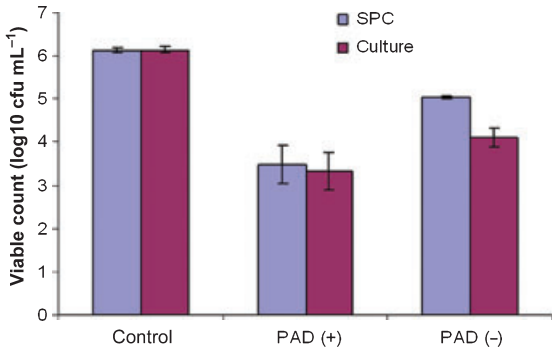

Figure 1 Effect of lethal photosensitization on the viability of *Enterococcus faecalis* cells *in vitro*. PAD (+) indicates the use of filter-sterilized photosensitizer, whilst in PAD (−), the photosensitizer was not sterilized. $n = 6$ in each group.

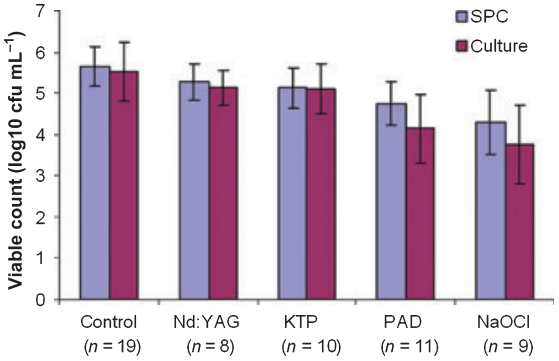

Figure 2 Effect of Nd:YAG and KTP laser irradiation, photo activated disinfection and sodium hypochlorite treatment on the survival of *Enterococcus faecalis* in the infected tooth model. Vertical bars represent the mean number of recovered cells ± SD.

light resulted only in one log reduction as indicated by SPC results. By contrast, the plate count revealed a higher reduction for suspensions treated identically (Fig. 1). Microscopic validation of the SPC results by means of an epifluorescence microscope showed the presence of rod shaped micro-organisms indicating nonsterility of the photosensitizer. Filtration of pure (nonlasered) dye solution over a polyester membrane filter, labelling and scanning confirmed this observation. The exact extent of contamination of the dye solution was batch-dependent (data not shown).

Ex vivo experiments

In the *ex vivo* experiments, no significant differences between SPC and plate count values were observed (Fig. 2). None of the two high power lasers reduced the number of *E. faecalis* (log reduction <1; $P > 0.05$). PAD application in prepared root canals diminished the SPC counts and the total plate count by approximately 1 and 1.5 log units respectively. This result was significantly different from that of the control group ($P < 0.001$). Largest reductions were obtained in the sodium hypochlorite treated group, i.e. 1.72 and 2.10 log reductions respectively, depending on the quantification method ($P < 0.001$). The negative controls yielded no culturable cells.

Discussion

Enterococcus faecalis was chosen as the test organism in this study because it is the species most often associated with persistent endodontic infections (Molander *et al.* 1998, Sundqvist *et al.* 1998, Peciuliene *et al.* 2000, Pinheiro *et al.* 2003) and it can also be found in primary root canal infections (Siqueira *et al.* 2002b). This Gram-positive, facultative anaerobe coccus has been used by many other investigators, probably due to its ease of growth and laboratory manipulation (Haapasalo *et al.* 2000, Hems *et al.* 2005, Tandjung *et al.* 2007).

The effectiveness of the laser applications and the NaOCl control against *E. faecalis* was tested on bacterial suspensions (*in vitro*) and in an infected tooth model (*ex vivo*). The results of the *in vitro* experiments show that both high-power lasers (Nd:YAG and KTP) did not affect the viability of planktonic *E. faecalis* cells. It has been demonstrated that laser-induced bacterial killing is due to different mechanisms: thermal heating of the bacterial environment above lethal values, local heating inside bacteria (by laser light-sensitive chromophores inside the bacteria) or light-induced modulation of enzymatic activity (Hellingwerf *et al.* 1996). As virtually all bacteria survived, none of the above-mentioned mechanisms took place, or the amount of energy delivered was insufficient. This might be due to poor absorbance of the Nd:YAG and KTP wavelengths in water. Probably, transmission of the laser beams rather than absorption by the bacterial suspension occurred, explaining the survival of bacteria.

Rooney *et al.* (1994) investigated the bactericidal effect of the Nd:YAG laser on *E. faecalis* suspensions in glass capillary tubes. No or a minimal bactericidal effect was observed at energy doses below 30 J. Above this value, they noted increasing bacterial killing with increasing doses of irradiation, with reductions of

>4 log units above 54 J. When a black dye was added, similar reductions were obtained at much lower energies (Rooney *et al.* 1994). In the present Nd:YAG experiment, energy doses of 37.5 J were delivered (1.5 W during 25 s) The latter parameters were chosen to be identical with those used in the *ex vivo* experiments, which were adopted from Moritz *et al.* (1999) and Schoop *et al.* (2006). The data obtained in this study are more or less in accordance with those from Rooney *et al.* (1994). The fact that in the latter study, *Enterococci* were suspended in broth compared with saline in this study, may have led to an increased absorbance of laser energy.

Application of the PAD system *in vitro* revealed some methodological problems. Many batches of the photosensitizer for the PAD application, as received from the manufacturer, proved to be nonsterile. Therefore, part of the PAD solution was sterilized by filtration over a 0.22 μm membrane filter. When using the filter-sterilized photosensitizer, PAD application *in vitro* led to a 2.7 log reduction of *E. faecalis* cells in both SPC and plate counts. This reduction was highly significant compared with the nontreated controls. The best results in the *in vitro* experiments were obtained in the NaOCl treated group, as no *Enterococci* survived this treatment. This is in accordance with results obtained by Radcliffe *et al.* (2004), who found that a 5 min contact time between an *E. faecalis* suspension and NaOCl 2.5% (w/v) eradicated all bacteria.

The results of the infected tooth model experiments show that it is hard to eradicate *E. faecalis* from the root canal. Comparison of the inoculum size (10^3–10^4) with the number of recovered cells (10^5–10^6) indicates that the *E. faecalis* cells did grow well in the teeth. Sealing of the apical foramen with composite resin did not seem to interfere with bacterial growth. This observation is substantiated by several studies that show that composite resins have minimal antibacterial effects (Karanika-Kouma *et al.* 2001, Beyth *et al.* 2007). Both high-power lasers gave a slight reduction of the intracanal flora, but the differences with the nontreated controls were not significant. Moritz *et al.* (1999), in a similar study, found a 99.16% (2 log units) reduction of bacteria in inoculated root canals after Nd:YAG irradiation. In this study, it was not possible to achieve such reductions. The Moritz group, however, used a suspension of *E. faecalis* and *E. coli* to inoculate their root canals. It has been shown that the susceptibility of the gram-negative rod *E. coli* to Nd:YAG laser irradiation is substantially higher than that of *E. faecalis* (Moritz *et al.* 2000). This might explain the higher log reductions achieved in the latter study.

Schoop *et al.* (2006) irradiated *E. faecalis* inoculated dentine disks at the bacteria free side with the KTP laser after 4 h of incubation. They observed minor changes in bacterial count at a power setting of 1 W. Using the higher setting of 1.5 W, no growth was observed in 10 out of 20 samples. However, the lower level of detection was rather high (5×10^2 CFU mL^{-1}), so that the effect may have been overestimated. In this experiment, the power setting of 1 W appeared to be too low to affect the viability of the *E. faecalis* cells.

In the PAD treated teeth, a 1.42 log reduction was observed with the culture-based method, but the difference from the control group was not significant. Williams *et al.* (2006) used the same PAD device in a comparable set-up and obtained a mean log reduction of 2.01 in teeth. However, other test bacteria were used in their study. Although the results were statistically different, the observed log reductions are not clinically relevant for the treatment of endodontic disease.

The counts in the NaOCl treated group were significantly different from those in the nontreated control teeth. Nevertheless, only an average 2 log reduction was obtained. The poorer results in the infected tooth model compared with those of the tests on suspensions (100% bacterial kill) could be attributed to the inactivation of NaOCl by the organic component in root dentine and the small volume of irrigant used. The root canal was completely filled with NaOCl, so the volume of irrigant that was able to act on the canal content was not more than a few 100 μL, which is rather low compared with the volumes that are used during root canal treatment.

The exposure times in the various treatments (KTP and Nd:YAG laser for 25 s; PAD for 150 s and NaOCl for 15 min), but also the total energy delivered in case of laser treatment differ. These conditions were chosen according to the best available evidence and because they are clinically relevant. The differences demonstrated in the killing of *E. faecalis* may therefore not only be due to the different methods but also the different exposure times used. Consequently, the use of other laser settings in each group can influence the outcome of the experiment.

It has been demonstrated that *E. faecalis* is able to form a bacterial biofilm in the root canal (George *et al.* 2005). Biofilm-grown (sessile) cells differ from their planktonic counterparts in a number of aspects,

including the presence of an extracellular polymeric substance, cell wall composition, growth rate, metabolic activity and gene expression (Costerton *et al.* 1999). Since at the end of the inoculation time, all liquid content was removed from the canals using paper points, planktonic cells were also removed. The presence of an *E. faecalis* biofilm in the teeth probably explains the poor efficacy of PAD and NaOCl in the *ex vivo* model.

Solid phase cytometry proved to be a fast and valid technique for enumeration of *Enterococci*. In this study no difference between SPC and plate counts was observed, except when using PAD. Application of PAD on the *in vitro* model led to a 2 log reduction of bacteria as indicated by plate count results, but only a 1 log reduction as obtained with SPC. However, visual inspection of the filter by epifluorescence microscopy revealed that other micro-organisms than the inoculating species were present, as evidenced from the different morphology of the cells. It was found that rod-shaped micro-organisms were present next to cocci. This observation raised the assumption of a contaminated photosensitizer, which was subsequently confirmed. It also shows that not all micro-organisms detected by SPC could be cultured using standard culture conditions.

Neither of the three laser systems yielded a clinically relevant reduction of the number of intracanal bacteria. Highest reductions were obtained after NaOCl irrigation but still this 2 log reduction of a 6 log population in the infected root canal is insignificant in practice. Overall, the effectiveness of the treatment modalities in this study to eliminate root canal infection was disappointing. It can be concluded that the root canal areas that escape mechanical cleaning cannot be effectively disinfected with the present treatment protocols. These findings do not necessarily mean that lasers cannot be beneficial in the field of root canal disinfection. The antibacterial effect of Nd:YAG has been proven in nonendodontic settings (Ward *et al.* 1996). Further research is necessary to establish the appropriate laser parameters permitting adequate antimicrobial action without harmful thermal side effects. Also, there is a need for randomized, blind, clinical trials. Besides, other applications such as laser activation of root canal irrigants have been reported (Blanken & Verdaasdonk 2007) that could benefit root canal treatment.

Conclusion

The laser systems, at the current settings, were less effective than NaOCl in reducing *E. faecalis* both *in vitro* and in the infected tooth model. The search for laser applications in endodontics should however be continued and different laser wavelengths and settings should be examined.

Acknowledgement

This work was supported by the European Society of Endodontology annual research grant in 2005.

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