light system of a specific wavelength (635 nm) to activate the aqueous solution. The solution selectively targets and tags bacteria when introduced into the root canals. The photosensitizer used in endodontic therapy is a pharmaceutical grade chemical substance. It releases nascent oxygen when exposed to low power light at its peak absorption. The nascent oxygen can cause oxidative injury to the bacterial cell wall and thus kill the microorganism. However, neither the photosensitizer nor the light has any significant antibacterial action when used alone.[10] The advantage of PAD is that it selectively eliminates bacteria. Furthermore, it does not affect any other normal tissue and causes no damage to the surrounding tissues.[11] There is no staining on the gingiva or restorations. It also does not encourage the development of any resistant species. PAD therefore seems a promising method to eradicate bacteria, even the resistant strain such as EF, in the root canal systems. In the previous study, there is limited knowledge of the bactericidal effect of various irradiation energy doses of PAD on EF and the relationship between the PAD power used and the irradiation time. The aims of this study were to examine the bactericidal effect of PAD on EF in a glass tube model to investigate the relationship between the PAD power used, the irradiation time and the energy toward its bactericidal effect, and an infected root canal model, to investigate the bactericidal effect of PAD against EF in the root canal system.

Materials and Methods

Laser devices

The laser irradiation was delivered by a small diode laser designed for clinical use (Denfotex, Denfotex Light Systems Ltd, Inverkeithing, Fife, U.K.). It produced red light at 635 nm with the output power ranging from 50 to 100 mW. The Denfotex handpiece had a 15 mm long endodontic emitter, which was equivalent to a size 40 endodontic file for root canal disinfection. Approximately 70% of the light was emitted from the full 15 mm tapered fiber, and 30% of the light was given out from the tip. This optical fiber was able to distribute uniform illumination of 360 degrees within the entire root canals.

Experiment 1: glass tube experiment

Preparation of bacteria. EF (American Type Culture Collection [ATCC] 29212) was cultured for 48 h at 37 C in brain heart infusion (BHI) broth (Oxoid CM225). A volume of 3,600 µL of bacterial broth were centrifugated with 800 rotations for 2 min, and the supernatant was discarded. Bacterial deposition was introduced into 3,300 µL of photosensitizer (12.7 µg/mL tolonium chloride), and vortexed. The cell suspension was adjusted spectrophotometrically to ensure that the amount of bacteria was $>10^{12}$ colony forming units (CFU)/mL.

Preparation of specimens. A total of 132 glass tubes with an internal diameter of 1.80

from every irradiated and control specimen was measured. The mean bacterial concentration of each group was calculated as the primary outcome measured in this study.

Statistical analysis. Statistical analysis was performed using SPSS version 17 software (SPSS Inc., Chicago, IL). All data were assessed for normal distribution using the Shapiro–Wilk test for normality. The differences in the mean EF concentration of the test (irradiated) and control group were assessed by Student's t test. Linear regression was used to study the relationship between the bactericidal effect (log reduction) of PAD and irradiation energy dose. The cutoff level of significance was taken as 5% for all analyses.

Experiment 2: root canal experiment

Preparation of bacteria. EF (American Type Culture Collection [ATCC] 29212) was cultured for 48 h at 37 C in BHI broth (Oxoid CM225).

Preparation of specimens. Sixty single-rooted teeth with straight canals were selected. The crowns and the coronal parts of the roots were removed, and the length of the roots was uniformed as 12 mm. The canals were enlarged to an apical size of 40# using Ni-Ti ProTaper instrumentation and sterilized with 10 mL of 5.25% sodium hypochlorite solution and 10 mL 17% EDTA solution between each endodontic file. The apical foramens and the surface of the roots were sealed with flowable composite resin and all the specimens were sterilized by autoclaving for 15 min 121 C.

Each of the specimens was incubated in a sterile centrifuge tube with 1 mL of the EF ATCC29212 at 37 C under anaerobic conditions for 21 days. The medium in each tube was refreshed every 3 days. After the incubation, the samples were collected by using three sterile paper points per canal, which were immediately placed in sterile centrifuge tubes. The extracted fluid was diluted in log 10 steps, and then 50 µL of each dilution was spread out on BHI agar plates, which were then incubated for 24 h at 37 C under anaerobic conditions. After incubation, the number of the CFU was counted on those plates containing between 20 and 200 colonies.

Test groups. All the specimens were randomly divided into three groups with 20 teeth in each group: (1) 20 root canals were disinfected by PAD, (2) 20 root canals were irrigated with 10 mL 5.25% sodium hypochlorite (NaOCl) solution for 5 min as the positive control, and (3) 20 root canals were irrigated with 10mL 0.9% sterile saline as the negative control.

The laser irradiations were done as the procedures below. Photosensitizer (12.7 micrograms / mL tolonium chloride)

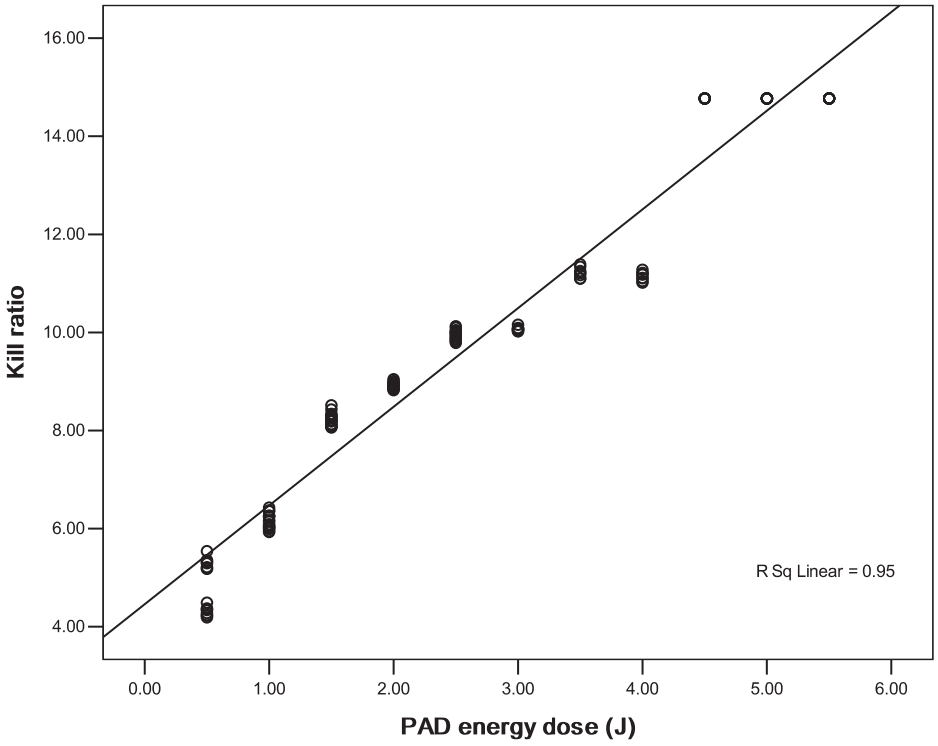

FIG. 1. Photoactivated disinfection (PAD) energy dose (J) and bactericidal effect (kill ratio).

11 samples in the NaOCl group. There was no significant difference between the PAD group and the saline solution group ($p > 0.05$) and the bacteria count in these two groups rebounded to the level before disinfection.

Discussion

PAD is an innovative approach for the disinfection of the root canal system. It involves the use of low-power lasers on photosensitisers to produce reactive oxygen species. The reactive oxygen species are short range free radicals that can disrupt bacterial membrane, which leads to rapid death of the microorganisms.[12] *In vitro*[13,14] and *in vivo*[9,15] studies were performed to explore PAD as an alternative approach to disinfection in endodontic therapy. Seal et al.[16] reported that PAD has the potential to eradicate a wide range of oral bacteria, including EF. However, the clinical parameters remained to be optimized.

NaOCl solution is considered by many to be the preferred irrigant for root canal treatment, because of its proteolytic effect.[17] In the experiment, no bacterium was detected after irrigation in the NaOCl group. It seems that 5.25% NaOCl was more effective in discriminating EF in root canals. However, the recovery of bacteria after 72 h was detected in 11 samples in the NaOCl group, which means that after irrigation by 5.25% NaOCl, there were still some bacteria remaining in the root canals, such as deep dentin tubules and canal irregularities. These results of the infected tooth model experiments show that it is hard to eradicate EF from the root canals. This is because of the complexities of root canal system, the deep invasion of microorganisms into dentinal tubules, and the formation of biofilms on the surface of the root canal walls.[18,19]

Unlike PAD, NaOCl is highly toxic to vital tissues. Heggers et al. suggested that the safe concentration of NaOCl for debridement of wounds should not be > 0.025%.[20] However, such a low concentration has no significant antimicrobial effect for endodontic treatment. At present, there is no consensus on the optimal concentration that is safe and effective for NaOCl use in endodontic therapy. A low concentration of 1% NaOCl and a high concentration of 5.25% NaOCl can all provides tissue dissolution and antimicrobial effects.[21,22]

Table 2. Bacteria Controls in the Three Groups (CFU/mL)

Groups n = 20	Before bacteria counts (Mean, SD)	After		72 h recovery	
		Positive number	Bacteria counts (Mean, SD)	Positive number	Bacteria counts (Mean, SD)
NS	7.14E + 06 (7.39E + 06)	20	3.11E + 05 (1.56E + 05)	20	4.66E + 06 (5.26E + 06)
5.25%NaOCl	1.14E + 07 (1.30E + 07)	0	0	11	5.66E + 04 (8.27E + 03)
PAD	6.07E + 06 (8.83E + 06)	20	1.67E + 04 (1.92E + 04)	20	3.81E + 06 (3.64E + 06)

CFU, colony forming units; PAD, photoactivated disinfection.

However, NaOCl accidents could occur between 1 and 5.25% concentrations of NaOCl; therefore, if a perforation or open apex exists, great care should be exercised to prevent an NaOCl accident, or an alternative irrigation solution should be considered.[23] Unlike NaOCl, the photosensitizers used in PAD are nontoxic to vital tissues.[24] PAD is also harmless to periodontal tissues because the increase in temperature is far below the threshold level to cause periodontal injury.[25] Common photosensitizers in PAD include tolonium chloride and methylene blue. They are organic dyes belonging to the phenothiazine family. Tolonium chloride was chosen as the photosensitizing agent in this experiment because it absorbs light at wavelengths ranging from 620 to 660 nm, and the red light irradiated from the device in this study was 635 nm. In another aspect, tolonium chloride is unchanged by the process, in which activity ceases as irradiation stops.[10]

EF is often associated with persistent endodontic infections, and is commonly found in the root canals of failed endodontic therapy patients.[2] EF can survive long periods of time in root canals without nutrient support.[3] In an in vitro study, EF

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