VERIFIABLE CPD PAPER

IN BRIEF

- Bacteria are responsible for the most commonly encountered dental diseases including pulpal pathology.
- The success rate of endodontics relies on the root canal system being rendered bacteria free.
- Conventional chemo-mechanical canal preparation techniques are unable to disinfect the canals predictably and consistently.
- PAD offers potential to eliminate bacteria from the root canals especially where conventional techniques have failed to do so.

Microbiological evaluation of photo-activated disinfection in endodontics (An *in vivo* study)

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Objective To determine the microbiological effect of photoactivated disinfection (PAD) as an adjunct to normal root canal disinfection *in vivo*.
Design A randomised trial carried out in general dental practice.
Subjects and methods Patients presenting with symptoms of irreversible pulpitis or periradicular periodontitis requiring endodontic therapy were selected at random. A microbiological sample of the canal was taken on accessing the canal, after conventional endodontic therapy, and finally after the PAD process (photosensitiser and light) had been carried out on the prepared canal. All three samples from each canal were plated within 30 minutes of sampling and cultured anaerobically for five days. Growth of viable bacteria was recorded for each sample to determine bacterial load.
Results Thirty of the 32 canals were included in the results. Cultures from the remaining two did not reach the laboratory within the target time during which viability was sustained. Of the remaining 30, 10 canals were negative to culture. These were either one of the canals in multi rooted teeth where the others were infected or where a pre-treatment with a poly-antibiotic paste had been applied to hyperaemic vital tissue. Sixteen of the remainder were negative to culture after conventional endodontic therapy. Three of the four which had remained infected cultured negative after the PAD process. In the one canal where culturable bacteria were still present, a review of the light delivery system showed a fracture in the fibre reducing the effective light output by 90%.
Conclusions The PAD system offers a means of destroying bacteria remaining after using conventional irrigants in endodontic therapy.

INTRODUCTION

It is well established that the elimination of pathogens from root canals during endodontic treatment is difficult[1,2] and current endodontic techniques are unable to consistently disinfect the canal.[3,4] Mechanical preparation of the canal leaves a smear layer on the surface of the canal wall occluding the entrances to any patent dentinal tubules.[5] Furthermore it is a site for accumulation of bacteria within the layer itself. This together with the irregular cross section of the canal and complex internal anatomical morphology[6] result in areas where residual bacteria can accumulate and adversely affect the outcome of any endodontic therapy.[7] To assist in the cleaning and debridement of the canal a range of irrigating and disinfecting solutions have been used. The one which predominates is sodium hypochlorite used in concentrations ranging from 0.2-5%.[8] Chelating agents such as EDTA and citric acid are used as adjuncts to remove the smear layer allowing access to the tubules.[9] Additionally disinfectants such as iodine potassium iodide (IKI) are also known to penetrate into the dentine.[10]

The choice of sodium hypochlorite as an irrigating solution is in part because of its effect on any residual soft tissue, as well as destroying bacteria with the free chlorine in the solution. However since this free chlorine is used up during this process, the volume of solution required is large, the process slow[11] and there is still a substantial risk of bacterial contamination.[12]

There are only a limited number of references to the presence of bacteria leading to higher incidence of failed endodontic treatments: this is primarily because of the difficulty of carrying out microbiological culture work. However, studies have shown that in those cases where negative cultures have been obtained at time of obturation, there is a 94% success rate. When obturation is performed and the cultures are positive, the success rate is reduced to 68%[4] confirming previous studies showing that failure of healing is more likely when the canals are obturated in the presence of persistent infection.[13,14]

Recently a novel method of disinfection for use in both caries and endodontics has become available. This is photo activated disinfection – PAD™. The principle on which it operates is that photosensitiser molecules attach to the membrane of the bacteria. Irradiation with light at a specific wavelength matched to the peak absorption of the photosensitiser leads to the production of singlet oxygen, which causes the bacterial cell wall to rupture killing the bacteria.[15] Extensive laboratory studies have shown that an important aspect of this system is that the two components when used independently of one another produce no effect on bacteria or on normal tissue. It is only the combination of photosensitiser and light which produces the effect on the bacteria.[15-17]

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Table 1 Pooled scores for levels of bacterial load in each canal at commencement of treatment (A) after conventional treatment (B) and after PAD application (C)

Microbiological score	Before treatment Sample A		After preparation Sample B		After PAD Sample C	
	No.	%	No.	%	No.	%
5	0	0	0	0	0	0
4	1	3.1	0	0	0	0
3	7	21.9	0	0	0	0
2 ($1.5x10^8$. bacteria.)	8	25	1	3.3	0	0
1	7	21.9	3	10	1	3.3
0	9	28.1	26	86.7	29	96.7
Total number of samples	**32**		**30**		**30**	

In two cases Samples B and C arrived outside the time limit and the results were excluded

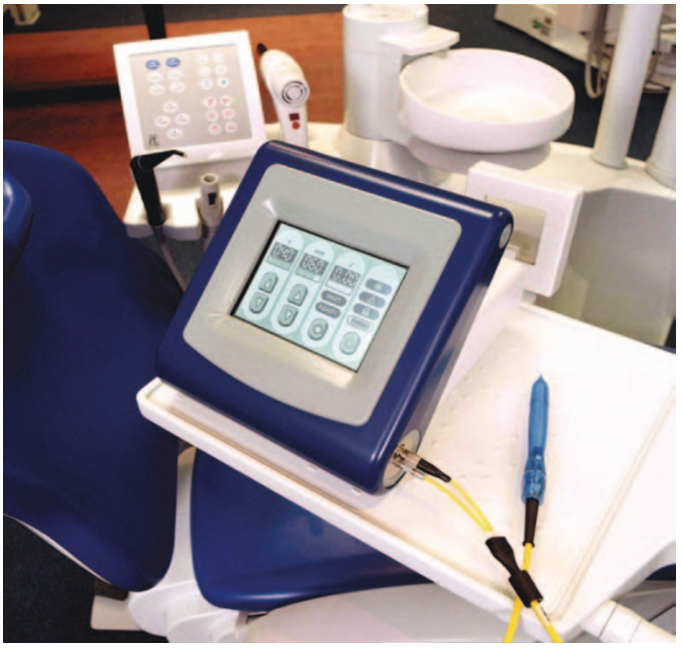

Fig. 1a A diode laser and an endodontic handpiece

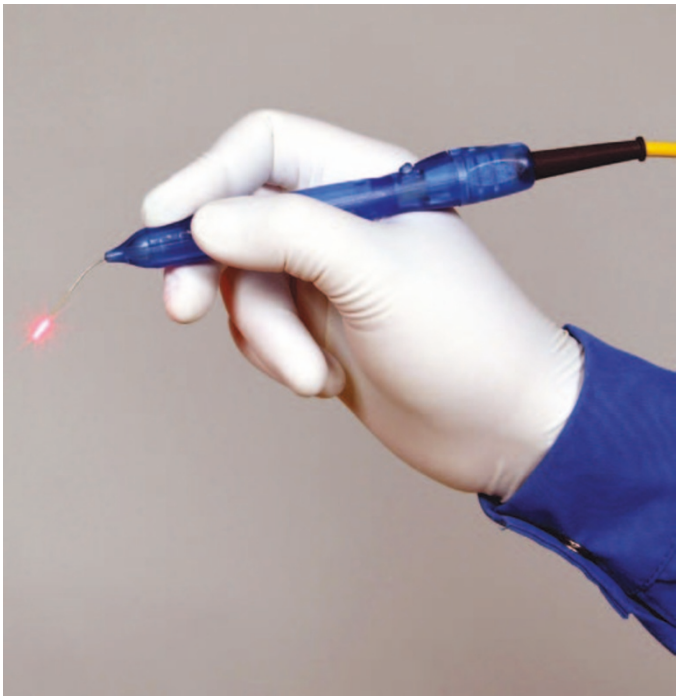

Fig. 1b Emitter handpiece and emitter

Using the principles described above, a system has been developed for endodontic use consisting of a small diode laser (Fig. 1a) connected to a delivery fibre, disposable hand piece and emitter (Fig. 1b). This is used in conjunction with a 12.7mg/l solution of the photosensitiser, tolonium chloride. This is a pharmaceutical grade of the vital stain, Toluidene blue O. This system has been evaluated in the laboratory and bacterial kills of the order of 10^9 have been achieved under conditions comparable to those found clinically.[16,17] It has been shown to be effective in killing the common bacteria associated with endodontic infections such as *Fusobacterium nucleatum*, *Prevotella intermedia, Streptococcus intermedius* and *Peptostreptococcus micros*. It has also been shown that the PAD system will kill *Enterococcus faecalis* which is regarded as one of the contaminants associated with canals which have recurrent infections.[18]

The emitter is a flexible hollow tube coated internally with a light diffusing material (Fig. 1b) of a comparable size to the tip of an ISO standard #40 file. The light is emitted over a 15 mm length of the tip with a uniform energy density. This energy density is increased by 30% at the tip. After completion of canal preparation, the canal is inoculated with the photosensitiser solution which is left *in situ* for a fixed period of time (60 seconds) to permit the solution to come into contact with the bacteria and diffuse through any biofilm structure. The emitter is then placed in the root canal and irradiation carried out for 120 seconds. This has been demonstrated in the laboratory study to kill high concentrations of bacteria generally found in root canals.[19]

The aim of the present study was to evaluate this device and determine microbiologically the levels of bacterial load within the root canal walls at three time intervals in the preparations of the canal with specific reference to facultative anaerobes and anaerobes, the common pathogens in endodontics:

- after initial access
- immediately after conventional preparation had been completed
- subsequently after treating the canal using photo activated disinfection.

In all cases, the dentine of the walls of the canal was sampled.

MATERIALS AND METHOD

Clinical procedure

The trial was carried out in a private general dental practice in Scotland by the same operator. Having obtained ethical approval, patients were selected at random. They were in good general health and between the ages of 16 and 70. Pregnant and nursing females and patients undergoing photodynamic therapy were excluded from the trial. The patients presented with symptoms of irreversible pulpitis or periradicular periodontitis. They all required root canal therapy on teeth with closed apices. Informed written consent was obtained

Thirty-two canals were studied. In each case, a pre-operative periapical radiograph was taken in a double pack film (Kodak, UK) using a long cone technique with an EndoRay film holder (Rinn Corporation, USA) to determine approximate canal length and canal morphology. After local anaesthetic had been administered, access to the pulp chamber was gained and then rubber dam placed. This was sealed with OraSeal Caulking (Ultradent Products Inc, USA.). In order to ensure the crown of the tooth was bacteria free, it and the surrounding dam were irrigated with PAD solution provided by the manufacturer (Tolonium chloride, Denfotex Ltd, Inverkeithing, UK) and this was irradiated with a SaveDent light (Denfotex Ltd, Inverkeithing, UK) for 60 seconds at 100mW. The emitter tip was held adjacent to the crown of the tooth and moved slowly around the crown circumference, at least two rotations being completed in the first 30 seconds. The remaining time was used to irradiate the area surrounding the access cavity. Labo-

ratory studies have shown that this type of treatment effectively kills bacteria in a biofilm.[20]

Once the canals had been identified and accessed and canal patency ascertained, a size 15 .02 sterile nickel-titanium hand file (Dentsply Maillefer, Ballaigues, Switzerland) was placed within the lumen of the canal at a point where resistance to the instrument's progress was just felt. It was filed backwards to remove debris from the canal walls and this instrument together with the swarf sample (dentine debris) obtained was placed in a sterile bottle labelled Sample A. Great care was taken in multi-rooted teeth to ensure that no cross contamination occurred between the canals during the sampling process as each canal was regarded as a test unit. Sodium hypochlorite irrigating solution was not used before this stage. This sample was transported immediately to the Medical Microbiology Department at the local general hospital (Aberdeen Royal Infirmary, Grampian University Hospitals Trust) for culturing.

The canal working length was then determined using an apex locator (AFA Analytic, Kerr Corporation, USA). The canals were prepared using GT Rotaries (Dentsply Maillefer) using a crown down approach working to 2 mm short of the working length. Profiles .04 (Dentsply Maillefer) were then used to prepare the apical two millimetres. Copious irrigation (more than 20 ml of each irrigant per canal) was used between instrumentations alternating between 20% Citric Acid solution (Western Infirmary Glasgow, UK) and 2.25% sodium hypochlorite solution in the form of 4.5% commercial thin bleach (Tesco, UK) diluted 50:50 with water. The solution was injected down the canal using an endodontic micro-needle (Monoject, Tyco Healthcare, Gosport, UK). All irrigants were used at ambient temperature.

When the endodontic emitter fibre could be placed down the canal with the tip within 4 mm of the working length, the canal was washed thoroughly with sterile water to remove any residual irrigants. The canal wall was then sampled as before using a .02 nickel-titanium hand file one size larger than the master apical file. This file with the dentine sample was deposited in a fresh sterile bottle (Sample B) and the canal dried with sterile paper points (Dentsply, DeTrey, Konstanz, Germany).

The PAD solution was injected into the canal using a sterile endodontic micro-needle (gauge 27) ensuring that the fluid passed to the working length. The liquid was agitated in each canal for 60 seconds using a .02 nickel-titanium hand file, two sizes smaller than the master apical file (MAF.) The endodontic emitter was then inserted until it was within 4 mm of the measured working length. The laser was then activated at 100mW for a period of 120 seconds. The emitter was moved up and down about 3 mm at 20 second intervals during the irradiation time.

Following withdrawal of the probe, a new sterile .02 nickel-titanium hand file two sizes larger than the MAF was inserted and a further sample of swarf was obtained in the same manner. This was transferred to a fresh sterile bottle (Sample C). The canal was then thoroughly irrigated using 2.25% sodium hypochlorite solution using an endodontic micro-needle to remove the PAD solution and the canals dried with sterile paper points. Samples B and C were again transported immediately to the Microbiology Department for culturing. The maximum time between collecting the samples and plating in the microbiological laboratory was 30 minutes.

A non-setting calcium hydroxide paste (UltraCal, Ultradent Products, USA) was placed into the canal, a cotton wool pledget (Roeko, Langenau, Germany) placed in the pulp chamber and the tooth was dressed with either IRM (Dentsply, DeTrey) in the case of posterior teeth or Chemfil Superior (Dentsply, DeTrey) in the case of anterior teeth.

At a subsequent visit, each canal was sealed using conventional techniques and formed a cohort of teeth for review at a later stage.

Microbiological assessment of swarf samples

The method of culture was selected to assess the bacterial load of the common facultative anaerobes and anaerobes such as *Fusobacterium nucleatum*, *Prevotella intermedia, Streptococcus intermedius* and *Peptostreptococcus micros* found in root canals. However no attempt was made to identify the specific bacterial flora during the culturing process. On arrival at the microbiological facility, a sterile swab was moistened with sterile nutrient broth (Oxoid Code CM1) and excess fluid expressed. This swab was rubbed against the full length of the cutting blade of the file and rolled onto a fresh blood Agar plate (Oxoid Columbia Blood Agar base + 5% sterile horse blood.) A sterile loop was used to streak five lines from the well. This streak pattern was repeated a further three times giving five growth areas in total (the well area plus the four streaked areas). This ensured standardisation of plating (Fig. 2).

The plates were incubated in a Don Whitley Anaerobic Workstation in an atmosphere of 10% hydrogen, 10% carbon dioxide and 80% nitrogen. The use of a palladium catalyst ensured that the oxygen level was less than 1% within 20 minutes and less than 0.55% within three hours. They were then examined and scored. If growth occurred in the well area, a score of one was allocated. If the growth occurred in both the well and the first five streaked lines, this was scored two and so on up to a maximum score of five.

To provide a semi quantitative method of evaluating the bacterial load, a separate experiment was carried out by inoculating a known concentration of oral streptococci in similar fashion. This was carried out using NCCLS methods (an internationally accepted method of standardising inocula). The inoculum of a Streptoccal strain was prepared using a colorimeter (Biomerieux Ltd). A suspension was made in saline and adjusted using the instrument to a 0.5 McFarland standard. This equates to the level of 1.5×10^8 colony forming units. A sterile endodontic file was placed in the suspension, allowed to drain, swabbed and inoculated onto the agar. Plating out in the described manner showed this growth to give a score of two. These studies were performed in triplicate.

RESULTS

Fourteen patients participated in the trial producing 32 canals available for culture. The scores for levels of infection at the three sample stages are set out in Table 1. Of the 32 root canals evaluated, only 30 had completed microbiological scores as two post preparation cultures and two PAD cultures arrived at the laboratory outside the time regarded as acceptable for maintenance of viability of micro-organisms. Ten canals were negative on culture. Of these, three canals were in multi-rooted teeth where other canals exhibited high bacterial scores. In a further three cases, the pulpal tissue was inflamed and had been treated initially using a corticosteroid and topical poly-antibiotic (Ledermix, Lederle) prior to extirpation. While these were treated in the same way, neither the use of the conventional irrigants nor PAD would have any further effect as the canals were already negative to culture. It did however confirm that cross-contamination of the canals did not occur during preparation. Twenty canals were initially infected with scores ranging from 1-4. No canal had the maximum level of measurable infection, a score of five (Fig. 3). Even after the use of the potent irrigating fluids, sodium hypochlorite and citric acid, there remained four canals (20% of the original canals with positive culture) which remained infected. With one exception, the level of infection was reduced to zero after the use of the PAD technique.

DISCUSSION

Culturing of root canal microflora is a particularly difficult and time consuming technique and requires microbiological facilities in close proximity to the dental surgery to ensure that bacteria

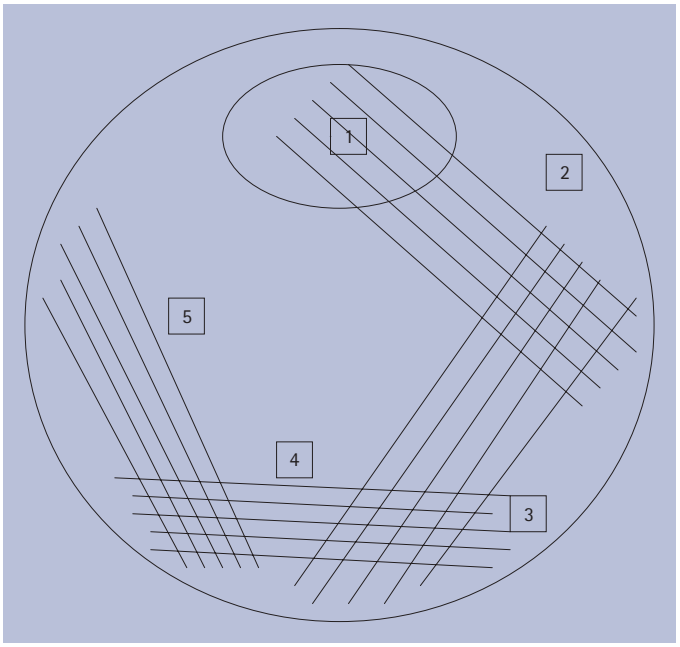

Fig. 2 Diagrammatic representation of plating of microbial growth. Numbers indicate sequence of streaking the culture: where bacteria are present on second streak this is equivalent to 1.5x 10^8 bacteria

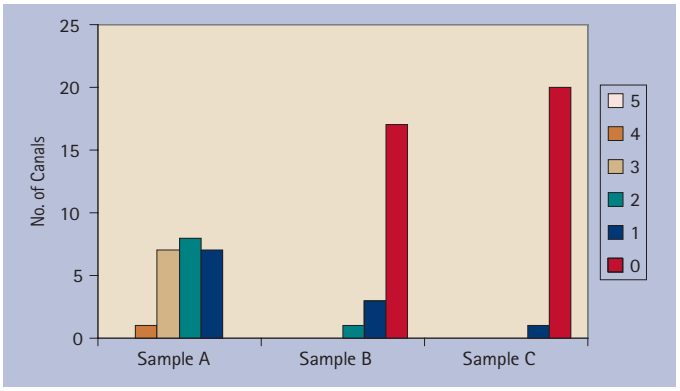

Fig. 3 Bar chart of all teeth with positive microbiological scores at commencement of treatment (A) together with scores after conventional treatment (B) and after PAD application (C). Score of 5 equivalent to heavy bacterial load, score of 2 equivalent to approximately 1.5x 10^8 bacteria, score of 0 equivalent to no culturable bacteria

(particularly the anaerobes) do not die in transit. However it is probably the only effective short term means of evaluating the various techniques for disinfecting root canals. The improvement in success rate by ensuring absence of bacterial contamination in endodontics, particularly when periradicular periodontitis is present, has been shown by a number of workers.[1,13,14]

In this study, canal samples were cultured within 30 minutes of the sample being taken. The viability of the cultures was checked initially before the study was started by transmitting test samples between clinic and laboratory to determine the survival of the bacteria. It was considered that anything cultured within 30 minutes of being sampled would not have been degraded using appropriate transporting conditions. To this end, any sample which arrived outside this time limit at the laboratory was discarded. In the light of laboratory evaluations which demonstrated quantitatively the effectiveness of the PAD process[15,16,20] it was decided that a semi-quantitative method of assessment would be suitable since the requirement was to determine if any culturable bacteria were present after either conventional or subsequent PAD treatments. For the same reason the decision to culture in an anaerobic atmosphere was made since the organisms involved are primarily either strict anaerobes or facultative anaerobes.

A further concern in culturing is the risk of canal cross contamination. The results in this study do demonstrate the reliability of the technique adopted since:

- it was possible to identify canal walls initially free of culturable bacteria in multi-rooted teeth
- there were no culturable bacteria detected in these canals after the process of cleaning and shaping and also after the PAD process.

It is well established that it is difficult to ensure that root canals are bacteria free when sealed. The conventional irrigants such as sodium hypochlorite are not effective at bacterial killing unless used in copious quantities and even then, it has been reported that a proportion of canals will still contain culturable bacteria after treatment.[8] Previous work on culturing of canals using hypochlorite has shown that of 20 canals treated with 0.5% NaOCl, only nine produced a negative culture.[1,12] Increasing the concentration to 5% reduced the positive cultures by one so that half the canals remained with a level of infection. However at this concentration of hypochlorite, the level of tissue toxicity is increased.[11]

A further limiting factor is the introduction of any fluid into a canal leading to air entrapment and inadequate wetting of the canal walls. This is a particular problem with sodium hypochlorite as it does not wet the dentine walls well, small narrow canals being particularly difficult to irrigate.

In the current study, using sodium hypochlorite solution in conjunction with citric acid to remove the smear layer, the number of canals which contained culturable bacteria after irrigation was 20% of those which were initially contaminated. This however is still a high proportion of canals, particularly as Sjögren's work[4,14] suggests that there is a 25% fall in success rate where canals are obturated when contaminated and are potentially likely to require remedial work at a later stage.

The introduction of the PAD solution and delivery of the prescribed energy dose resulted in three of the four contaminated canals being rendered culturable bacteria free. In these three cases, non wetting of the canal walls could not have been a significant problem. In fact, the photosensitiser solution has better wetting capabilities when compared to sodium hypochlorite, having a lower surface tension. Care must however be taken to ensure maximum wetting of the surroundings as it is important that the PAD solution contacts the bacteria otherwise the photosensitisation process will not occur.

In the one case which did not respond to PAD treatment, the emitter tip appeared to be slightly kinked. The handpiece and emitter was returned to the manufacturers who measured the light output at the tip and found it to be only 10% of the expected output. Further examination showed that the fibre had fractured. Thus, the bacteria did not receive a sufficient energy dose to kill them. The procedure of moving the fibre up and down the canal has now been discontinued as it was considered that binding of the fibre in the canal was the cause of the kinking.

CONCLUSIONS

The results of the study show that the PAD technique was successful in eliminating all the culturable bacteria when the correct combination of photosensitiser and correct energy dose are used and where both the light and the photosensitiser reach the bacteria. It highlighted the need for care in the use of the emitter to ensure that it is not bent too tightly or trapped in the canal.

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