Evaluation of antibacterial action of photosensitizer solution activated by diode lamp and three intracanal medicaments (in vitro study)

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ABSTRACT

Background: The elimination of the microorganisms from the root canal systems, an important step for the successful root canal treatment. This study was conducted to evaluate the antibacterial effectiveness of the photoactivated disinfection by using the toluidine blue O and a low- energy light emitting diode (LED) lamp .

Materials and method: Sixty single rooted extracted teeth were decoronated, instrumented, irrigated, sealed at the apex and contaminated with endodontic anaerobic bacteria for 7 days to form biofilms in prepared root canals. Group I. Twelve teeth were medicated by photosensitizer (toluidine blue O) solution activated by diode lamp (FotoSan; CMS Dental, Copenhagen, Denmark).Group II. Twelve teeth were medicated by the tricresol formalin. Group III. Twelve teeth were medicated by the camphorated monochlorophenol (CMCP). Group IV. Twelve teeth were medicated by calcium hydroxide ($Ca(OH)_2$) paste. Group V. Without the intracanal medication (control group). The bacterial swabs were taken before and after medication and following the photoactivated disinfection procedure immediately and after 7days. The canal contents were swabbed by paper points inserted to the root canals, serially diluted and cultured on blood agar. Survival fractions were calculated by counting colony-forming units.

Result: Treatment of the root canals with PAD (fotosan) caused a high significant reduction of the bacterial count, resulting in a 96.39% elimination of root canal bacteria, followed by root canal treated by tricresol formalin (group II),then CMCP (group III) and Ca(OH)2 (group IV) respectively.

Conclusion: Light activated disinfection possesses potent antibacterial action against the anaerobic bacteria cultivated in root canals.

Key words: Photoactivated disinfection, FotoSan, anaerobic bacteria. (J Bagh Coll Dentistry 2013; 25(3):43-48).

INTRODUCTION

Microorganisms are the primary an etiologic factor in the development of pulp and periapical diseases [1]. Endodontic pathogens have developed a variety of strategies to survive in adverse conditions. They may invade dentinal tubules and persist in superficial layers of dentin adjacent to the canal lumen [2], and they may organizeas biofilms, complex sessile communities performing numerous adaptive changes in behavior that increase their resistance to a variety of chemotherapeutic agents compared with their planktonic counterparts [3].

Conventionally, disinfection of the root canal is sought by a "chemomechanical" approach that involves cleaning and shaping of the root canal system by the application of a chemical disinfectant and mechanical instrumentation [4]. Nonetheless, this technique often fails to eradicate bacterial biofilms completely, mostly because of various microbiological and anatomical factors [5].

Chemo-mechanical preparation of the root canal reduces endodontic infection, but microorganisms are able to survive within the complex anatomy of the root canal system [6]. Antimicrobial intracanal medicaments are used to complement the disinfection of the root canal system[7].

Phototactivated disinfection (PAD) is an antimicrobial strategy that combines a nontoxic photosensitizer and low-energy light to produce highly reactive singlet oxygen species, which results in microbial elimination [8]. PAD has emerged as a promising approach to eradicate endodontic pathogens [9].

MATERIALS AND METHOD

Preparation of Tooth Specimens

Sixty human single-rooted extracted teeth were used. The tooth specimens were selected for the experiments after inspecting for any signs of cracks or damages on the cementum .The teeth were decoronated to obtain root segments with a standard length of 12 mm [10-12]. The apical patency and the glide path presence were confirmed with a size 15 K-file .The working length was established with the same file, subtracting 1 mm from the file measurement at the point where it was just visible at the foramen.

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The cleaning and shaping of root canals were performed in a crown-down manner [12] , using the Protaper hand nickel titanium system. The canals were irrigated with 3 ml 6% NaOCl [12] throughout the instrumentation sequence (SX,S1,S2,F1,F2,F3) until finishing file F4 (40/ 0.6). After the preparation, each apex was sealed with the wax and the external surface coated with two layers of nail varnish to prevent the contamination. The teeth were mounted vertically in the addition silicon impression material blocks**.** Prior to the inoculation, the specimens were sterilized by autoclaving for 15 minutes at 121 °C.

Contamination of the tooth specimens

The bacterial suspension was prepared by adding 1 ml of isolated bacteria, cultivated in BHI-B for 24 hours, to 5 ml of fresh Brianheart infusion broth. Each root canal was completely filled with the bacterial suspension using the sterile 1-ml insulin syringes without overflowing. The sterile K-files #15 were used to carry the bacterial suspension to the entire root canal length by one inward and outward movement. Then the roots incubated at 37°C for 7 days. The fresh culture medium was added to the canal at 2, 4, and 6 days after the initial inoculum **.**[14]

Sample Grouping

The teeth specimens were divided according to the intracanal medicament:

Group I. Twelve teeth were medicated by photosensitizer (toluidine blue O) solution activated by diode lamp (FotoSan; CMS Dental, Copenhagen, Denmark)**.**

Group II. Twelve teeth were medicated by tricresol formalin; by the cotton pellet technique.

Group III. Twelve teeth were medicated by camphorated monochlorophenol (CMCP);by the cotton pellet technique.

Group IV. Twelve teeth were medicated by calcium hydroxide $Ca(OH)_2$ paste; by condensing it in the root canal.

Group V. Twelve teeth were leaved without the intracanal medication (control group).

Light Source and Photosensitizer

An LED lamp emitting in the red spectrum with a power peak at 628 nm was the light source used throughout all the experiments of group I (FotoSan; CMS Dental, Copenhagen, Denmark). The energy output was measured to be 1 J/s. A long tapered tip, referred to as the endodontic tip, was mounted to the device **.**With its apical size of 500 µm and a 0.03 taper in the apical part, it was inserted into the canals up to 3 mm short of the working length and guide the light to the apical parts**.**

A liquid solution of the toluidine blue O (TBO; Sigma-Aldrich, St. Louis, MO), a thiazine dye of the quinone-imine family, was used as photosensitizer in the experiments [15]**.**

Procedure of Medication

1. Group I. TBO solution (0.1 mg/mL) was injected into the root canal using a sterile endodontic micro-needle (gauge 27) ensuring that the fluid passed to the working length. The endodontic tip was inserted into the canal 3 mm short of working length, and 30 seconds of irradiation followed [15]. After swabbing, the canals were dried by paper points until they are dry, closed by dry cotton pellets and sealed by the temporary filling.

2. Group II and III. The intracanal medicaments of 0.025 ml volume were placed in canal by cotton pellet technique by mean of a micropipette and then the teeth were sealed by the temporary filling**.**

3. Group IV. The teeth were treated by calcium hydroxide which was placed by a fine needled syringe then the excess material was removed by cotton pieces, then the teeth were sealed by the temporary filling.

Microbiological samples were collected before and after medication with the sterile paper points (F3) inserted once, placed for 5 seconds to full working length then withdraw and transferred to tubes containing 1ml of 0.85% NaCl solution and agitated in vortex for 1 minute. After serial dilutions in saline, aliquots of 0.1 ml were cultivated onto a petri dish containing the blood agar culture media [14]. One cultured petri dish was incubated anaerobicaly at 37°C. After 24 hours, the bacterial colonies that grown on the culture medium were counted by the colony counter to verify the number of microorganisms present in the inoculums.

RESULTS

The count of the root canal bacteria was expressed as colony forming unit(CFU)×10^3.The number of colonies recorded were multiplied by reverse of the dilution factor which had been selected by performing a pilot study to have a readable count of bacteria on the agar surface .

The findings showed that the root canals treated by Fotosan exhibited the least viable count of bacteria immediately after 30 second from treatment and after 7 day(1.75±0.75,0.25±0.05) respectively, followed by the root canals treated by tricresol formalin

(group II),then CMCP (group III) and $Ca(OH)_2$ (group IV) respectively (Table 1).

The results of this study showed that the root canals treated by PAD exhibited high percentage of reduction of the bacteria count immediately after 30 second from treatment and after 7 day (96.36,99.48) respectively, followed by tricresol formalin (group II), then CMCP (group III) and Ca(OH)2 (group IV) respectively. Table (2)

Using LSD test, the results indicated that the light activated disinfection (group I) exhibited non significant difference between mean of the bacteria count after 30 second and after 7 day. The results also showed non significant difference between the light activated disinfection (after 30 second) and the tricresol formalin group but there were significant difference between the light activated disinfection (after 7 day) and tricresol formalin group.

At the same time ,there were high significant differences between mean of the bacteria count after 30 second and after 7 day of the light activated disinfection (group I) when compared with other groups. Table (3)

DISCUSSION

Antibacterial Efficiency of Photoactivated Disinfection (PAD).

The results of this study showed that the root canals treated by photoactivated disinfection exhibited high percentage of reduction of bacteria count immediately after 30 second from treatment and after 7 day (96.36, 99.48) respectively.

These results coincide with Rios *et al* [12], who found that root canals treated with photoactivated disinfection for 30 seconds alone exhibited a 2.9% survival rate of E. faecalis, whereas the combination of NaOCl followed by photoactivated disinfection lowered the survival rate to 0.1%. These results are in accordance with another study that reported a 97% reduction of E. faecalis viability in extracted human teeth using photoactivated disinfection with methylene Blue and a laser but with longer treatment times (5 minutes). [16]

Soukos *et al* [16] reported that oral endodontic pathogens exposed only to methylene blue (25µg/ml) for 5 minutes in planktonic phase demonstrated high cytotoxicity .This toxicity, led to 79% to 100% reduction in cell numbers. The addition of red light of 665 nm with a fluence of 30 J/cm2 resulted in complete eradication of those species that were incompletely eliminated by methylene Blue. Methylene blue alone exhibited 83.2% reduction of *E. faecalis* biofilm species in the root canal system of extracted human teeth. The combined effect of methylene blue and red light did not lead to complete eradication of *E. faecalis* biofilms (97% reduction). These findings are in accordance with the results of this study.

The results of the present study are in agreement with Bergmans *et al* [17] who stated that the treatment of root canals with photoactivated disinfection (15 J) caused a significant reduction of the bacterial load, resulting in a 93.8% reduction of S. anginosus ($P < 0.0001$), a 88.4% reduction of E. faecalis ($P < 0.05$) and a 98.5% reduction of F. nucleatum ($P < 0.0001$), but no sterilization.

The results of this study disagreed with Seal *et al* [18] and Lee *et al* [19] , who used phenothiazine-based photosensitizer and low-intensity red lasers against gram-positive bacteria but did not use an optical fiber to access the root canal lumen. Seal et al found that 3% sodium hypochlorite irrigation reduced more *Streptococcus intermedius* in the endodontic biofilms than photoactivated disinfection with 100 _g/mL toluidine blue and 21J of 632 nm laser light.

The results of the present study are in agreement with Schlafer *et al* [15] who found that the mean post treatment reduction of 95.82% (1.38 log10) showed that the effect of the light is again weaker on adherent organisms than on bacteria in suspension inside the canal lumen, which showed 99.7% (2.51 log10) killing, but Foschi *et al.* in 2007[20] who demonstrated that sensitization of E. faecalis microorganisms colonizing the root canal of single rooted extracted human teeth with 6.25 mg/ml methylene blue for 5 minutes followed by exposure to red light with energy fluence of 60 J/cm2 light energy fluence at a power density of 100 mW/cm2 led to approximately 78% killing.

Prolonged Antibacterial Efficiency of Photoactivated Disinfection(PAD) after 7 day.

In the present study, the sampling of the root canals treated by photoactivated disinfection after 7 days showed that the percentage of reduction is increased from 96.36% to 99.48 % and lack the bacterial regrowth ,this results in agreement with Garcez *et al* [21] who concluded that the use of photoactivated disinfection as an adjuvant to the conventional endodontic treatment leads to a statistically significant further reduction of bacterial load ($P<0.05$) and in particular reduces the amount of bacterial regrowth after 24 hours compared to either treatment alone ($P<0.0001$).

The present results are coincide with George and Kishen [22] who found that when the biofilm was subjected to photoactivated disinfection using methylene blue dissolved in water, there was a difference of 1.5log10 in the mean viable count that corresponded to 96.89% reduction in viable bacteria compared with the control group. Complete killing of bacteria was observed when the root canals were subjected to root canal treatment, photoactivated disinfection using (perfluorodecahydronaphthalene) oxygen carrier, and treatment comprising Root Canal Treatment combined with photoactivated disinfection using oxygen carrier. Photoactivated disinfection using oxygen carrier alone or in combination with conventional disinfection technique showed the absence of bacteria even after 24 hours of incubation, suggesting complete bacterial inactivation..

Antibacterial Efficiency of Intracanal Medicaments.

Formaldehyde containing compound achieved high percentage of bacterial reduction 95.4%.This conclusion agrees with the result of Nunn *et al* [23] who stated that the formaldehyde has good and long distance antibacterial action permitting the vapor to reach the most distance places in root canal.

Formaldehyde containing compound possess antibacterial action superior to CMCP. Tricresol formalin vapor action eradicated the microflora in necrotic root canal effectively, achieved high percentage of bacterial reduction 95%.This is very good result may be because presence formaldehyde in these compounds.[24]

The present study showed that the CMCP achieved high percentage of bacterial reduction 90.6%.

Al-Huwaizi [24].found that CMCP gained a negative culture of 70% which indicated that its vapor antibacterial action is not as efficient as the formaldehyde containing compound. CMCP vapor action had mild antibacterial action on necrotic root canal. This results in agreement with our finding.

Tanriverdi *et al* [25] reported that the CMCP still better than calcium hydroxide, and they concluded that when CMCP used in the root canal the interappiontment time should not exceed the first 3 day. Antibacterial action of CMCP was found to be more on the anaerobic than on aerobic bacteria.

The present study showed that the calcium hydroxide achieved 83.4% percentage of bacterial reduction. this findings are in agreement with Shuping *et al* [26] who stated that the addition of calcium hydroxide as an intracanal medication for at least 1 week produced 92.5% of canals void of bacteria. There was a statistically significant decrease in bacterial numbers between the final instrumentation samples and the samples taken after calcium hydroxide therapy. These results are coincide with Athanassiadis in 2007[27] who found that the calcium hydroxide has been widely accepted as an intracanal medicament because of its antimicrobial properties, especially because of its action on gram-negative bacteria**.**

A significant decrease in the number of CFUs and the percentage of viable E. faecalis was observed after treatment with $Ca(OH)_2$**.**[28]

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Table 1. The effect of fotosan, tricresol formalin, CMCP, $Ca(OH)_2$ and control groups on root canal bacteria values expressed in $CFU\times10^3$

Groups	State	Descriptive statistics		
		Mean	S.D.	S.E.
Group I Fotosan	Before	47.92	7.39	2.13
	After 30 second	1.75	0.75	0.22
	After 7day	0.25	0.05	0.01
Group II Tricresol formalin	Before	49.67	6.14	1.77
	After	2.33	1.07	0.31
Group III CMCP	Before	46.33	7.04	2.03
	After	4.33	0.98	0.28
Group IV $Ca(OH)_2$	Before	42.75	8.28	2.39
	After	7.00	1.86	0.54
Group V Control group	Before	50.67	5.42	1.56
	After	45.33	5.09	1.47

Table 2. Percentage of reduction of bacteria count of groups.

Groups	Percentage of reduction
Group I Fotosan	96.39 (30 S)
	99.48 (7 D)
Group II Tricresol formalin	95.41
Group III CMCP	90.62
Group IV $Ca(OH)_2$	83.44
Group V Control group	10.47

Table 3. LSD test comparing the differences in mean bacteria count value after treatment between groups.

Groups		Mean Difference	p-value
I after 30 S	I after7D	1.500	0.118 (NS)
	II	-0.583	0.540 (NS)
	III	-2.583	0.008 **
	IV	-5.250	0.000 ***
	V	-43.583	0.000 ***
I after7 D	II	-2.083	0.031 *
	III	-4.083	0.000 ***
	IV	-6.750	0.000 ***
	V	-45.083	0.000 ***
II	III	-2.000	0.038 *
	IV	-4.667	0.000 ***
	V	-43.000	0.000 ***
III	IV	-2.667	0.006 **
	V	-41.000	0.000 ***
IV	V	-38.333	0.000 ***

***=significant **=highly significant ***=very highly significant**