

ORIGINAL ARTICLE

# Comparison of antibacterial activity of diode laser 980 nm and double antibiotic paste during regenerative endodontic therapy of mature necrotic teeth

## ABSTRACT

**Aim:** This study compared the antibacterial effect of diode laser and double antibiotic paste (DAP) on the response of mature teeth with necrotic pulp and apical periodontitis to regenerative endodontic therapy (RET) in a dog model.

**Methodology:** Pulp necrosis and periapical pathosis were induced in 30 mature double rooted premolars in three mongrel dogs aged between 2 to 3 years. These teeth were classified according to the method of root disinfection into three equal groups (10 teeth each), group I: DAP; group II: diode laser (DL) 980 nm and group III: without disinfection (control). Bacterial samples were collected before and after one month of disinfection. The colony forming unit (CFU) and percent of reduction in colony count (%RCC) were evaluated. Revascularization techniques were performed using induction of bleeding and platelet rich fibrin (PRF). The pulp chamber was sealed with mineral trioxide aggregate (MTA) and the coronal cavities were filled with glass ionomer cement. Statistical analysis was done utilizing ANOVA, Tukey's post hoc and paired t tests.

**Results:** The highest mean value of CFU was recorded in the control group followed by group I and group II. The difference in CFU between before and after treatment within each group was statistically significant ( $P \leq 0.05$ ). Group II showed the highest %RCC, followed by group I, while the control group showed a percent increase. Percentage of RCC was statistically significant between all groups ( $P \leq 0.05$ ).

**Conclusion:** Diode laser 980 nm exhibited more antibacterial effect than double antibiotic paste during regenerative endodontic therapy of mature necrotic teeth in a dog model.

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## Introduction

**R**egenerative dentistry has growing and promising role in the management of endodontic and periodontal lesions. It aims to stimulate the renewal and regeneration of damaged structures like the root, dentin structures and dental pulp complex cells (1). The RET was first suggested for the management of immature teeth with necrotic pulp (2-9).

Recently, regenerative endodontic treatment also shows promising results in mature teeth (10, 11). The idea of pulp regeneration provides biological seal rather than mechanical seal obtained by artificial obturating materials. The subsequent healing reaction to regenerative endodontic in the apical third of the root canal had better resistance to infection (2).

A major prerequisite to achieve RET is to achieve efficient root canal disinfection (8,10). Sodium hypochlorite, mechanical preparation and calcium hydroxide were traditionally advocated for root canal disinfection; however in situations involving biofilm-related infection, they were ineffective (12). As a result, further intracanal medications were suggested as substitutes or adjuncts in root canal disinfection such as triple antibiotic paste (TAP).

One of the main disadvantages of TAP is discoloration of young mature teeth due to minocycline that is one of the components of TAP, consequently double antibiotic paste (DAP) was proposed (12,13). Higher concentrations of DAP eliminate microbes but it has cytotoxic effect on viability of stem cells. DAP with lower concentration (0.1 mg/ml) has a significant effect on bacteria and negative effect on viability of stem cells (13).

Recently the use of laser power has gained an increased interest in the endodontic field due to its bactericidal effect, bio-stimulation, improving the success and longevity utilizing the thermal effect of laser on surrounding tissues (14, 15). This may lead to save the patients from the invasive surgical intervention, so it will save both the patient and the dentist time. The range

of laser wavelengths for dental applications ranges between 800 and 1,064 nm (16). A recent study assessed the effectiveness of diode laser in maturogenesis of immature teeth with necrotic pulps and endorsed using it (17). However, there are few studies on the antibacterial effect of diode laser in regenerative endodontic. This section of the research compared the antibacterial effect of diode laser and DAP on the response of mature teeth with necrotic pulp and apical periodontitis to RET in a dog model.

## Materials and Methods

### *Ethical approval*

The research was approved by the Research Ethics Committee at Faculty of Dental Medicine for Girls, Al Azhar University, Egypt (P-EN-21-03). Additionally, all the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines were followed up.

### *Animal model*

Three 2 to 3-year-old mongrel dogs were enrolled in this study. The dogs were obtained commercially from Al-Fahad Trading Company for Animals (Abu Rawash, Giza, Egypt). These dogs were kept in separate cages under optimum conditions of ventilation, cleanliness standards, and a healthy diet in the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Cairo University, Egypt. They had complete set of permanent dentitions and mature teeth with no sex predilection (11). In each dog, 10 premolars were used to sum 30 teeth constituting 60 root canals. These teeth were divided into three groups (10 teeth each). Each group was randomized equally in the upper and lower jaws in each animal (10 teeth: 4 teeth in upper jaw and 6 teeth in lower jaw). The right side included the control and laser groups while the left side included the control and DAP groups.

### *Sample size calculation*

Sample size calculation was done using alpha ( $\alpha$ ) level of 0.05 (power = 80%) by IBM® SPSS® Sample Power® Release 3.0.1.

The calculation was estimated using CDC Epi Info program version 7.2.0.1 (Atlanta, USA). A total sample of a minimum 28 teeth from two dogs (10 each experimental group; and 8 for the control group) was needed based on an estimated mean bacterial count of  $1.712 \pm 0.848$  in experimental group using diode laser compared to  $0.552 \pm 0.097$  in experimental group using double antibiotic paste (17).

#### *Classification of samples*

Thirty mature premolars were divided into two major experimental groups and one control group (10 teeth each) according to the disinfection protocol; Group I: Double antibiotic paste, Group II: Diode laser 980 nm and control group that represented teeth with induced infection and without treatment.

#### *Induction of periapical pathosis*

General anesthesia was administered after fasting the dogs for 12 hours. The dogs were premedicated by 0.05 mg/kg body weight atropine sulphate (Atropine sulphate 1%®, ADWIA, Egypt) injected subcutaneously and 1 mg/kg weight xylazine HCl (Xylaject 2%®, ADWIA, Egypt) injected intramuscularly. The anesthesia was induced by using ketamine HCl (Ketamine hydrochloride®, Rotexmedica Co., Germany) injected intravenously through a cannula in the cephalic vein at a dose of 5 mg/kg body weight. The anesthesia was maintained by 2.5% thiopental sodium (Thiopental sodium®, EIPICO, Egypt) at a dose of 25 mg/kg body weight solution injected intravenously. Coronal cavity was made in all experimental and control teeth. Exposing the pulp chamber was made using #2 diamond stone with high-speed handpiece mounted on a portable unit. A sterile file #15 was used to disrupt the pulp. Supra gingival plaque from the dog's teeth was mixed with sterile saline solution; sterile sponge was soaked in the plaque suspension and inserted into the pulp chamber. A piece of cotton was put into the entrance of each canal, and the coronal cavities were filled by cotton for one month. Dogs were given soft diet and Carprofen tablet (Rimadyl tablet®, Zoetis,

USA) orally for 10 days at a dose of 4.4 mg/kg once daily as postoperative analgesia (5-8).

#### *Preparation of double antibiotic paste*

To prepare diluted concentration of DAP, equal portions of ciprofloxacin (Ciprocin 250 mg Capsule®, EIPICO, Egypt) and metronidazole (Flagyl 250 mg Tab®, Sanofi Aventis, Egypt) were mixed with distilled water to obtain a concentration of 0.1mg/mL, then, 2.5 g of methyl cellulose powder was added to 100 mL of DAP with concentration (0.1 mg/mL) under magnetic stirring to achieve a homogenous gel that was used in this study (18).

#### *Pretreatment bacterial samples and colony-forming units (CFU /mL)*

Under the same general regimen of anesthetics, proper aseptic conditions and rubber dam isolation, the previously infected experimental teeth were entered again. The soaked cotton was pulled and each root canal was filled with sterile saline solution as a transport fluid. Bacterial samples were taken by introducing 4 sequential absorbent paper points of size compatible with root canal diameter up to the working length, which was delimited 1 mm from the radiographic apex. After 30 seconds, the paper points were removed from the canals and placed in a sterile test tube containing 2 mL of sterile saline. One mL of water was quantitatively cultured (using 100 and 10-1 dilutions) on Mueller Hinton culture media. After the incubation period, the developed colonies of bacteria were counted and the number of colony-forming units per mL (CFU/mL) was determined. The CFU/mL value was calculated using the following formula:  $CFU/mL = \text{average number of colonies} \times \text{inverse of dilution}$  (19).

#### *Root canal preparation and disinfection*

After the infection period and under general anesthesia, periapical lesions had been verified by periapical radiographs. All previously infected teeth were accessed again under aseptic conditions and rubber dam isolation. The working length to the anatomical apex was determined for REP



using an apex locator (E-CONNECT, E-PEX Pro, Eighteeth, China). The root canals were instrumented to the desired length using ProTaper Universal system (Dentsply Maillefer, Ballaigues, Switzerland) up to #F4. All canals were irrigated using 2 mL of 1.5% sodium hypochlorite (NaOCl) between each file (Clorox Co., Egypt) with a 27-gauge side vented needle during all steps of preparation. Canals were rinsed finally with 0.9% normal saline (20 mL/canal, 5 min), dried with paper points and dressed with DAP in group I. The DAP mixture was put intra canals by injecting through the canal using a 20-gauge sterile plastic syringe. The access cavities were filled with the glass ionomer as a temporary filling material and teeth were left for one month before the second intervention. On the other hand, disinfection by diode laser in group II was held after mechanical preparation. The root canals were filled with 2 mL of 1.5% sodium hypochlorite, the laser fiber (980 nm, 2.5w) was entered inside the canal 1 mm before apex and, then the fiber was moved up and down inside the canal to perform lasing.

The root canals were dried, and then the laser fiber tip (980 nm, 2.5w) was reinserted 1 mm shorter than the actual working length. The fiber was moved in a circular motion from the apex to the coronal end and from the coronal end to the apex (5 passes with 5 seconds in-between where the pass was from the apex to the coronal end). The internal wall of the canal wasn't touched with the fiber tip (20).

The coronal cavities were filled using glass ionomer restoration (Riva Self Cure, SDI Limited, Australia).

#### *Treatment modalities*

After one month and under the same general anesthesia as well as aseptic techniques, the experimental teeth were entered again, the glass ionomer restoration was removed using diamond stone. The root canals were treated according to different treatment protocols for experimental groups as follows.

In group I, DAP was removed with copious irrigation with distilled water followed by 6mL of 1.5% NaOCl solution and 17%

EDTA solution (Prevest Denpro, Digiana, Jammu, India) (5 mL/canal, 5 min). Distilled water with paper point dryness was used in between irrigations.

In group II, the canal was irrigated with 2 mL of 1.5% NaOCl solution, then activated by diode laser 980 nm at power of 1.5Watt with Ton=10milliseconds; T off=10milliseconds 50Hz (50% pulse mode). The fiber optic tip with size 200 micron diameter was applied intra the canal 1 mm short of the working length moved in helical movement along the root walls at speed of 2mm/sec. Distilled water with paper point dryness was used in between irrigation. Irrigation with 17% EDTA solution and irradiation with the laser were performed and followed again by 2mL of 1.5% NaOCl irrigation. Final irrigation with EDTA 17% solution for 1 minute was carried out and followed by final rinse with distilled water and paper point dryness (21).

#### *Post treatment bacterial samples and bacterial count*

When root canal disinfection procedure was done, post treatment bacterial samples and count were performed by the previously described method. The root canals were then dried with sterile paper points for regeneration protocol.

For bacterial count, visible colonies produced before and after treatment were counted in every plate. The number of colonies/plates was multiplied by the corresponding dilution factor and by 10 to determine the total colony forming units (CFU) per mL of sample. Antibacterial effectiveness was assessed by determining the percentage of reduction in colony counts (%RCC) before and after treatment (8).

Calculation of the change's percentage as:

$$\frac{(\text{CFU base line (before treatment)} - \text{CFU a month (after treatment)})}{(\text{CFU baseline (before treatment)})} \times 100$$

#### *Procedure of pulp regeneration*

A sterile size #20 hand K-file was inserted 2 mm beyond the canal terminus until bleeding was induced to fill the canal space. The blood clot was formed at several minutes. A total of 20 mL venous blood

was taken from the dog's cephalic vein. The blood sample was put into a test tube without anti-coagulant and centrifuged immediately using a tabletop centrifuge (REMI Laboratories, Mumbai, India) at 3,000 revolutions per minute for 10 minutes. Three separate layers were formed in the tube: platelet-poor plasma at the top, platelet-rich fibrin clot (PRF) at the middle and red blood cells at the bottom. Sterile instrument was used to gently remove the PRF clot. Sterile dry gauze was used to squeeze the PRF clot. Cut pieces of the freshly constructed PRF membrane were added incrementally in the canal using a finger plugger up to the level of the cemento-enamel junction (CEJ). Orifices were plugged using MTA, and the coronal cavities were sealed by glass ionomer filling (6).

#### *Statistical analysis*

Data management and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 18. The summaries of numerical information used mean, standard deviation and confidence intervals. Data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between groups with respect to normally distributed numeric variables were done by one-way analysis of variance (ANOVA) test, followed by Tukey's post hoc test. Comparison of total bacterial count within the same group was performed by paired t test. All P-values were two-sided. P-value  $\leq 0.05$  was considered significant.

## Results

#### *Comparison of total bacterial count between groups*

The data are collected in table (1). Before treatment, the highest mean value was recorded in group II (Diode laser), followed by group I (DAP), while the least value was recorded in control group. The difference between groups was statistically significant ( $P \leq 0.05$ ). Tukey's post hoc test revealed no significant difference between group I and group II.

After treatment, the highest mean value was recorded in control group, followed by group I (DAP), while the least value was recorded in group II (Diode laser). The difference between groups was non-statistically significant ( $P > 0.05$ ).

#### *Comparison of total bacterial count within the same group*

The data are shown in (Table 1). The difference between before and after treatment was statistically significant in all groups ( $P \leq 0.05$ ).

#### *Percentage of reduction in colony count*

The data are shown in (Table 2). Group II (DL) showed the greatest percent decrease and followed by group I (DAP), while the control group showed a percent increase. The difference between all groups was statistically significant ( $P \leq 0.05$ ).

## Discussion

Endodontic therapy is a time-consuming procedure. It involves multiple visits, high fees and fracture risk. RET of necrotic mature teeth could face these drawbacks because it induces a biological obturation of the root canal in the form of newly developed tissues ingrowth (10, 11).

One of the main and crucial steps for successful RET is the disinfection of the root canal. So, this study compared the antibacterial effectiveness of DL and DAP as intra-canal disinfectants. This experiment proved that DL has better antibacterial effectiveness than DAP for disinfection of the root canal during RET of necrotic mature teeth in a dog model. Moreover, the DAP's known drawbacks as development of the bacterial resistance can be excluded by using diode laser for disinfection in regenerative endodontic.

In the present study, dogs were chosen as an animal model because dogs have similar apical repair compared with humans in shorter time (average one sixth of human) due to increased growing rate (5). The double rooted premolars were chosen to sum up 10 teeth in each dog with 20 root canals in order to increase the whole number of samples for a reliable statistical



**Table 1**

The mean  $\text{Log}_{10}$ , standard deviation (SD) for comparison between  $\text{Log}_{10}$  CFU of bacterial counts in all groups before and after treatment

Groups		Mean	SD	Difference				t	P
				Mean	SD	C.I. Lower	C.I. upper		
Group I (DAP)	Before	4.40 <sup>a</sup>	.787	.85	.06	.82	.88	56.91	.000*
	After	3.549	.797						
Group II (DL)	Before	4.60 <sup>a</sup>	.946	1.35	1.07	.73	1.97	4.73	.000*
	After	3.254	.739						
Control group	Before	3.55 <sup>b</sup>	.073	-.04	.01	-.05	-.03	-14.38	.000*
	After	3.585	.079						

\*Significance at  $P \leq 0.05$ . C.I.=95% confidence interval. Tukey's post hoc test: sharing the same superscript letter are not significantly different.

**Table 2**

Comparison between groups regarding percentage of reduction in the number of colony forming units (%) of microorganisms

Groups	Mean	SD	95% Confidence Interval for Mean		Min	Max	F	P
			Lower Bound	Upper Bound				
Group I (DAP)	-85.74 <sup>b</sup>	1.82	-86.79	-84.69	-88.00	-81.75	7287.393	.000*
Group II (DL)	-92.77 <sup>c</sup>	2.59	-94.27	-91.27	-95.77	-86.86		
Control group	9.40 <sup>a</sup>	2.17	7.85	10.95	7.00	13.00		

\*Significant at  $P \leq 0.05$ . Tukey's post hoc test: with different superscript letters are significantly different.

analysis. Additionally, premolars are available for endodontic treatment and have appropriate-sized canals (5, 6, 8).

The choice of 1.5% NaOCl solution as an irrigant in this study is due to its potent antimicrobial action, preservation of stem cells and growth factor release that are necessary in RET (22). Also, using of 17% EDTA solution for conditioning was based upon its demineralizing effect on the superficial dentin layer with release of growth factors and removal of loosely attached smear layer (4, 10, 23).

Because of its convex triangular cross-section, ProTaper Universal rotary files were used to instrument canals because they

reduced friction between the blade and the canal wall and improved cutting efficiency. Additionally, their design facilitates cutting, enables the blades to clear debris from the canal, and avoids accidental screwing into the canal. To guarantee that the root canals were completely cleaned and shaped, the apical preparation of the canals was finished with an F4 ProTaper Universal file (24).

Because 1 mg/mL DAP in a prior trial did not demonstrate significant changes in direct antibacterial activity (10), 0.1 mg/mL DAP for one month was preferred in the current study. Additionally, DAP at this lower concentrations has a consid-

erable impact on microorganisms and a detrimental impact on stem cell survival (13). Nevertheless, DAP at low concentrations (0.1 mg/mL) makes its use hard therefore; methyl cellulose was employed as a vehicle in order to achieve a consistency suitable for clinical use and to lengthen the duration of the therapeutic agent. Previously, the intracanal medications have been delivered using methyl cellulose, a synthetic polymer of cellulose (18, 25). Regarding the disinfection period (one month), previous study concluded that dentin pretreatment with 5 mg/mL of DAP or higher for a month induced significantly higher residual antibiofilm effects in comparison to one-week pretreatment with the same concentrations (1).

Due to its three-dimensional architecture, autologous nature, and bioactive compounds that promote regeneration (7, 26), PRF was utilized in this investigation to display the multipotent stem cell (MSC) markers and to show significant mineralizing differentiation potential. The MTA was applied to the blood clot due to its biocompatibility and superior mechanical and sealing qualities, it is the drug of choice for scaffold covering in RET (2, 7, 27).

To avoid reinfection inside the canals that could impair the outcome, final coronal restoration was completed following endodontic therapy. According to reports, coronal leakage is a factor in root canal therapy failure, and success rates are higher for teeth with high-quality restorations than for teeth with poor restorations (28). In this study, diode laser was used as it is effective in reducing intra canal bacterial count and penetration in the depth of 500 microns in dentin. Compared to other kinds of lasers, it is smaller and significantly less expensive (20).

In the current work, intracanal irradiation was carried out in a pulsed mode to reduce the possibility of thermal harm to the underlying bone, periodontal tissues, or external root surface, which decreases the postoperative pain and promotes periapical healing. As previously noted, a diode laser evenly spreads the beam throughout the root canals to ensure a successful photoreaction (14, 15). In a recent *in vitro*

investigation, using optical fiber to disinfect the root canal led to a greater antibacterial impact (29). While a different *in vitro* investigation found a reduced antibacterial impact, this could be explained by the varied experimental methodologies (30). Similarly to the results of the present study, diode laser 980 nm could reduce the bacterial infection up to 88.38% with a distal output of 0.6 watts in continuous wave (CW) mode. A 980 nm diode laser induced antibacterial effect in root canals infected with *Enterococcus faecalis* at 77 to 97% (31). Lasers are used in wet canals to worm the irrigating solution and increase its disinfectant capacity. To make sure the laser covered the whole internal wall of the canal, the fiber was moved in a circular motion from the apex to the coronal end and back again. Touching the internal canal wall with the fiber tip was avoided to avoid melting the dentin and transferring the thermal impact to the area around the periodontal ligaments. Additionally, the fiber tip was maintained clean to produce an effective laser beam (20).

While microbiological sample (S2) was taken after laser application using paper points as in (S1) but after scrubbing the canal wall with the master apical file to detach microorganisms attached to the canal wall, microbiological sample (S1) was taken after irrigation of the root canals with 1 mL saline to allow adequate collection of microorganisms on the paper point. The similar idea was applied to a study that separated the surface biofilm before cultivating in order to prevent misleading negative results (32).

Removal of cultivable microorganisms remains the major goal and can be utilized as a surrogate outcome of the clinical studies, whereas the microbiological reduction following root canal cleaning is regarded as an immediate outcome. Therefore, the microbiological analysis of the samples was used for this study in order to assess the reduction in the number of CFU of microorganisms. The difference in CFU at baseline was taken into account because this discrepancy could lead to errors in over- or underestimating the differences between the two groups (33).



Regarding diode laser group, the result of this study is in agreement with a previous study that compared the antibacterial effect of diode laser 980 nm and triple antibiotic paste and concluded that diode laser could be used instead of triple antibiotic paste for disinfection of root canals in revascularization (17).

Regarding DAP group, the result of this study is in agreement with a prior study that looked at the impact of several dilutions (0.125, 0.25, 0.5, 1, and 10 mg/mL) of antibiotic medicines (DAP and TAP) used in endodontic regeneration to see how they affected the growth of an *Enterococcus faecalis* biofilm. It concluded that *Enterococcus faecalis* was resistant to all tested dilutions' antibacterial effects. However, DAP and TAP at 0.125 mg/mL had a notable antibacterial impact without having cytotoxic effects on stem cells (13).

The decrease in CFU between before and after treatment was statistically significant in groups I and II due to the antibacterial effect of both DAP and diode laser. While the result of control group showed significant increase in the CFU due to the absence of disinfection. These findings support those of a prior investigation (8). In contrast, the rate of RCC in various groups deviates from the findings of other studies (13). This variation could be explained by the employment of various approaches in each study.

Further studies are recommended to compare the antibacterial effect of diode laser 980 nm and 1.5% NaOCl in regeneration, to assess 980 nm diode laser on the disinfection of mature teeth in endodontic regeneration of human teeth, to use 980 nm diode laser on the disinfection of retreated root canals in case of failed root canal treatment, and to evaluate the antibacterial effect of 980 nm diode laser on different microbial endodontic species.

## Conclusions

Diode laser 980 nm showed more antibacterial effect than double antibiotic paste during RET of mature teeth with necrotic pulp and periapical pathosis.

## Clinical Relevance

Using of diode laser 980 nm induces immediate root canal disinfection during RET of mature teeth with necrotic pulp and periapical pathosis that will save both the patient and the dentist time and allow to make RET in one visit.

## Conflict of Interest

There are no conflicts of interest.

## Acknowledgments

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