

Research Report

## EFFECTIVENESS OF TIME IRRADIATION BY LASER DIODE 650 NM AND PHOTSENSITIZER *Methylene Blue* ON DECREASING THE NUMBER OF MIXED BACTERIAL COLONIES FROM NECROTIC ROOT CANAL

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### ABSTRACT

**Background.** Pulp necrosis is defined as the irreversible death of pulp tissue. It clinically observed by the destruction of its own tissue. The primary etiology of pulp necrosis is irritation due to bacterial infection. The treatment for pulp necrosis is root canal treatment with a success percentage ranging from 40-93% based on cavities with minimal bacteria that can be sterilized. One method of sterilization using Antibacterial Photodynamic Therapy (aPDT), which uses laser diodes is currently being developed in the field of conservative dentistry, but there are still many differences of opinion regarding the ideal amount of time of laser radiation or photoactivation used to reduce the number of bacteria, especially in root canals. **Aim.** To determine the effective radiation time of the 650 nm wavelength laser diode with Methylene Blue Photosensitizer in reducing the number of bacterial colonies from necrotic root canals. **Method.** This research is a laboratory experimental study with 30 samples and 6 groups with different durations of irradiation namely 45, 60, 75 and 90 seconds. **Results.** It was found that there was a decrease in the number of mixed bacterial colonies within necrotic root canals with the obtained p-value of the ANOVA test results being  $<0.05$ . This shows that there is a significant difference between the amounts of mixed bacterial colonies from necrotic root canals in each treatment group. **Conclusion.** Diode laser radiation with a wavelength of 650 nm with duration of 90 seconds of radiation and Methylene Blue Photosensitizer is an effective time in reducing the number of bacterial colonies from necrotic teeth compared to the duration of radiation of 45, 60 and 75 seconds.

*Key words:* Pulp Necrosis, ANOVA

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### BACKGROUND

Pulp necrosis is defined as the irreversible death of pulp tissue; it can be

clinically observed by the destruction of its own tissue. The primary etiology of pulp

necrosis is irritation due to bacterial infection<sup>1</sup>. Failure of root canal treatment is caused by several factors, the most common of these factors is the presence of microleakage and bacterial infections, especially bacterial infections from within the root canal. 73% of root canal treatment failure is caused by inadequate preparation and obturation. In addition, failure of root canal treatment can also occur due to the inaccessibility of the root canal due to its complex anatomy, iatrogenic factors, root fracture, or a root canal re-infection<sup>2</sup>.

The most common bacteria found in necrotic root canals are *Actinomyces spp.* (30%), *Streptococcus spp.* (25%), *Staphylococcus aureus* (20%), *Pseudomonas aeruginosa* (10%), *Proteus vulgaris* (5%), *Acinetobacter calcoaceticus* (5%), and *Klebsiella pneumoniae* (5%)<sup>3</sup>. Bacterial virulence factors within the root canals depend on the identity and characteristics of each type of bacteria. These pathogenic bacteria have the ability to stick, colonize, survive, propagate, and also fight the body's immune system; which includes neutrophils, complement proteins, and antibodies. These pathogenic bacteria can cause damage to surrounding tissue either directly or indirectly<sup>4</sup>.

Root canal treatment consists of three important steps, also named the Endodontics Triad, namely (1) biomechanical preparation including

cleaning and forming of the root canal, (2) sterilization which includes irrigation and disinfection, and (3) obturation or the hermetic filling of root canal. Sterilization is 2<sup>nd</sup> step of the Endodontic Triad and it is defined as the process of eliminating microbes found in the cavity; it is done before filling the root canal<sup>5</sup>. Some sterilizing agents that are commonly used are calcium hydroxide (Ca(OH)<sub>2</sub>), trichresol formalin, Chlorophenol-Kamfer-Menthol (ChKM), and metacresylacetate. Calcium hydroxide is the sterilizing agent most dentist use, but can be difficult to clean from the root canal<sup>6</sup>. Trichresol formalin has very toxic side effects on periapical tissue and if used excessively can cause periodontitis. ChKM has an unpleasant odor and taste<sup>7</sup>.

Antibacterial Photodynamic Therapy (aPDT) is currently being developed in the field of conservative dentistry, especially the treatments that are related to endodontic. aPDT is a two-stage procedure which combines of the use of photosensitive antibacterial agents and light rays which have been used in recent years to eliminate bacteria<sup>8</sup>. aPDT is a light activated disinfection which consists of two components, namely the source of light as photoactivation and a photosensitizer liquid. This photosensitizer liquid is activated by a light source that produces a reactive oxygen species called singlet

oxygen and also a free radical that can damage the structure of bacterial cells. This chemical reaction can damage proteins, lipids, nucleic acids, and other components<sup>9</sup>.

## MATERIALS AND METHOD

Ethical Clearance Certificate: 226/HECC.FODM/VIII/ 2018. Bacterial culture preparations were taken from newly extracted necrotic teeth. The bacterial cultures on these necrotic teeth were extracted using paper points and inserted into a test tube containing Brain Heart Infusion (BHI) broth I. Then stirred and incubated at a temperature of 37°C in an incubator for 48 hours with an anaerobic atmosphere.

After incubating for 48 hours, 0.5 ml of the bacterial culture in the BHI broth I tube was taken using a micropipette and then put into a test tube containing BHI broth II and equated with the Mc Farland scale to obtain a bacterial suspension of  $1.5 \times 10^8$  CFU/ml.

From the BHI broth II test tube, as much as 0.5 ml of bacterial suspension was taken with a micropipette and inserted into each eppendorf tube. The eppendorf tube consists of 30 tubes which have been coated with black cloth-tape. Samples of 30 eppendorf tubes were grouped into six groups. Each group consisted of five

eppendorf tubes. Group I is a control group (without a photosensitizer and without laser diode radiation). Group II was given a photosensitizer in the form of Methylene Blue (MB) as much as 0.5 ml but it was not given photodynamic therapy using 650 nm laser diode. Group III was given photosensitizer MB and radiated with a laser diode for 45 seconds. Group IV, group V and group VI were treated like group III with the duration of laser diode radiation being 60, 75, and 90 seconds.

**Table 1.** Mean and standard deviation of mixed bacterial colonies from root canal

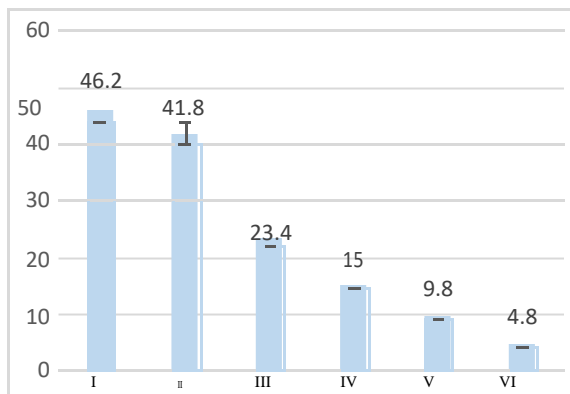
Group	N		SD
I	5	46.2	1.48324
II	5	41.8	1.92354
III	5	23.4	1.51658
IV	5	15.2	0.83666
V	5	9.8	0.83666
VI	5	4.8	0.83666

About 0.1 ml of bacterial culture was taken from each eppendorf tube (group I to VI) with a micropipette and planted in different petridishes containing nutrient agar. The petridishes containing nutrient agar media were incubated for 48 hours at 37°C in an anaerobic atmosphere. After

incubation, the number of bacterial colonies on petridish were calculated using the Total Plate Count method. The calculation results are in the form of the percentage of bacteria that are still alive, which is calculated using the formula :

$$\% \text{ living colony} = \frac{\bar{x} \text{ Treatment colonies}}{\bar{x} \text{ Control colonies}} \times 100\%$$

## RESULTS



**Gambar 1** Grafik persentase koloni bakteri campur akar gigi nekrosis

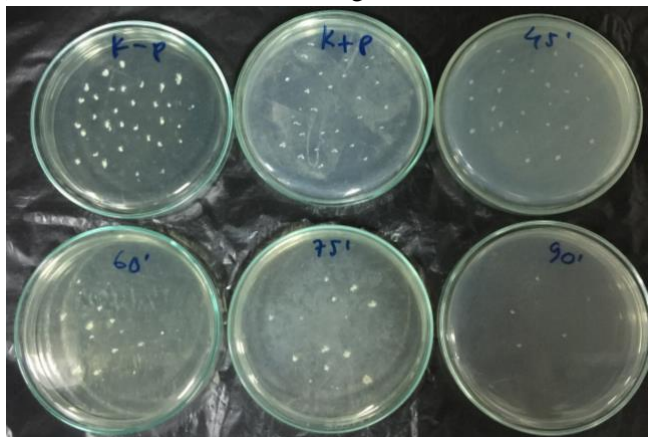
**Table 1.** and **Figure 1.** show the mean and standard deviation decreasing from group I to group VI; which means of the number of mixed bacterial colonies within necrotic root canals from group I to group VI are also decreasing. The data obtained are then analyzed and processed using the normality test (Kolmogorov-Smirnov), and the homogeneity test (Levene Test) and the results show that  $p > 0.05$  which can be interpreted that the data is normal and homogenous. Difference tests (ANOVA) shows that the p value is

$< 0.05$ , which means that there were significant differences between the number of mixed bacterial colonies within necrotic root canals in each treatment. Following the ANOVA test, a Post-Hoc Test was done after.

**Tabel 2.** Hasil uji Tukey HSD antar kelompok

Kelompok	1	2	3	4	5	6
1	-	.000*	.000*	.000*	.000*	.000*
2	-	-	.000*	.000*	.000*	.000*
3	-	-	-	.000*	.000*	.000*
4	-	-	-	-	.000*	.000*
5	-	-	-	-	-	.000*
6	-	-	-	-	-	-

Keterangan : \*) Perbedaan bermakna atau signifikan



**Gambar 2.** Koloni bakteri campur saluran akar gigi nekrosis pada media *nutrient agar* states when releasing energy or becoming triplet states if they continue to get enough

Colonies from 46.2 to 41.8; this infers that there is a significant difference in those two groups.

By giving radiation, photosensitizers that have diffused into the bacterial wall will transfer energy and produce a reactive compound. After absorbing light, the electron configuration becomes unstable (excited state). From excited states, photosensitizers can return to being ground

energy. A triplet state is a reactive state, in this situation chemical interactions occur between electrons from molecules with oxygen which have an electron configuration in a stable state. Because of this interaction, the electron configuration of the oxygen molecule is unstable<sup>12</sup>.

In the triplet state, radical ions called ROS (Reactive Oxygen Species) will be produced, these consist of anion superoxide ( $O_2^{\bullet -}$ ), radical hydroxyl ( $\bullet OH$ ), hydrogen peroxide ( $H_2O_2$ ), and oxygen singlets ( $^1O_2$ ).

These radical ions can interfere with the integrity of the cell membrane which causes irreversible damage to bacteria<sup>13</sup>. This whole process explains why there was a decrease in the number of bacterial colonies in the group given radiation compared to the group not given radiation.

The next groups, which were group IV, group V and group VI were treated like group III, but with the duration of laser diode radiation being 60, 75, and 90 seconds. It can be seen in **Table 1** that the mean/average of living bacteria from group IV to VI are decreasing. This happens because the longer duration of laser radiation causes more photosensitizers to be in an excited state. Therefore, with the increasing number of photosensitizers in excited states, the longer the triplet state phase will be. The increased duration of the triplet state phase will cause the number of

bacteria that experience death or lysis to increase<sup>11</sup>.

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