

Research Report

Differences in photodynamic therapy exposure time and *Staphylococcus aureus* counts

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ABSTRACT

Background: The success of endodontic treatment can be achieved when pathogenic bacteria are eliminated from the root canal and periapical tissue resulting in healing of such tissue. One of the bacteria located in root canals is *Staphylococcus aureus* (*S. aureus*) reportedly found to be in severe periapical abscesses. Photodynamic therapy is one current technology that can help eliminate microorganisms without causing damage to human body cells. Average of research has been conducted using different tools and bacteria to evaluate the effects of exposure time used in photodynamic therapy on the number of bacteria. **Purpose:** The research reported here aimed to determine the correlation between the exposure time of photodynamic therapy and the number of *S. aureus* bacteria. **Methods:** The *S. aureus* bacteria used in this research were divided into seven treatment groups: a control group and six treatment groups with respective exposure times of 10, 20, 30, 40, 50 and 60 seconds. All of the bacteria were administered a photosensitizer and radiated according to the treatment intended for each group. They were then planted in nutrient agar and incubated for 48 hours. The colonies of bacteria formed were calculated using the Quebec colony counter and subsequently analyzed by means of both Kruskal Wallis and Mann Whitney U tests. **Results:** After calculating the number of bacterial colonies, the average number of *Staphylococcus aureus* bacteria in the non-irradiated group was 119 CFU/ml, 29 CFU/ml in the group with a 10-second exposure time, 20 CFU/ml in the group with a 20-second exposure time, 13 CFU/ml in the group with a 30-second exposure time, 7 CFU/ml in the group with a 40-second exposure time, but none in the groups with exposure times of 50 or 60 seconds. **Conclusion:** The longer the photodynamic therapy exposure time, the greater the number of *S. aureus* bacteria eliminated. An exposure time of 50 seconds was found to be sufficient to exterminate all *S. aureus* bacteria present.

Keywords: Photodynamic therapy; *Staphylococcus aureus*; exposure time

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium considered to be a facultative anaerobe. Concentrations of *S. aureus* bacteria varying between 0.7% to 15% are present in acute dental abscesses.¹ Thus, successful endodontic treatment may occur when these pathogenic bacteria have been eliminated from the root canal and periapical tissue, resulting in healing of the latter.²

Various procedures have actually been undertaken to achieve successful endodontic treatment, such as root

canal preparation, irrigation material application and intra-channel medication. Nevertheless, they fail to ensure the eradication of bacteria present in the root canal system which, if allowed to remain there, will trigger re-infection and render root canal treatment ineffective.³

Consequently, root canal preparation and irrigation are performed to remove dead and infected vital tissue as well as forming the root canal in order that it can be cleared easily and obturated effectively. The processes of preparation and microbiological irrigation are intended to exterminate and eliminate microorganisms in root canals.⁴ The most widely-used irrigation materials include sodium

chloride (NaOCl) and chlorhexidine. However, they are unable to destroy all pathogenic microorganisms. The smear layer formed after root canal preparation can actually reduce the effectiveness of the disinfectant agent, thereby allowing potential re-infection and causing the failure of root canal treatment. Moreover, the formation of narrow root canals makes it difficult for all dental surfaces to be irrigated.² Therefore, new methods of endodontic treatment are required to facilitate and improve treatment success.

Photodynamic therapy (PDT) is the latest technology employing photoactivated disinfection to destroy pathogenic microorganisms during endodontic treatment. PDT was first introduced for cancer treatment. However, given the increasing cases of bacterial resistant to antibiotics, PDT technology has subsequently been developed to eliminate bacteria. Various microorganisms can be destroyed by PDT without proving toxic to the surrounding tissue^{2,5} and this form of therapy has also recently been used as a root canal disinfectant.⁶

PDT consists of three components, namely: light source (photoactivation), photosensitiser material and oxygen.⁷ This therapy is used after mechanical preparation and chemical irrigation and prior to obturation, while it may or may not be accompanied by intracanal medication.⁸ PDT is also known to have the advantages of greater selectivity in the destruction of bacteria, with the result that it does not induce bacterial resistance, and ease of use. Several cases of research have even shown a dose of photoactivation administered to destroy bacteria to be lower than one causing damage to keratinocyte cells and fibroblasts.⁵

Extensive research has been conducted to determine the ability of PDT to destroy various bacteria. Fotosan, a photosensitiser tool with a wavetime of 380–450 nm, is known to be capable of killing Gram-positive and negative bacteria, such as *S. mutans* and *E. faecalis*.³ Moreover, the research conducted by Arneiro suggests that PDT is effective in reducing *E. faecalis* bacteria in the root canal. Consequently, PDT is considered an appropriate disinfectant material in endodontic treatment.⁹ For these reasons, the research reported here aimed to investigate the correlation between PDT exposure time and *S. aureus* counts in order to reveal the effects of PDT exposure time on the number of *S. aureus* bacteria.

MATERIALS AND METHODS

S. aureus bacteria were employed as samples for the purposes of this research, being divided into seven groups: a control group and six treatment groups with respective exposure times of 10, 20, 30, 40, 50 and 60 seconds. Each group consisted of six samples. The *S. aureus* bacteria culture provided by the Laboratory of Microbiology, Faculty of Dentistry, Universitas Airlangga was standardized using Mc Farland 1.5 × 10⁸ CFU/ml. Then, 0.5 ml of the culture was drawn by means of micropipette and inserted into each of the 42 endodontic tubes whose walls had been coated

Table 1. The average number of *S. aureus* bacterial colonies after exposed to PDT

Groups	n	\bar{x}	SD
Control	6	119.17	8.976
10 Seconds	6	28.67	1.633
20 Seconds	6	19.83	1.472
30 Seconds	6	12.67	1.862
40 Seconds	6	6.50	1.871
50 Seconds	6	0.00	0.000
60 Seconds	6	0.00	0.000

with black insulation in order to approximate the conditions inside the tubes to those within opaque root canals.¹⁰

This research utilised a PDT tool manufactured by Fotosan 630 (CMS Dental APS, Copenhagen Denmark) consisting of activation rays and a photosensitiser liquid. In group I (control), the test tubes were not subjected to the photosensitiser and radiation, while for the other groups the test tubes were exposed to the photosensitiser for one minute, before being radiated for a precise exposure time based on the specific treatment designed for each group. Subsequently, each group was inoculated and grown in petridish nutrients while incubated for 48 hours at 37°C in an anaerobic atmosphere. The number of bacterial colonies in each group was then calculated using the CFU method with a Quebec colony counter. The results obtained were tested for their normality (distribution of abnormal data) and the differences between groups evaluated by means of Kruskal Wallis and Mann-Whitney U tests.

RESULTS

The effects of PDT exposure time on the number of *S. aureus* bacteria studied during this research were illustrated by the average number of *S. aureus* colonies as shown in Table 1 and the bacterial colony growth as presented in Figure 1. In order to evaluate the difference between groups, a Kruskal Wallis test was performed the results of which indicated there to be a significant difference in the number of *S. aureus* bacteria colonies among all treatment groups with a p value of 0.000 (p<0.05) Furthermore, a Mann Whitney U test was conducted to determine the differences between two groups within the entire research population. The results of the Mann Whitney U test are contained in Table 2.

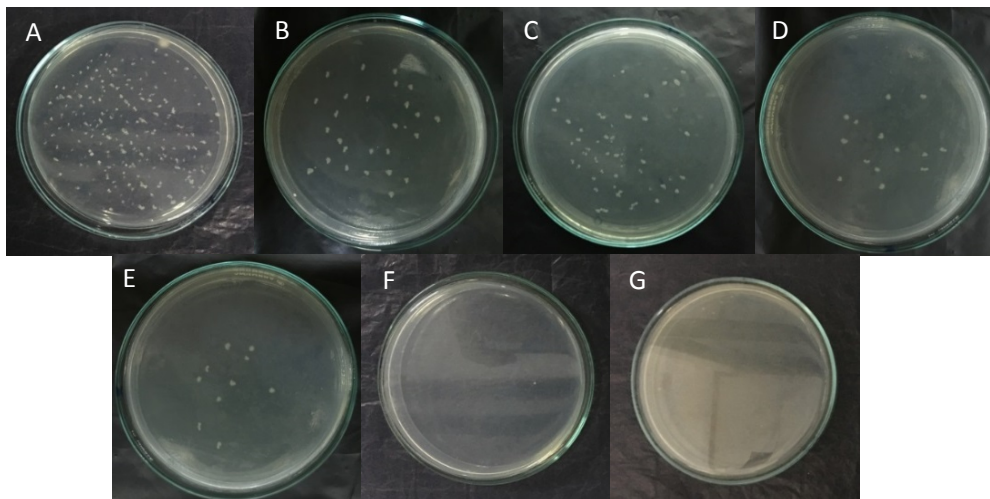
DISCUSSION

A variety of measures have been taken to ensure the success of endodontic treatments, such as root canal preparation, irrigation material usage and intra-channel medication. However, such measures still do not guarantee the elimination of bacteria in the root canal

Table 2. Results of the Mann Whitney U between the groups

Groups	10 Seconds	20 Seconds	30 Seconds	40 Seconds	50 Seconds	60 Seconds
Control	0.004*	0.004*	0.004*	0.004*	0.002*	0.002*
10 Seconds	-	0.004*	0.004*	0.004*	0.002*	0.002*
20 Seconds		-	0.004*	0.004*	0.002*	0.002*
30 Seconds			-	0.004*	0.002*	0.002*
40 Seconds				-	0.002*	0.002*
50 Seconds					-	1.000

Note: *) significant difference

**Figure 1.** *S. aureus* bacterial colonies in nutrient agar media after exposed to PDT.

Note: A. Control group; B. Group II (10 seconds); C. Group III (20 seconds); D. Group IV (30 seconds); E. Group V (40 seconds); F. Group VI (50 seconds); G. Group VII (60 seconds).

system.¹¹ Furthermore, the use of chemicals such as NaOCl or chlorhexidine cannot eradicate all pathogenic microorganisms. A smear layer formed after root canal preparation can reduce the effectiveness of the disinfectant to the extent that re-infection by the bacteria and consequent failure in root canal treatment remain as possibilities. Small root canals can also produce toughness in all tooth surfaces to be irrigated.² Technological developments in endodontic treatment have involved the use of PDT as a disinfectant. PDT is applied after mechanical and chemical irrigation preparation which serves to eliminate pathogenic bacteria and consists of three components: a light source (photoactivation), photosensitiser material and oxygen.^{5,7} If the photosensitiser or photoactivation are used separately, there will be no antimicrobial effect.⁸

The mean value obtained indicated the number of bacteria capable of surviving PDT application. The results of the difference test subsequently revealed there to be a significant difference between the treatment groups ($p < 0.05$). This result is consistent with research conducted by de Oliveira which posited that PDT can help to eliminate

microorganisms in root canals.⁵

When photosensitiser is administered to the root canal, the photosensitiser containing phenothiazines will bind to the bacterial cell wall. This occurs because phenothiazines are positively charged (cation), while the bacterial cell wall is negatively charged (anion). Both will bind to produce an electrostatic interaction causing the release of Ca^{2+} and Mg^{2+} ions located on the bacterial wall which, in turn, results in increased permeability of the bacterial cell wall. Such increased permeability will cause the photosensitiser to diffuse into the plasma membrane and the cytoplasm into bacterial DNA. Consequently, on completion of this process, photoactivation is conducted which provokes formation reactions of ROS and singlet oxygen. The ROS and singlet oxygen generated from the process can then produce cytotoxic effects in the bacteria, while also causing various problems, including: elongation of crosslink plasma membrane proteins, inactivation of succinate NaDH enzymes and lactate dehydrogenase, reduced balance between K^+ ions and other ions, as well as that destruction of bacterial cell DNA that leads to death

of the bacterial cells.¹²

As a result of this research, 10 seconds exposure to PDT radiation was known to produce an antibacterial effect, yet numerous bacteria survived. Similarly, after 20, 30, and 40 seconds of irradiation, although the number of bacteria decreased, not all were eradicated. In contrast to these results, Fotosan's protocol states that 30 seconds of irradiation is required during endodontic treatment. Indeed, the research reported here demonstrated that this period of irradiation still proved insufficient to destroy all the bacteria. These results also differ from those of a 2014 study conducted by Xhevdet which stated that exposure to radiation for 60 seconds did not provide significantly contrasting results compared to those of the control group. However, during this research 50 seconds of irradiation proved sufficient to destroy all bacteria.

Many factors can influence the differences between these research results and those of previous investigations. Variations in the tools used may have caused differences in the power and wavelength produced. The use of large-capacity photoactivations may also have produced side-effects such as thermal injuries on periodontal tissue, and they should, therefore, be used carefully.⁹ Moreover, the photosensitivity agents used in this research varied, consisting of photosensitisers containing toluidine blue which were derived from Fotosan, a class of phenothiazines that can kill bacteria at low concentrations without causing toxicity in the surrounding tissue.¹³ Another potential factor is the use of optical fiber. Fiber in photoactivation can produce a superior effect because it can help to reach difficult-to-access areas.⁵ Thus, this research used fiber optics since they are able to irradiate the bottom of the eppendorf tube.

At exposure times of 50 and 60 seconds, no live bacteria survived. This suggests that the concentrations of ROS and singlet oxygen formed as a result of photosensitiser activation reactions had proved capable of eradicating all bacteria present. In other words, when photoactivation of a longer duration is performed, the reaction between the formation of ROS and singlet oxygen that occurs will increase in intensity resulting in the destruction of a greater number of bacteria. This means that a longer PDT

exposure time triggers the decrease in bacteria. Similarly, research conducted by Xhevdet confirmed that the longer the PDT exposure time, the greater the decrease in the number of bacteria.² Finally, it can be concluded that longer photodynamic therapy exposure will decrease the number of *S. aureus* bacteria and an exposure time of 50 seconds can destroy all *S. aureus* bacteria.

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