
A PCR based study to evaluate the effectiveness of photodynamic therapy in extraction socket disinfection

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ABSTRACT

The purpose of this study was to assess the efficacy of (Light-Activated) Photodynamic therapy in extraction socket disinfection. The goal is to assess the quantitative and qualitative changes in microbial load following the use of photodynamic therapy in extraction socket disinfection. This study included 20 patients ranging in age from 18 to 55 years who required extraction of non-restorable teeth or those with periapical lesions. Extraction was done under local anesthesia, following the principles of atraumatic extraction for socket preservation. Pre-operative samples were collected with paper points for real-time polymerase chain reaction (PCR) analysis. A photosensitizer, Methylene blue dye, was applied for 60 seconds. The PDT laser (660nm) was used for a total of 3 minutes, with 20 second intervals. Following PDT application, repeat samples were collected for PCR analysis to determine the bactericidal effect in socket disinfection. The results showed a predominance of *P. gingivalis*, a potential periodontal pathogen, and a significant reduction in the same following the application of light activated PDT. Photodynamic therapy has demonstrated promising bactericidal effects, which can improve postoperative outcomes and be considered as one treatment option for immediate implants in infected sockets.

Key words: photodynamic therapy, infected sockets, photosensitizer, disinfection.

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Introduction

The presence of infection at a potential implantation site is often seen as a reason to avoid implantation. This is typically due to the harmful effects of microorganisms in sockets with chronic infections, which can negatively impact healing and bone formation, especially in cases of immediate implantation. Some researchers have carried out studies on both humans and animals have shown that successful implant placement is possible in sites that were previously infected after thorough mechanical debridement following extraction.¹

Anneroth and colleagues were the first to publish a study in an animal model (monkeys).² Later In 1989, Lazzara first reported immediate implant placement in an extraction socket in humans.³ Photodynamic therapy or light-activated disinfection is a technology based on the production of free oxygen radicals capable of affecting the membranes of microorganisms.⁴ The method involves a photosensitizer substance, such as methylene blue, which can be triggered by light of a specific wavelength. The photosensitizer after its activation produces energy capable of transforming the surrounding oxygen into free radicals; the free radical which then attacks the exposed microorganisms leading to bactericidal effect.⁵ Photodynamic therapy may be used in dentistry to reduce the bacterial load especially in cases of periodontal lesions, peri-implantitis and during root canals disinfection.⁶ Within the oral cavity, there exist over 700 prokaryotic species, with the majority of them constituting the normal flora of a healthy oral environment. Some of these microorganisms are responsible for oral pathologies.⁷ Bacteria such as *Aggregatibacter actinomycetemcomitans*, *Pasteurella*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* are responsible for common forms of periodontitis and periimplantitis.⁸

Presence of bacteria can alter the process of wound healing. *P. gingivalis* and to a lesser extent important oral bacteria impaired re-epithelialization this occurred through mechanisms that involved enhanced apoptosis, reduced migration and decreased proliferation.⁹ The *P. gingivalis* strains ATCC 33277, W83, and W50 significantly inhibited wound healing. The presence of a capsular polysaccharide lowered significantly the inhibition of epithelial cell migration, while gingipain activity significantly increased the inhibition of cell migration.¹⁰

One of the methods to lower the amount of microbes is

antimicrobial Photodynamic Treatment (PDT), which uses red light, infrared light, and diode lasers with a wavelength of 660nm. Free oxygen radicals are released as a result of the energy transfer, and they kill bacteria and their byproducts. Based on multiple studies, it was observed that the populations of *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythia* decreased following PDT treatment.

Two proposed mechanisms underlie the lethal harm inflicted on bacteria through Photoactivated Disinfection (PAD): i) DNA damage and ii) damage to the cytoplasmic membrane, leading to the release of cellular contents or the inactivation of membrane transport systems and enzymes. Observations of DNA damage, including breaks in both single-stranded and double-stranded DNA, as well as the reduction of plasmid supercoiled fractions, have been reported in both gram-positive and gram-negative species following PAD. There is also some indication that the photosensitizer may have a greater affinity for intercalating into double-stranded DNA, potentially leading to more pronounced damage. Thus inactivation of membrane enzymes and receptors is also possible.¹¹

Since extraction socket preservation and disinfection is a preceding step towards implant placement. Thus PDT may be considered as one of the modality for optimal socket disinfection thus aim was to evaluate the effectiveness of PDT in disinfecting extraction socket.

Materials and Methods

This clinical study was undertaken on 20 patients requiring the extraction of one tooth that were beyond restoration and afflicted with periodontal disease or periapical lesions. The patients age ranged from 18 to 55 years, and they were chosen for treatment at the Department of Periodontology and Implantology, M.A. Rangoonwala Dental College and Research Centre in Pune.

The inclusion criteria were: i) age of the patients ranging between 18-55 years; ii) patients ready to provide an informed consent; iii) systemically healthy patients; iv) patients with teeth earmarked for extraction, such as those with fractured teeth, non-restorable teeth necessitating extraction, nonvital teeth lacking potential for endodontic therapy, or those with failed endodontic treatment, as well as those with a hopeless periodontal prognosis; v) the extraction socket, characterized by intact walls capable of containing the photosensitizer dye, with all four walls re-

maintaining intact, was evaluated immediately after the tooth extraction.

The exclusion criteria: i) patients who are smokers; ii) patients with any history of alcohol abuse; iii) pregnant and lactating women; iv) patients with long term antibiotics.

Surgical procedure

Patient was administered with 2% lignocaine hydrochloride (1:1,00,000 adrenaline). On the selected sides extraction was performed using principles of atraumatic extraction (Figure 1). Immediately after extraction pre-operatively sterile paper points were utilized to collect samples, which were subsequently transferred into Eppendorf tubes filled with transport media. Mechanical debridement of the socket was carried out. After mechanical debridement, photosensitizer methylene blue dye was injected with the concentration of 0.0005% in the extracted socket for a period of 60 seconds, after which the excess dye was washed out with normal saline. The residual dye was activated and socket was disinfected using low level laser PDT (HELBO 660nmTM) with power density >60mW/cm (Figure 2) with a 3D optic probe. Each socket was disinfected for total 3 minutes. Each cycle was performed for 1 minute with an interval of 20 seconds, with the fluence of 4-5 J/cm². Following irradiation post

-operative sample was collected using sterile paper points for PCR analysis (Figure 3).

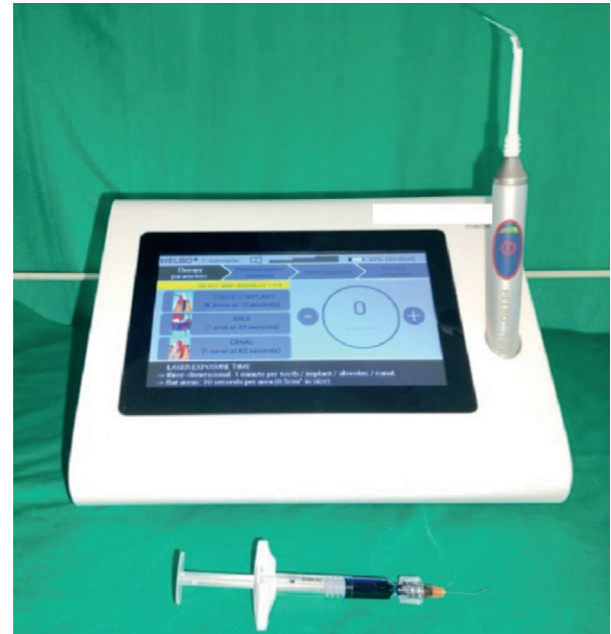


Figure 2. Laser (660nm).

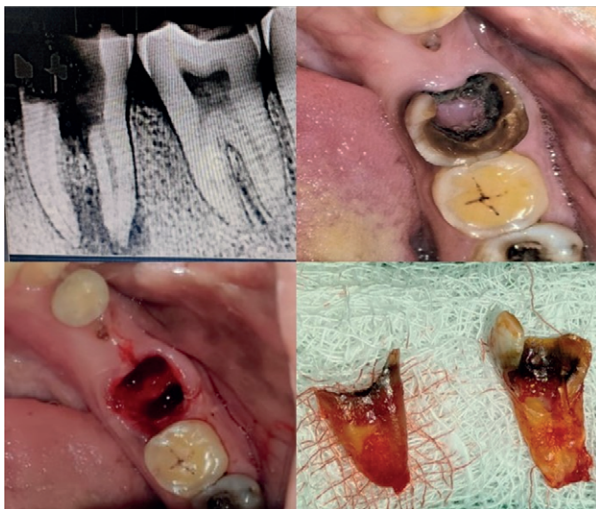


Figure 1. A) Intraoral periapical radiograph with 36; b) intraoperative clinical picture; c) socket after extraction; d) extracted tooth.

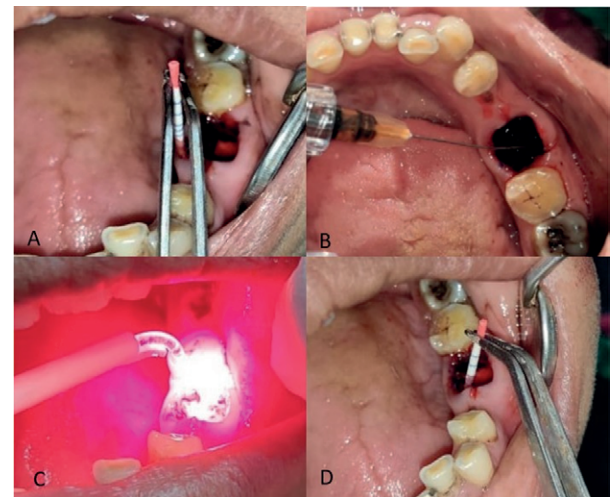


Figure 3. A) Sample collection prior to application of PDT with sterile paper points; B) application of photosensitizer methylene blue dye for 60 sec; C) PDT laser (660nm) was applied for total 3 minutes with an interval of 20 seconds with a 3D optic probe; D) post-operative samples were collected using sterile paper points for PCR analysis.

Microbiological analysis

P. gingivalis is putative pathogen found in extraction sockets. PCR analysis aims to evaluate qualitative and quantitative evaluation.

PCR procedure for *P. gingivalis* is as follows.

Real-time qPCR amplification and detection were performed with the Realplex master cycler (Eppendorf) using a 96-well format. To limit contamination, reactions were set-up in a Laminar air flow, and the reactions were run and analyzed in another laboratory where DNA manipulation was not performed.

Reagents used for PCR analysis were as follows: i) PCR primers (stock: 25 pmole concentration); ii) DNA extracts; iii) PCR master mix (with SYBER green dye); iv) molecular grade water.

Following set of PCR primers were used which are specific to 16SrRNA gene of *P. gingivalis*. *P. gingivalis* primers:

1. Forward primer:

AGG CAG CTT GCC ATA CTG CG

2. Reverse primer:

ACT GTT AGC AAC TAC CGA TGT

PCR reactions were performed in a total volume of 25 μ l containing 2 μ l of template DNA, 12.5 μ l of TB Green Premix Ex Taq (Tli RNaseH Plus) (Takara Bio inc., Kusatsu, Japan), 8 pmol of each of the *P. gingivalis*-specific primers. (TB Green Premix Ex Taq (Tli RNaseH Plus) PCR master mix was used which contains TaKaRa Ex Taq HS, dNTP Mixture, Mg²⁺, Tli RNase H, and TB Green) Stepwise preparation of master mix was as follows: i) PCR master mix was gently vortex and briefly centrifuge after thawing; ii) a thin walled PCR tube was placed on ice and the following components were added for each 25 μ l reaction; TB Green Premix master mix: 12.5 μ l; *P.gingivalis* (Forward primer): 0.5 μ l (8 pmole); *P.gingivalis* (Reverse primer): 0.5 μ l (8 pmole); template DNA: 2 μ l (<1 μ g/ reaction); water was added to make final volume 25 μ l; iii) the samples were Gently vortex and spin down; iv) the tubes were placed in Real time thermal cycler (Eppendorf, Germany).

qPCR reaction conditions were 95°C for 3 min, and 35 cycles of 95°C for 20 s and 60°C for 30 sec and 72°C for 30 sec.

Serial dilutions of the DNA extracted from the standard strain of *P. gingivalis* ATCC No. 33277 (Known quantity, 10⁸ to 10³ CFU/ml) were run to plot the standard graph. Deionized water served as negative control.

The cycle number at which amplification was initiated is called cycle threshold (Ct value). Standard curve was plotted using Ct values of standard DNA samples (Known quantity). Unknown samples were run in real time PCR to get Ct values for each sample and then these Ct values were plotted on the standard curve to get the corresponding quantity.

The data on categorical variables was shown as n (% of cases) and the data on continuous variables was presented as mean and Standard Deviation (SD) along with 95% confidence interval of mean difference. The pairwise statistical comparison of means of continuous variables was done using paired t test. The underlying normality assumption was tested before subjecting the study variables to t test. All results were shown in tabular as well as graphical format to visualize the statistically significant difference more clearly.

In the entire study, the p-values less than 0.05 were considered to be statistically significant. All the hypotheses were formulated using two tailed alternatives against each null hypothesis (hypothesis of no difference). The entire data was statistically analyzed using Statistical Package for Social Sciences (SPSS ver 24.0, IBM Corporation, USA) for MS Windows.

Results

In the present study, Ct (cycle threshold) value was calculated. Therefore, it was observed that there was increased Ct value post-operatively. Hence, there was satisfactory reduction in the total *P. gingivalis* count was observed after the application of PDT.

Comparison of pre-op and post-op mean Ct value

Distribution of mean \pm SD of pre-operative and post-operative Ct value was 29.44 \pm 2.17 and 30.82 \pm 2.02 respectively. The mean difference (pre – post) along with 95% CI of mean difference in Ct value was -1.38 (-2.63 to -0.13). Distribution of mean post-operative Ct value is significantly higher compared to mean pre-operative Ct value (P-value<0.05; Table 1, Figure 4).

Comparison of pre-op and post-op mean Log of quantity of number of microorganisms

Distribution of mean±SD of pre-op and post-op Log of quantity of number of microorganisms was 6.75±0.71 and 6.29±0.66 respectively. The mean difference (pre – post) along with 95% CI of mean difference in the log value was 0.45 (0.042 to 0.863).

Distribution of mean post-operative Log value of quantity of number of microorganisms is significantly lower compared to mean pre-operative Log value of quantity of number of microorganisms (P-value <0.05; Table 2, Figure 5)

Discussion

An accidental discovery marked the beginning of photodynamic therapy at the dawn of the 20th century, which subsequently led to its application in the medical field for

light-induced inactivation of cells, microorganisms, or molecules.¹² The term "photodynamic therapy" was initially introduced by John Toth in 1981, upon his observation of the "photodynamic chemical effect." Photodynamic therapy basically involves three non-toxic ingredients: visible harmless light; a nontoxic photosensitizer; and oxygen. Its foundation lies in the concept where a photosensitizer attaches itself to the target cells and can be triggered by light of an appropriate wavelength. Following activation of the photosensitizer through the application of light of a certain wavelength, singlet oxygen and other very reactive agents are produced that are extremely toxic to certain cells and bacteria.¹³ The imperative demand for alternative, effective, and cost-efficient treatments for infections and illnesses has emerged as fungal, bacterial, and viral pathogens increasingly develop resistance to conventional antibiotics and therapies. In recent decades, photodynamic therapy has gained traction and shown efficacy in treating various diseases. Success stories documented in medical reports across different ail-

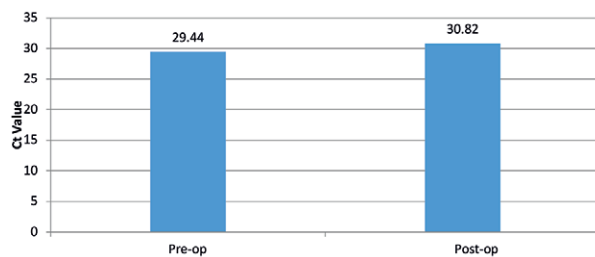


Figure 4. Bar graph showing distribution of pre-op and post-op mean Ct value.

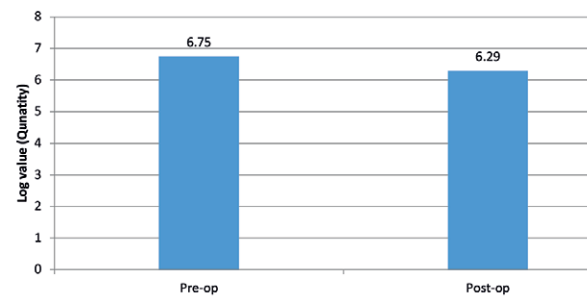


Figure 5. Distribution of pre-op and post-op mean log of quantity of number of microorganisms (*P. gingivalis*).

Table 1. Distribution of pre-op and post-op mean Ct Value.

	Pre-op (n=20)		Post-op (n=20)		Difference (Pre – Post)		P-value
	Mean	SD	Mean	SD	Mean	95% CI	
Ct Value	29.44	2.17	30.82	2.02	-1.38	-2.63 to -0.13	0.032*

P-value by paired t test. P-value<0.05 is considered to be statistically significant. *P-value<0.05.

Table 2. Distribution of pre-op and post-op mean log of quantity of number of microorganisms (*P. gingivalis*).

	Pre-op (n=20)		Post-op (n=20)		Difference (Pre – Post)		P-value
	Mean	SD	Mean	SD	Mean	95% CI	
Log (No. of microorganism)	6.75	0.71	6.29	0.66	0.45	0.042 to 0.863	0.032*

P-value by paired t test. P-value<0.05 is considered to be statistically significant. *P-value<0.05.

ments, coupled with studies investigating its effects, have sparked a rapidly growing interest in this approach. Several factors contribute to its widespread adoption, including its bactericidal action, immunostimulating properties, analgesic effects, bioenergetic impact, ease of administration, favourable patient tolerance, absence of side effects or adverse reactions, and its substantial medical, social, and economic efficacy. It is evident that thorough debridement and decontamination of the hard and soft tissue aspects of infected sockets as well as the removal of microbial debris are prerequisites for successful immediate implant placement.¹⁴ In a meta-analysis of 7 studies including 1586 implants and 25 failures, Zhao *et al.* found a 116% higher risk of implant failure amongst implants placed in infected sites, as compared to those placed in non-infected sites with borderline statistical significance.¹⁵

In our research, we observed a noteworthy decrease in total *P. gingivalis* count as a result of photodynamic therapy. The study's findings revealed a notable alteration in the post-operative Cycle Threshold value after employing photodynamic therapy for socket disinfection.

Similar study was carried out by Munteanu *et al.* (2022) about the efficiency of photodynamic therapy in the bacterial decontamination of periodontal pockets which resulted PDT as an effective tool in terms of reducing specific periopathogens.¹⁶ Another study carried out by Andre *et al.* (2022) was on open flap debridement compared to repeated application of photodynamic therapy in the treatment of residual pockets and concluded that there was reduction in PPD and lowered levels of *P. gingivalis*.¹⁷ Furthermore, the extent of heterogeneity appears to be associated with the specific method of mechanical debridement carried out and the choice of dye. The absorption coefficient of bacteria depends on the specific photosensitizer and the exact laser wavelength employed, resulting in diverse effects. The lack of a definitive "gold standard" procedure for antimicrobial photodynamic therapy (aPDT) perpetuates uncertainty regarding minor clinical improvements. The inability to assess the potential cost-benefits of PDT therapy stems from insufficient data.¹⁸

Conclusions

Photodynamic therapy in socket disinfection has shown better bactericidal effects in multiple applications and has shown significant results. The primary outcome of the study following Photodynamic therapy (660nm) was a

significant decrease in the *P. gingivalis* count, as evaluated through real-time PCR. Since this study was conducted as a one-time event, samples were collected just once from each patient. A more extensive sample size, an extended follow-up period, and additional data would undoubtedly provide valuable guidance to practitioners regarding the optimal frequency of PDT for managing the growth of *P. gingivalis*. Before incorporating lasers into regular practice, factors like equipment cost and availability must be taken into account. It's important to note that there are no definitive contraindications for laser use.

Contributions: all authors contributed equally to the work. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare no potential conflict of interest, and all authors confirm accuracy.

Ethics approval and consent to participate: the study was registered with reference number MCES/EC/856, registration number ECR/511/INST/ MH2014/RR-20 by the ethics committee of M.C.E Society. The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights. All patients provided written informed consent to participate.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

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References

1. Naves MM, Horbylon BZ, Gomes CF, et al. Immediate implants placed into infected sockets: a case report with 3-year follow-up. *Braz Dent J* 2009;20:254-8.
2. Anneroth G, Hedström KG, Kjellman O, et al. Endosseous titanium implants in extraction sockets. An experimental study in monkeys. *Int J Oral Surg* 1985;14:50-4.
3. Lazzara RJ. Immediate implant placement into extraction sites: surgical and restorative advantages. *Int J Periodontics Restorative Dent* 1989;9: 332-43.

4. Esposito M, Grusovin MG, De Angelis N, et al. The adjunctive use of light-activated disinfection (LAD) with FotoSan is ineffective in the treatment of peri-implantitis: 1-year results from a multicentre pragmatic randomised controlled trial. *Eur J Oral Implantol* 2013;6:109-19.
5. Andrade PF, Garlet GP, Silva JS, et al. Adjunct effect of the antimicrobial photodynamic therapy to an association of non-surgical and surgical periodontal treatment in modulation of gene expression: a human study. *J Photochem Photobiol B* 2013;126:119-25.
6. Arweiler NB, Pietruska M, Skurska A, et al. Nonsurgical treatment of aggressive periodontitis with photodynamic therapy or systemic antibiotics. Three-month results of a randomized, prospective, controlled clinical study. *Schweiz Monatsschr Zahnmed* 2013;123:532-44.
7. Aas JA, Paster BJ, Stokes LN, et al. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 2005;43:5721-32.
8. Romano F, Barbui A, Aimetti M. Periodontal pathogens in periodontal pockets and in carotid atheromatous plaques. *Minerva Stomato* 2007;56:169-79.
9. Bhattacharya R, Xu F, Dong G, et al. Effect of bacteria on the wound healing behavior of oral epithelial cells. *PLoS One* 2014;9:e89475.
10. Laheij AM, van Loveren C, Deng D, de Soet JJ. The impact of virulence factors of *Porphyromonas gingivalis* on wound healing in vitro. *J Oral Microbiol* 2015;7:27543.
11. Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). *J Antimicrob Chemother* 1998;42:13-28.
12. Nicolae V, Chiscop I, Cioranu VSI, et al. The use of photoactivated blue-O toluidine for periimplantitis treatment in patients with periodontal disease. *Revista De Chimie* 2015;66:2121-3.
13. Takasaki AA, Aoki A, Mizutani K, et al. Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. *Periodontol* 2000 2009;51:109-40.
14. Corbella S, Taschieri S, Tsesis I, Del Fabbro M. Postextraction implant in sites with endodontic infection as an alternative to endodontic re-treatment: a review of literature. *J Oral Implantol* 2013;39:399-405.
15. Kakar A, Kakar K, Leventis MD, Jain G. Immediate implant placement in infected sockets: a consecutive cohort study. *J Lasers Med Sci* 2020;11:167-73.
16. Munteanu IR, Luca RE, Mateas M, et al. The efficiency of photodynamic therapy in the bacterial decontamination of periodontal pockets and its impact on the patient. *Diagnostics* 2022;12:3026.
17. Andere NMRB, Castro Dos Santos NC, Araújo CF, et al. Open flap debridement compared to repeated applications of photodynamic therapy in the treatment of residual pockets: A randomized clinical trial. *J Periodontol* 2022;93:1671-81.
18. Chambrone L, Wang HL, Romanos GE. Antimicrobial photodynamic therapy for the treatment of periodontitis and peri-implantitis: An American Academy of Periodontology best evidence review. *J Periodontol* 2018;89:783-803.