**In vitro** Photodynamic Antimicrobial Activity of Protoporphyrin IX in the Presence of Hydrogen Peroxide against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Author FA did the antimicrobial experiments, survival assay and wrote the manuscript. Author CR performed the bacterial growth. Authors FA and KU performed the fluorescence measurements. Authors FA and CR managed the literature searches. Authors NM and PRA helped in interpretation of results. Author SG designed the study, coordinated the group study (Head of Group) and corrected manuscript. All authors read and approved the final manuscript.

**ABSTRACT**

**Aims:** This study reports on *In vitro* investigation of photodynamic antimicrobial activity of protoporphyrin IX (PPIX) in the presence and absence of Hydrogen peroxide (H\(_2\)O\(_2\)) against *S. aureus* and *P. aeruginosa*.

**Place and Duration of Study:** Department of Medical Physics, Anna University, Chennai between December 2013 and February 2014.

**Methodology:** A light-emitting diode (LED) was used as a light source to irradiate PPIX. The antibacterial effect was analyzed by standard plate counting method. Steady-state fluorescence spectroscopy technique was used to monitor the damage at protein level.

**Results:** We found that the antibacterial effect is dependent on PPIX concentration as well as H\(_2\)O\(_2\) concentration and light dose. PPIX-H\(_2\)O\(_2\) combination showed higher bacterial
reduction of $6.5 \log_{10}$ and $2.7 \log_{10}$ for S. aureus and P. aeruginosa respectively, when the light dose increased to 70 J/cm$^2$. Fluorescence spectroscopic characterization showed a considerable change in the intensity of emission of tryptophan present in the microorganisms between pre- and post-APDT.

**Conclusion:** PPPIX-H$_2$O$_2$ is a promising combination for APDT against Gram positive and Gram negative bacteria. The LED seems to be a very good option for PDT because of its low cost and miniature in size.

**Keywords:** Photodynamic therapy; light-emitting diode; hydrogen peroxide; protoporphyrin ix; Staphylococcus aureus; Pseudomonas aeruginosa; antimicrobial photodynamic therapy.

1. **INTRODUCTION**

The worldwide increase in antibiotic resistance among different classes of Gram-positive and Gram-negative bacteria has led to a search for alternative antimicrobial therapies. *Staphylococcus aureus* is considered as one of the most common human pathogens, which causes a wide range of diseases such as wound infections, syptic arthritis, osteomyelitis and endocarditis [1,2]. This bacterial pathogen has increasing ability to resist many antibiotics such as tetracycline, erythromycin, penicillin, methicillin and vancomycin [3]. *Pseudomonas aeruginosa* is an opportunistic pathogen and it causes infections with a high mortality rate due to its resistance to many antimicrobials [4]. This bacterial pathogen excretes many extracellular virulent factors that contribute to degradation of the immune system and destroy the tissue integrity of the host [5]. In this context, extensive research for alternative antimicrobial treatment was carried out against the multidrug resistant pathogens such as S. aureus and P. aeruginosa. Numerous published reports have shown that pathogenic bacteria that are resistant to antibiotic treatment can be inactivated successfully with Photodynamic Therapy (PDT), which is referred as Antimicrobial Photodynamic Therapy (APDT) [6,7].

APDT involves non-toxic components, which include light activated dye known as photosensitizer (PS) and harmless visible light of an adequate wavelength in the presence of molecular oxygen [8]. Excitation of the PS produces reactive oxygen species (ROS) that affect the integrity and function of bacterial cell walls, nucleic acids or enzymes, which result in cell death [9-12]. Although PDT has been considered for treatment of certain cancers [13], it is also used for the treatment of some oral diseases such as oral candidiasis [14] and root canal infections [15]. Other applications of PDT at a less developed stage include treatments for arthritis [16], psoriasis [17], Barretts’s esophagus [18], atherosclerosis [19], and restenosis [20]. Furthermore, PDT is increasingly being used in dermatology for a wide range of neoplastic, inflammatory, and infectious cutaneous conditions [21].

In many studies, it has been shown that Gram positive bacteria are susceptible to APDT when compared to their Gram negative counterparts [22]. In Gram positive bacteria, the cytoplasmic membrane is surrounded by a relatively porous layer of peptidoglycan and lipoteichoic acid, which allows the photosensitizer (PS) to cross easily [23]. However, the Gram negative bacteria are surrounded by outer membrane and inner cytoplasmic membrane separated by peptidoglycan-containing periplasm. The Gram negative bacteria can be destroyed only by increasing the permeability of the outer membrane using different chemicals or biological agents as suggested by Bertoloni et al. [24]. In this, it has been
shown that APDT using PPIX is not effective against Gram negative bacteria such as *P. aeruginosa* [25].

In PDT, it is required to use a stable, wavelength-unique, homogeneous, and large-area irradiation light source. Currently, various laser and non-laser light sources have been considered for PDT [26]. Although, laser light sources are not only very expensive, but also a specially designed optical beam delivery system is needed to broaden the beam for the irradiation of wide area. On the other hand, conventional light sources can be easily coupled with appropriate optical filters to irradiate the target area uniformly [27]. However, these conventional lamps may lead to serious thermal effect, which should be avoided during PDT. With the recent advances in optoelectronic devices, Light Emitting Diodes (LEDs) have been considered as an alternative light source for PDT. This is because, LEDs offers many advantages compared to other conventional light sources for PDT such as, less hazardous, less expensive, thermally nondestructive, and readily available [28].

In PDT it is also worth to note the role of Hydrogen peroxide (H$_2$O$_2$). H$_2$O$_2$ an oxidizing agent used widely in removing dead tissue and cleaning wounds [29]. It is reported that, H$_2$O$_2$ can be used successfully in APDT to improve the effectiveness of the PS [30,31]. Garcez et al. [32] reported the APDT effect of methylene blue (MB) in the presence of H$_2$O$_2$ against *S. aureus*, *Escherichia coli* and *Candida albicans*. They found that H$_2$O$_2$ is an interesting approach to improve the antimicrobial activity of MB. However, *P. aeruginosa*, a pathogen resistant to traditional chemotherapy and involved in many infections [33] was not included in their study. Furthermore, to the best of our knowledge, there is no published study on the use of PPIX combined with H$_2$O$_2$ in APDT against *S. aureus* or *P. aeruginosa*.

In this context, this paper aims to study the influence of several experimental conditions on APDT mediated by PPIX in the presence and absence of H$_2$O$_2$ against *S. aureus* and *P. aeruginosa*. A light-emitting diode was used as a light source for irradiation of the PS. Standard plate counting method was used to determine the number of surviving bacteria before and after the different APDT treatments. It was aimed also to investigate the LED as a light source in APDT. Attempts were also made to study the steady state fluorescence spectroscopic characterization of tryptophan from *S. aureus* and *P. aeruginosa* to understand the molecular changes in particular at protein level due to APDT.

### 2. MATERIALS AND METHODS

#### 2.1 Bacterial Strains and Growth Conditions

Methicillin-sensitive *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 10145) were provided by the Centre for Advanced Studies in Botany, University of Madras. Both strains were grown in Muller-Hinton broth (MHB) (Himedia, Mumbai, India) for 16 h at 37°C with shaking at 200rpm in LS 500 incubator/shaker (Neolab, Mumbai, India). Subsequently, the bacterial pellet was harvested by centrifugation at 10,000 rpm for 10 min and washed three times using normal saline (Na Cl 9g/L). Bacterial cells were then resuspended in normal saline to an optical density (OD) of 0.05 and 0.01 at 600 nm for *S. aureus* and *P. aeruginosa*, respectively, which corresponded to 10$^6$-10$^7$ colony-forming units (cfu)/mL.

#### 2.2 Photosensitizer and Light Source

Protoorphyrin (PPIX) was obtained from Sigma Aldrich (St. Louis, USA). The molecular characteristics of PPIX and molecular structure are shown in Fig. 1. Stock solutions were
prepared before each experiment in sterile normal saline and kept in the dark. Before each experiment, the photosensitizer was allowed to warm up to room temperature. Hydrogen peroxide (H$_2$O$_2$) was obtained from Merck (Mumbai, India). PPIX was used at concentrations of 30, 60, 100 and 200μM while H$_2$O$_2$ was used at 1, 10, 50 and 100mM. All illuminations were carried out with a portable LED (XR-C, Cree Inc., Germany) with a wavelength of 637 nm (Fig. 2). The output of the LED was measured by Field Maser GS power meter (Coherent Inc., USA) and it was found 20mW. The emission spectra of the LED were measured using spectrometer (USB 4000-VIS-NIR, Ocean Optics, USA) and are shown in Fig. 3 together with the absorption spectrum of PPIX, which was measured using UV/VIS absorption spectrophotometer (Lambda 35, Perkin Elmer, USA).

![Fig. 1. Molecular characteristics and molecular structure of PPIX](image1)

![Fig. 2. The LED used in this experiment: XR-C, Cree Inc., Germany, with a wavelength of 637 nm](image2)
2.3 Effect of APDT with PPIX Alone or in the Presence of Increasing Concentrations of H₂O₂

To investigate the photodynamic effect of PPIX (30µM) alone or in the presence of increasing concentrations of H₂O₂, aliquots (100µl) of a suspension of *S. aureus* or *P. aeruginosa* was added to 100µl of PPIX-H₂O₂ in 96 wells plate (Tarsons, Kolkata, India) and incubated at 37°C in the dark for 15 min. H₂O₂ was used at concentrations of 1, 10 and 100 mM. Samples were irradiated separately for 15 min in the dark under aseptic conditions in a laminar flow. The light dose that reached the samples surface was 18 J/cm². Samples used as controls include: bacteria alone without PS and light irradiation, bacteria illuminated with light and bacteria mixed with PPIX or H₂O₂ alone without light illumination and bacteria mixed with PPIX irradiated with light.

![Absorption spectrum of PPIX and emission spectrum of LED](image)

2.4 Effect of APDT with Increasing Concentrations of PPIX Alone or in the Presence of H₂O₂

In order to know whether increasing the PS concentration in the presence of H₂O₂ can kill more bacteria compared to when the PS is used with increasing H₂O₂ concentrations; 100µl of *S. aureus* or *P. aeruginosa* was added to 100µl of PPIX-H₂O₂ in 96 wells plate and incubated in the dark at 37°C for 15 min. PPIX used at concentrations of 30, 60, 100 and 200 µM while H₂O₂ was used at 50mM. Samples were irradiated separately for 15 min in the dark under aseptic conditions in a laminar flow. The light dose that reached the samples surface was 18 J/cm². Control group includes: bacteria alone without PS and light irradiation and bacteria photosensitized with PPIX alone (30, 60,100 and 200µM).

2.5 Effect of Increasing the Light Dose in APDT with PPIX Alone or in the Presence of H₂O₂

The effect of increasing the light dose for PPIX alone or in the presence of H₂O₂ was investigated. 100µl of *S. aureus* or *P. aeruginosa* was added to 100µl of PPIX-H₂O₂ in 96 wells plate and incubated in the dark at 37°C for 15 min. The PS and H₂O₂ concentrations...
used were 30μM and 50mM respectively. Each sample was irradiated separately in the dark under aseptic conditions inside a laminar flow. Each bacterium was irradiated to light from the LED for 15, 30, 45 and 60 min which equivalent to light doses of 18, 36, 54 and 70 J/cm², respectively. Control samples include: bacteria alone without PS and light irradiation and bacteria photosensitized with PPIX alone at light doses of 18, 36, 54 and 70 J/cm².

2.6 Bacterial Survival Assay

Standard plate counting method was used to determine the numbers of CFU of S. aureus and P. aeruginosa. After irradiation, 100μl aliquots were taken from each sample, 1:10 serially diluted six times in normal saline, spreaded on nutrient agar plates and incubated for 24 h at 37°C.

2.7 Measurement of Tryptophan Fluorescence

Steady-state fluorescence of tryptophan from the pre- and post- treated S. aureus and P. aeruginosa was acquired using spectrofluorometer (FluoroMax-2, ISA Jobin Yvon-Spex, Edison, NJ). The excitation source (ozone-free xenon arc lamp) connected to the excitation monochromator to obtain the light of the required wavelength. The fluorescence emission was collected using the emission monochromator connected to a photomultiplier tube (R928P, Hamamatsu, Shizuoka-Ken, Japan). Both excitation and emission monochromators gratings have a groove density of 1200 grooves/mm. The slit widths for excitation and the emission were adjusted at 5 nm during fluorescence data acquisition with an integration time of 0.1s. The generated signal is transferred to the PC through an RS232 interface. Data Max (Windows-based data acquisition program) powered by GRAMS/386® was used for processing the data. To investigate the APDT effect of PPIX (30μM) alone or in presence of H₂O₂ (100mM) on tryptophan fluorescence, 200 µl of S. aureus or P. aeruginosa suspended in normal saline (10⁶ – 10⁷ cell/mL) was added to 200µl of PPIX-H₂O₂ in 12 wells plate. Samples were irradiated separately with LED for 15 min. The light dose that reached the samples surface was 18 J/cm². Samples used as controls include bacteria alone without PS or light illumination and bacteria photosensitized with PPIX (30μM). Appropriate volume of normal saline was added to bring the total volume of each sample in the cuvette to 1.50mL. Samples were excited at 280 nm and emission spectra were collected in the wavelength range from 300 nm to 540 nm.

2.8 Statistics

Each experiment was performed twice and at least in triplicate. Values are expressed as means ± standard deviation. Differences were tested for statistical significance by Student’s t test. Probability values less than 5% were considered significant.

3. RESULTS AND DISCUSSION

3.1 Effect of APDT with PPIX Alone or in the Presence of Increasing Concentrations of H₂O₂

It was reported that bacterial strains showed different sensitivities against exogenous H₂O₂ due to APDT. In all bacterial strains some physiological damage has been observed, particularly to their membrane permeability and the efficiency of APDT depended on the bacterial strains [32]. The authors also found that, a high concentration of H₂O₂ has
exhibiting considerably change in membrane potential, esterase activity and intracellular pH. Funk & Krise [34] reported that, a single dose of hydrogen peroxide at lower concentration was found to produce dramatic increases in the apparent intracellular accumulation of fluorophores with different physicochemical properties in different cell types. The results were reliable with changes in lateral membrane diffusion induced by H₂O₂.

In the present study, APDT with PPIX as the function of H₂O₂ concentrations was carried out and from the results it is observed that a significant reduction in the CFU of S. aureus and P. aeruginosa (Figs. 4 and 5). The highest CFU reduction (P<0.005) is found to be about 2.5 log₁₀ and 1.5 log₁₀ for S. aureus and P. aeruginosa, respectively when PPIX irradiated in the presence of H₂O₂ (100mM). As it is shown in Figs. 4 and 5, the two groups: PDT and PDT+ H₂O₂ (1mM), only about 0.5 log₁₀ and 0.2 log₁₀ bacterial reductions were obtained for S. aureus and P. aeruginosa respectively. However, when H₂O₂ concentration was increased to 10mM, the antibacterial effect was also increased by 1.0 log₁₀ and 0.5 log₁₀ for S. aureus and P. aeruginosa, respectively. These data demonstrated that the Gram positive bacterium, S. aureus, seems to be more sensitive to APDT with PPIX alone or in the presence of H₂O₂ compared to the Gram negative bacterium P. aeruginosa. Furthermore, neither light, nor photosensitizer or H₂O₂ alone showed significant reduction in the CFU of S. aureus and P. aeruginosa. The antibacterial effect of PPIX activated with light is due to the formation of singlet oxygen and reactive oxygen species such as superoxide anions (O₂⁻) and hydroxyl radicals (•OH). The enhanced antibacterial effect of PPIX in the presence of H₂O₂ may be due to the changes in the membrane permeability and hence the probability of more accumulation of the PS in the cell, or it may be due to the fact that the photoreaction would cause membrane disruption which then facilitate H₂O₂ penetration into the cell [35,36].

Garcez et al. [32] reported that, there is a possibility of ROS formation due to H₂O₂. Using 60 µM of MB and H₂O₂ of 100mM with 60 J/cm² light dose from diode laser, the authors obtained about 1.2 log₁₀, 0.9 log₁₀ and 1.3 log₁₀ reductions in the viability of S. aureus, Escherichia coli and Candida albicans respectively. However, in this study, 30µM of PPIX activated with 18 J/cm² light dose from LED in the presence of H₂O₂ (100mM) higher inactivation (2.5 log₁₀) in the viability of S. aureus was observed.

3.2 Effect of APDT with Increasing Concentration of PPIX Alone or in the Presence Of H₂O₂

As the PS represents the main component in the photosensitization process, the dependency of PS concentration as the function of light dose and H₂O₂ was also investigated. Results of lethal photosensitization of S. aureus and P. aeruginosa as the function of PPIX concentrations in the presence or absence of H₂O₂ (50mM) are shown in Figs. 6 and 7, respectively. The antimicrobial effect of PDT proportionally increases (P<0.005) with PPIX concentration and these results are consistent with that of previous reports [37]. It is noted that, there is a considerable reduction (2.5 log₁₀) in S. aureus CFU using PPIX at 200µM. However, APDT in the presence of 50mM of H₂O₂ exhibited a more reduction (3.4 log₁₀) even at 30µM of PPIX. Under similar concentration of PPIX (alone) and PPIX in presence of H₂O₂, it is observed that the APDT efficiency is lesser for P. aeruginosa. For example it is noted that 200 µM PPIX at a light dose of 18 J/cm², P. aeruginosa has exhibited only 0.5 log₁₀ reduction and 1.0 log₁₀ reduction with H₂O₂ (50mM). The differences in susceptibility of the Gram positive and Gram negative bacteria to the APDT in this study may be attributed to the differences in the structure of the cell wall. P. aeruginosa have an outer membrane, which acts as a barrier and reduce reactive oxygen species taken by the cell [38].
Fig. 4. The effect of increasing concentrations of H$_2$O$_2$ on lethal photosensitization of *S. aureus* using PPIX (30µM), Incubation time 15 min and the light dose 18 J/cm$^2$. Data are means ± standard deviation of three experiments. Columns marked with different letters were significantly different (P<0.005).

Fig. 5. The effect of increasing concentrations of H$_2$O$_2$ on lethal photosensitization of *P. aeruginosa* using PPIX (30 µM), Incubation time 15 min and the light dose 18 J/cm$^2$. Data are means ± standard deviation of three experiments. Columns marked with different letters were significantly different (P<0.005).

It’s well known that neutral PSs like PPIX are not able to photosensitize the Gram negative bacteria [39,40]. However, the results obtained in this study revealed that, the APDT effect of PPIX against *S. aureus* and *P. aeruginosa* can be enhanced in the presence of H$_2$O$_2$.
Fig. 6. Effect of increasing PPIX concentrations (30, 60, 100, 200 µM) alone or in the presence of H$_2$O$_2$ (50 mM) on the viability of S. aureus. Incubation time 15 min and the light dose 18 J/cm$^2$. Data are means ± standard deviation of three experiments (P<0.005)

Fig. 7. Effect of increasing PPIX concentrations (30, 60, 100, 200 µM) alone or in the presence of H$_2$O$_2$ (50 mM) on the viability of P. aeruginosa. Incubation time 15 min and the light dose 18 J/cm$^2$. Data are means ± standard deviation of three experiments (P<0.005)
3.3 Effect of Increasing the Light Dose in APDT with PPIX Alone or in the Presence of H₂O₂

Exposure time represents a fundamental quantity in APDT. Figs. 8 and 9 shows the dependence of APDT against *S. aureus* and *P. aeruginosa* respectively on light dose. For each bacterium, the most effective bacterial kills were seen when PPIX alone (30µM) or in the presence of H₂O₂ (50 mM) was irradiated with light dose of 70 J/cm². When 70 J/cm² light dose had been delivered to activate PPIX alone, approximately 2.0 and 1.0 log₁₀ CFU reduction (P<0.005) was achieved for *S. aureus* and *P. aeruginosa*, respectively. However, when the same treatment was applied with adding H₂O₂ (50mM) with PPIX to the bacteria and irradiated with 70 J/cm² light dose, 6.5 log₁₀ and 2.7 log₁₀ CFU reductions was obtained for *S. aureus* and *P. aeruginosa*, respectively.

Previous study by Ganz et al. [37] reported that APDT effect was less at low concentration of PPIX. However, they observed higher reduction when PPIX illuminated for 20 min. The study of the light irradiation time demonstrates that the balance between PPIX concentration and illumination time can control the efficiency of PDT. Furthermore, for non-perfused tissues where the accumulation of PS is less, longer irradiation time should be considered to allow identical therapeutic effect.

![Fig. 8. Effect of increasing the light dose (18, 36, 54, 70 J/cm2) using PPIX (30µM) alone or in the presence of H₂O₂ (50mM) on the viability of *S. aureus*. Incubation time 15 min. Data are means ± standard deviation of three experiments (P<0.005)](image)

3.4 Measurement of Tryptophan Fluorescence

All bacteria cells own proteins with the aromatic amino acid, tryptophan, which is the dominant cellular fluorophore in the ultraviolet spectral region [41]. Tryptophan shows a broad emission from 320 to 400 nm. However, the maximum emission wavelength is greatly
dependent on its local environment and protein structure [42]. Fluorescence spectra of tryptophan from bacteria can give further information about the mechanism of antimicrobial action.

![Fluorescence spectra of tryptophan from bacteria](image)

**Fig. 9.** Effect of increasing the light dose (18, 36, 54, 70 J/cm$^2$) using PPIX (30 µM) alone or in the presence of H$_2$O$_2$ (50 mM) on the viability of *P. aeruginosa*. Incubation time 15 min. Data are means ± standard deviation of three experiments (P<0.005)

The APDT effects on tryptophan fluorescence from *S. aureus* and *P. aeruginosa* are presented in Figs. 10 and 11 respectively. The overall fluorescence from post-treated *S. aureus* and *P. aeruginosa* exhibited a significant reduction (P<0.005) in the intensity. The emission peak at 340 nm is attributed to the key amino acid, tryptophan which is present in the bacteria [43]. From the inset of Figs. 10 and 11, it is found that there is a reduction in the peak emission at 340 nm due to APDT with respect to control group. The decreases in tryptophan fluorescence intensity may correspond to protein damage as suggested by Manpreet et al. [44]. The fluorescence spectra of *P. aeruginosa* exhibited a red shift after APDT; however this shift was not shown in the spectra of *S. aureus*. The noticed red shift may not relate to a change in tryptophan emission from *P. aeruginosa* but a contribution of the pyoverdine emission at 340 nm [45]. The intrinsic fluorescence of tryptophan for the control and APDT group for *S. aureus* and *P. aeruginosa* correlates with that of CFU results. Moreover, the results obtained in this study showed that APDT can have a significant effect on the fluorescence properties of bacteria.
Fig. 10. The effect of APDT using PPIX (30 µM) alone or in the presence of H₂O₂ (100 mM) on tryptophan fluorescence from *S. aureus*. Incubation time 15 min and the light dose 18 J/cm². Data are means ± standard deviation of three experiments (P<0.005).

Fig. 11. The effect of APDT using PPIX (30 µM) alone or in the presence of H₂O₂ (100 mM) on tryptophan fluorescence from *P. aeruginosa*. Incubation time 15 min and the light dose 18 J/cm². Data are means ± standard deviation of three experiments (P<0.005).
4. CONCLUSION

The results showed in this study suggest that PPIX-H₂O₂ is a promising combination for APDT against Gram positive and Gram negative bacteria. APDT with PPIX-H₂O₂ seems significantly effective at very high H₂O₂ concentration against S. aureus, but its only mildly to moderately effective against P. aeruginosa. We achieved higher bacterial killing when PPIX in the presence of H₂O₂ illuminated with increasing light doses from the LED. The LED seems to be a very good option for PDT because of its low cost and miniature in size. Steady state fluorescence spectroscopy may be considered to characterize the molecular changes at protein level due to APDT as well as to monitor APDT efficiency. The optimized properties of PS as well as specific delivery systems will determine if APDT for bacterial infection could be considered as an alternative approach to traditional antibiotic therapy. Although, after extensive well-designed preclinical and clinical trials, this novel therapeutic approach may be considered in clinical practices for the treatment of superficial infections.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chopra I. Antibiotic resistance in Staphylococcus aureus: concerns, causes and cures. Expert Rev Anti Infect Ther. 2003;1:45-55.
2. Gemmell GC. Glycopeptide resistance in Staphylococcus aureus: is it a real threat?. J Infect Chemother. 2004;10:69-75.
3. Chambers HF. The changing epidemiology of Staphylococcus aureus?. Emerg Infect Dis. 2001;7:178-182.
4. Cunha BA. Nosocomial pneumonia: Diagnostic and Therapeutic considerations. Med Clin North Am. 2001;85:79-114.
5. Van Delden C, Iglewski BH. Cell-to-cell signaling and Pseudomonas aeruginosa infections. Infect Dis. 1988;4:551-560.
6. Zanin ICJ, Goncalves RB, Brugnera-Jr A, Hope CK, Pratten J. Susceptibility of Streptococcus mutans biofilms to photodynamic therapy: An In vitro Study. J Antimicrob Chemother. 2005;56:324-330.
7. Vera DM, Haynes MH, Ball AR, Dai T, Astrakas C, Kelso MJ, Hamblin MR, Tegos GP. Strategies to potentiate antimicrobial photoinactivation by overcoming resistant phenotypes. Photochem Photobiol. 2012;88:499-511.
8. Jori G, Fabris C, Soncin M, Ferro S, Coppellotti O, Dei D, Fantetti L, Chiti G, Roncucci G. Photodynamic therapy in the treatment of microbial infections: Basic principles and perspective applications. Lasers Surg Med. 2006;38:468-481.
9. Michaeli A, Feitelson J. Reactivity of singlet oxygen toward amino acids and peptides. Photochem Photobiol. 1994;59:284-289.
10. Stark G. Functional consequences of oxidative membrane damage. J Membr Biol. 2005;205:1-16.
11. Ravanat JL, Di Mascio P, Martínez GR, Medeiros MHG, Cadet J. Singlet oxygen induces oxidation of cellular DNA. J Biol Chem. 2000;276:40601-40604.
12. Spesia MB, Caminos DA, Pons P, Durantini EN. Mechanistic insight of the photodynamic inactivation of Escherichia coli by a tetracationic zinc(II) phthalocyanine derivative. Photodiagn Photodyn Ther. 2009;6:52-61.
13. Wilson BC. Photodynamic therapy for cancer: Principles. Can J Gastroenterol. 2002;16:393-6.
14. Mima EG, Pavarina, AC, Dovigo, LN, Vergani, CE., Costa, CAD, Kurachi C, Bagnato, VS. Susceptibility of Candida albicans to Photodynamic Therapy in a Murine Model of Oral Candidosis. Oral Surg. Oral Med Oral Pathol Oral Radiol Endod. 2010;109:392-401.
15. Garcez AS, Ribeiro MS, Tegos GP, Nunez SC, Jorge AOC, Hamblin MR. Antimicrobial Photodynamic Therapy Combined with Conventional Endodontic Treatment to Eliminate Root Canal Biofilm Infection. Lasers Surg Med. 2007;39:59-66.
16. Trauner KB, Hasan T. Photodynamic treatment of rheumatoid and inflammatory arthritis. Photochem. Photobiol. 1996;64:740–750.
17. Boehncke WH, Elshorst-Schmidt T, Kaufmann R. Systemic photodynamic therapy is a safe and effective treatment for psoriasis. Arch. Dermatol. 2000;136:271–272.
18. Barr H. Barrett's esophagus: treatment with 5-aminolevulinic acid photodynamic therapy. Gastrointest. Endosc. Clin. North Am. 2000;10:421-437.
19. Rockson SG, Lorenz DP, Cheong WF, Woodburn KW. Photoangioplasty: An emerging clinical cardiovascular role for photodynamic therapy. Circulation. 2000;102:591–596.
20. Jenkins MP, Buonaccorsi GA, Raphael M, Nyamekye I, McEwan JR, Bown SG, Bishop CC. Clinical study of adjuvant photodynamic therapy to reduce restenosis following femoral angioplasty. Br. J. Surg. 1999;86:1258-1263.
21. Lee Y, Baron ED. Photodynamic therapy: Current evidence and applications in dermatology. Semin Cutan Med Surg. 2011;30:199-209.
22. Hamblin MR, Hasan T. Photodynamic therapy: A new antimicrobial approach to infectious disease. Photochem Photobiol Sci. 2004;3:436-450.
23. Malik Z, Ladan H, Nitzan Y. Photodynamic inactivation of Gram- negative bacteria: Problems and possible solutions. J Photochem Photobiol B. 1992;14:262-266.
24. Bertoloni G, Rossi F, Valduga G, Jori G, van Lier J. Photosensitizing activity of water- and lipid-soluble phthalocyanines on Escherichia coli. FEMS Microbiol Lett. 1990;71:149-156.
25. Wainwright, M. Photodynamic antimicrobial chemotherapy. J Antimicrob Chemother.1998;42:13-28.
26. Brancaleon L, Moseley H. Laser and non-laser light sources for photodynamic therapy. Lasers in Medical Science. 2002;17(3):173-186.
27. Wilson BC, Patterson MS. The physics, biophysics and technology of photodynamic therapy’. Physics in Medicine and Biolog. 2008;53(9):R61-R109.
28. Trindade FZ, Pavarina AC, Ribeiro AP, Bagnato VS, Vergani CE, Costa CA. Toxicity of photodynamic therapy with LED associated to Photogem®: An In vivo study. Lasers in Medical Science. 2012;27(2):403-411.
29. Feuerstein O, Moreinos D, Steinberg D. Synergic antibacterial effect between visible light and hydrogen peroxide on Streptococcus mutans. Journal of Antimicrobial Chemotherapy. 2006;57(5):872-876.

30. McCullagh C, Robertson PKJ. Photo-dynamic biocidal action of methylene blue and hydrogen peroxide on the cyanobacterium Synechococcus leopoliensis under visible light irradiation. Journal of Photochemistry and Photobiology B: Biology. 2006a;83(1):63-68.

31. McCullagh C, Robertson PKJ. Photodestruction of Chlorella vulgaris by methylene blue or nuclear fast red combined with hydrogen peroxide under visible light irradiation. Environmental Science & Technology. 2006b;40(7):2421-2425.

32. Garcez AS, Nunez SC, Baptista MS, Dahastanli NA, Itri R, Hamblin MR, Ribeiro MS. Antimicrobial mechanisms behind photodynamic effect in the presence of hydrogen peroxide. Photochemical & Photobiological Sciences. 2011;10(4):483-490.

33. Nordmann P, Naas T, Fortineau N, Poirel L. Superbugs in the coming new decade; Multidrug resistance and prospects for treatment of Staphylococcus aureus, Enterococcus spp. and Pseudomonas aeruginosa in 2010. Curr Opin Microbiol. 2007;10:436-440.

34. Funk RS, Krise JP. Exposure to hydrogen peroxide can increase the intracellular accumulation of drugs. Mol Pharmaceutics. 2007;4:154-159.

35. Caetano W, Haddad PS, Itri R, Severino D, Vieira VC, Baptista MS, Schroder AP, Marques CM. Photo-induced destruction of giant vesicles in methylene blue solutions. Langmuir. 2007;23:1307-1314.

36. Seaver LC, Imlay JA. Hydrogen peroxide fluxes and compartmentalization inside growing Escherichia coli. J Bacteriol. 2001;183:7182-7189.

37. Ganz RA, Viveiros J, Ahmad A, Ahmadi A, Khalil A, Tolkoff MJ, Nishioka NS, Hamblin MR. Helicobacter pylori in patients can be killed by visible light. Lasers Surg Med. 2005;36:260-265.

38. Jori G, Fabris C, Soncin M, Ferro S, Coppellotti O, Dei D, Fantetti L, Chiti G, Roncucci G. Photodynamic Therapy in the Treatment of Microbial Infections: Basic Principles and Perspective Applications. Lasers in Surgery and Medicine. 2006;38(5):468-4681.

39. Bonnett R, Buckley, DG, Burrow, T, Galia ABB, Saville B, Songca SP. Photobactericidal Materials Based on Porphyrins and Phthalocyanines. J Mater Chem. 1993;3:323-324.

40. Perria C, Carai M, Falzo M, Orunesu G, Rocca A, Massarelli G, Francaviglia N, Jori G. Photodynamic therapy of malignant brain tumors: clinical results of, difficulties with, questions about, and future prospects for the neurosurgical applications. Neurosurgery. 1988;23:557-563.

41. Ammor MS. Recent Advances in the Use of Intrinsic Fluorescence for Bacterial Identification and Characterization. J Fluor. 2007;17(5):455-459.

42. Chen Y, Barkley MD. Toward understanding tryptophan fluorescence in proteins. Biochemistry. 1998;37(28):9976-9982.

43. Fathi A, Chandrasekaran R, Shanmugam S, Narayanasamy M, Prakasa RA, Singaravelu G. Influence of Hydrogen Peroxide or Gold Nanoparticles in Protoporphyrin IX Mediated Antimicrobial Photodynamic Therapy Against Staphylococcus aureus. Afr. J. Microbiol. Res. 2013;7:4617-4624.
44. Manpreet B, Alexander MR, Sajeda M, Brian H, Michael W. A Study of the Uptake of Toluidine Blue by Porphyromonas gingivak and the Mechanism of Lethal Photosensitization. J Photochem Photobiol. 1998;68:370-376.

45. Alimova A, Katz A, Siddique M, Minko G, Savage HE, Shah MK, Rosen RP, Alfano RR. Native fluorescence changes induced by bactericidal agents. IEEE Sensors J, 2005;5:704-711.

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