Antimicrobial Photodynamic Effectiveness of Light Emitting Diode (LED) For Inactivation on *Staphylococcus aureus* Bacteria and Wound Healing in Infectious Wound Mice

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**Abstract.** This study aims to determine the effectiveness of the antimicrobial photodynamic therapy (aPDT) LEDs of 392 nm and 628 nm red LEDs for photoinactivation on *Staphylococcus aureus* in vitro and the photodynamic effectiveness of therapy for wound healing in mice in vivo. The sample used was *Staphylococcus aureus* bacterial isolate which was infected on mice incision wounds. The LED exposure was carried out at the energy density of 19.44 J/cm\(^2\), respectively. In vitro test samples were divided into three groups, namely (P0): control group, (P1): LED treatment group 392 nm, (P2): LED treatment group 628 and (P3): LED combination treatment group 392 and 628 nm. In the in vivo test, the mice of infectious wound models were divided into 5 namely (M+): positive control group, (M-): negative control group, (M1): LED treatment group 392 nm, (M2): LED treatment group 628 and (M3): LED combination treatment group 392 and 628 nm. The results of in vitro studies showed an increase in the photoinactivation effect on LED exposure, with the effectiveness of inactivation on the LED 392 nm at 67.10%. The effect of photoinactivation of bacteria on wounds in vivo due to photodynamic therapy in the red LED exposure group was 88%, blue LED exposure group was 94%, exposure combination group of red and blue LEDs was 95%. So, the antimicrobial photodynamic therapy LEDs are effective for bacterial inactivation and accelerate wound healing in mice.

1. **Introduction**

Injury is damage to the tissue structure and normal anatomical function of the skin due to pathological processes originating from internal or external. Most infected wounds are caused by bacterial colonization from parts of the body or the outside environment. The most common infection-causing bacteria is *Staphylococcus aureus* which caused complications on post-surgical wound infections. Complications of infected wounds can vary in range from local to systemic. The most severe local complication of an infected wound is stalled wound healing, resulting in a non-healing wound. This
often results in significant pain, discomfort and psychological detriment for the patient. Systemic complications can include cellulitis (bacterial infection of the dermal or subcutaneous layers of skin), osteomyelitis (bacterial infection of the bone or bone marrow) or septicemia (bacterial presence in the blood that can lead to a whole-body inflammatory state). Localized infections can often be treated with topical antibiotics. Drainage or debridement may be necessary to remove slough and devitalized tissue, as these slow wound healing and can affect the efficiency of topical antibiotics. In severe infections, oral or intravenous antibiotics are needed. Bacterial biofilm is a major barrier to wound healing. This is caused by slow penetration and changes in protein-DNA synthesis from antibiotics. Also, biofilms may have the ability to reduce the production of hydrogen peroxide ($\text{H}_2\text{O}_2$) which is a precursor of toxic molecules when DNA-protein synthesis is altered [1].

At previous research used a PDI-LED instrument with wavelengths of 430 nm and 629 nm for inactivation of $S$. aureus bacteria with endogenous photosensitizers causing a reduction of $S$. aureus bacteria by 75% [2]. Endogenous photosensitizers are photosensitizers produced naturally by bacteria called porphyrin. Hsu, et al. (2012) used a 640 nm LED lamp which produced an association response of 70% and 80% of the light source in hamsters that were given carcinogenic reactants [3]. The interaction of light, photosensitizer agents and oxygen to eliminate microbial cells by photogeneration of reactive oxygen species (ROS) is called photodynamic therapy [4]. Photodynamic has been applied in various photosensitizer agents including drug: doxycycline [5]; synthetic dye: methylene blue [6], toluidine blue [6-7]; Metal: silver nano particles [8-10], gold [11-12]; and nature: chlorophyll [13], curcumin [14-16], porphyrin [17].

The particles of photosensitizer agents would be diffused into the biofilm, that will be one of the keys to the success of photodynamic therapy [8]. The deeper the photosensitizer particle diffused into the biofilm, the level of reduction caused by PDT will be higher. In addition, the suitability between the wavelength spectrum of the light source and the photosensitizer absorption spectrum is the key to the success of photodynamic therapy to reduce biofilms [5]. That will provide enough photons to activate all the photosensitizer molecules available to allow the killing of most of the bacteria present [5]. Therefore, every light source such as laser and LED light source can be applied for photoinactivation on bacteria with porphyrin endogenous. Time exposure of light irradiation play role in the interaction with the target. Moreover, the photochemistry interaction on PDI occurs within the exposure time more than one seconds in low optical density power [2].

This study was made to the design of the instrument using endogenous photosensitizer and blue LEDs and red LEDs to create a photodynamic effect for the inactivation of $S$. aureus bacteria in vitro and in vivo. This study aims to analyze the effectiveness of the antimicrobial photodynamic therapy (aPDT) LEDs of 392 nm and 628 nm red LEDs for photoinactivation in $S$. aureus in vitro design and the photodynamic effectiveness of therapy for wound healing in mice in vivo design.

2. Materials and Methods

2.1. Design and Build Photodynamic Therapy Instruments

Stage of making Photodynamic therapy devices by combining the timer circuit and the LED probe, as well as the input adapter that functions as a power supply with a voltage of 15 volts and a current of 1.5 amperes. This tool is made portable so that it is lightweight and comfortable to use with the scheme shown in Figure 1.

![Figure 1. Scheme instrument of photodynamic therapy](image-url)
The LED used is a high-power LED meaning the power generated is greater. The probes use 2 kinds of LEDs, namely high-power LEDs with wavelengths of 391.76 nm and 627.73 nm. For each probe uses 4 for high power LEDs arranged in series where all current inputs for each high-power LED are the same. Figure 2 shows the LED driver Ic used, namely IC HY 3015 functions to regulate the voltage from the microcontroller to the LED. The resistor functions as a current resistor so that the LED used is not damaged, while the capacitor is used as a current store so that when the LED is turned off, the LED does not immediately die but there is a time-out for the LED to turn off.

![Figure 2. Scheme of LED driver circuit](image)

The microcontroller used is the AT-Mega 8 microcontroller which is an 8-bit CMOS microcontroller with AVR RISC architecture that has 8K bytes in the Programmable Flash system. Microcontroller with low power consumption can execute instructions with a maximum speed of 16MIPS at a frequency of 16MHz. AT-Mega 8 microcontroller is used for setting the timer and LED intensity (brightness). Figure 3 shows a schematic of the AT-Mega 8 microcontroller and regulator IC used to filter the microcontroller input voltage.

![Figure 3. (a) Microcontroller AT-Mega 8, and (b) IC 7805](image)

2.2. In vitro Design Preparation and Sample Treatment
One dose of culture was inoculated into 10 mL of sterile physiological solution (0.9% sodium chloride (NaCl)) and homogenized for a concentration of 0.5 McFarland (10^7 CFU / mL) or until reaching 30-300 colonies. The sample is placed on a glass tube for later treatment with irradiation. Treatments were divided into the following experimental groups: P0 was no treatment; P1 was the treatment with LED 391.76 nm irradiation; P2 was the treatment with LED 627.73 nm irradiation, and P3 was the combination treatment between LED 391.76 nm and LED 627.73 nm irradiation. The irradiation was done completely in the darkroom.

2.3. In vivo Design Preparation and Sample Treatment
The sample for in vivo design used white mice was obtained from the Integrated Research and Testing Laboratory (LPPT) of Gajah Mada University. White mice (Mus musculus L.) used are white Swiss male mice at the age of 10 weeks in a fertile state. The dorsal part is shaved around 9 cm^2. To make a cut about 20 mm after being anesthetized using ketamine at a dose of 0.01 ml. Incision wounds are infected with S. aureus bacteria and closed. Samples were then incubated for 24 hours at room
temperature and obtained samples with infection wounds caused by *S. aureus* bacteria. Treatments were divided into the following experimental groups: M was no infection and irradiation treatment; M + was infection without irradiation treatment; M1 was the treatment with LED 391.76 nm irradiation; M2 was the treatment with LED 627.73 nm irradiation, and M3 was the combination treatment between LED 391.76 nm and LED 627.73 nm irradiation. The irradiation was done completely in the darkroom and repeated for up to 7 days.

### 2.4. LED Irradiation

The light source used was LED 391.76 ± 27.86 nm with an output power of 0.1380 mW and LED 627.73 ± 15.79 nm with an output power of 0.0315 mW. Medium temperature is stable at room temperature. The energy densities were used in this treatment, i.e. 391.76 nm LEDs for 20 min of 19.44 J / cm², 627.73 nm LEDs for 20 min of 1.93 J / cm² and combination LEDs of 5.18 J / cm² for 10 min, respectively.

### 2.5. Bacteriological Analysis

After the treatment, samples were studied in Petri disks using TPC (Total Plate Counter) for in vitro models. Mannitol Salt Phenol-red Media Agar 10.8 g / 100 ml is sterilized at 121 °C for 15 minutes. *S. aureus* was growth in Mannitol Salt Phenol-red Agar media (Oxoid, UK) incubation for one night at 37 °C after irradiation treatment. After that, the number of colonies that grow on the agar media and compared with the control is calculated.

### 2.6. Macroscopic Analysis

In vivo group, the clinical assessment of wound length and appearance was performed by taking a digital image using a digital camera in the same lighting conditions on days 7. The length of the wound in the sample is measured and the healing rate compared with the control group.

### 3. Result and Discussion

Based on Figure 4a in vitro P1 test group with the blue irradiation treatment group provides high effectiveness in bacterial inactivation, this is due to the use of blue LEDs in accordance with the absorbance of *S. aureus* porphyrin bacteria. In previous studies, endogenous porphyrin test results for *S. aureus* bacteria showed that these bacteria accumulated the largest endogenous porphyrin types of coproporphyrin III by 57.83% and 20.17%. The successful photoinactivation of bacteria involves the process of photosensitization, which is the process of absorption of light by porphyrins which in turn activates the occurrence of chemical reactions that produce various reactive oxygen species [2]. The killing of bacteria with the light energy dose was limited by the concentration of the photosensitizer at or, more strictly, its ROS-generating capacity [5]. Each of these photosensitizer has their own absorbance value and will generate photo–physical process when they have enough energy. By using a light source, higher photon energy absorption by endogenous photosensitizers is an indication of the success of the PDT process [16].

**Figure 4.** Results of irradiation treatment graphs (a) in vitro design, and (b) in vivo design

In the photosensitization mechanism, the intersystem crossing process is an important key in producing reactive oxygen species. The intersystem crossing process has 2 types of possible changes
in compounds namely type I and type II. Type I, photosensitive molecules that are excited react directly with the substrate and transfer a proton or electron to form anions or radical cations. These radical compounds will react with oxygen to produce reactive oxygen (ROS) which will react with the substrate to produce hydrogen peroxide (H$_2$O$_2$) [18]. Type II, excited photosensitive molecules can transfer their energy directly to oxygen molecules (O$_2$) causing the molecular changes to be more reactive to form singlet oxygen (O$_2$). When electrons experience excitation to a higher energy level, electrons become unpaired orbitals. Compounds in the form of free radicals and singlet oxygen can cause deadly cell damage depending on intracellular localization, for example by damaging various cell membranes, DNA, mitochondria, and others.

Based on Figure 4b in vivo test, there is a difference between M + group with irradiation treatment group, so it can be concluded that the use of photodynamic therapy in wounds can help the process of inactivation of bacteria so that it will inhibit the infection process even worse. In the M1 group, the percentage of wound healing was higher than in the M2 group, this was comparable to the results of the in vitro test. The use of blue LEDs for photoinactivation of bacteria to help wound healing by accelerating cell regeneration [19]. Healing process from in vivo test it is shown in Figure 5.

The main goal of wound treatment is to restore the function and shape of skin tissue back to normal with minimal local complications. When a wound occurs, the tissue will experience a healing process which is a complex phenomenon and involves several processes. The mechanism of wound healing begins with the inflammatory phase which is characterized by the presence of vascular and cellular responses caused by injury in the soft tissue area [20]. This phase will stop the bleeding and clean the wound from foreign bodies, dead cells and bacteria to begin the healing process by performing a hemostasis and vasoconstriction reaction. Thrombocytes will come out of blood vessels, stick together, and it will freeze blood coming out of blood vessels with the help of fibrin called hemostasis [21].

![Figure 5](image_url)

**Figure 5.** Irradiation treatment in vivo design (a) M+ group, (b) M1 group, (c) M2 group, (d) M3 group

The inflammatory phase is usually characterized by a reddish color due to dilation of the capillaries (rubor), warm temperatures (heat), pain (dolor), and swelling (tumor). The next healing phase is the proliferation or epithelialization phase. Epithelialization is the process of forming new epithelium in wound tissue. Numerous studies show there are unique cell interactions between epithelium, endothelium, and fibroblasts in the process of wound healing. During the wound healing stage, angiogenesis plays a role in supplying food and oxygen in the wound area and increasing the formation of granulation tissue. Collagen is the main protein of the extracellular matrix and plays a major role in wound healing.

4. Conclusion
The highest effectiveness of photoinactivation in *S. aureus* bacteria was in the blue LED exposure group, whereas the effectiveness of wound healing in vivo due to *Staphylococcus aureus* bacterial
infection was greatest in the red and blue LED exposure group. So, the antimicrobial photodynamic therapy LEDs are effective for bacterial inactivation and accelerate wound healing in mice.

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