

A Critical Review on Antimicrobial Photodynamic Inactivation Using Light Emitting Diode (LED)

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Abstract

The light-emitting diode (LED) is an advanced technology with a wide range of applications in our day-to-day life. It has numerous advantages over conventional light, such as controlling the spectrum of light, the specificity of the wavelength, cool emitting surface, and cost-effectiveness. The novel technologies and developments have proved the efficacy of LEDs in eliminating microbes rather than being an effective lighting source. The LED employs Photodynamic Inactivation to eliminate micro-organisms with the help of various photo-sensitizers. Photodynamic inactivation is a non-chemical based technique that helps fight against the microbes without developing the resistant microbial strains. The illumination of LED at a specific narrow wavelength exhibits antimicrobial activity against a wide range of microbes, including resistant strains. Getting rid of harmful micro-organisms is one of the effective ways to reduce health risks and promote quality of life. Hence, the LEDs with specific narrow wavelengths can be employed to sterilize the medical equipment, healthcare environment, and food preservation without using chemicals. The Photodynamic Inactivation using LED as a light source will be a promising source for eradicating harmful micro-organisms, including nosocomial and foodborne pathogens.

Keywords: LED, Light, Photodynamic inactivation, Antimicrobial activity, Photosensitizers, Micro-organisms

Introduction

The lighting systems also play a vital role in the everyday life of people, and it can also improve safety, creates suitable spaces rather than illumination. The sun as the natural lighting source produces light in 400-700 nm, which possess sterilizing properties. Many biological studies also have reported the significance of lighting systems in influencing the psychological and behavioral activities of humans. Besides that, innovations and technologies have also proved the potential of light to eliminate micro-organisms. It is one of the advanced technologies which uses the specific wavelength of the narrow spectrum of visible to exhibit the disinfecting property. The light exhibits antimicrobial activity according to the wavelength which it is emitted. The visible light of the narrow wavelength can be used to eliminate the wide variety of microbes, including bacteria, fungi, viruses, yeast, etc. (Gwynne and Maurice, 2018). The light-emitting diode (LED) is considered to be safer and effective disinfect than ultra-violet rays. Many studies have demonstrated the photodynamic inactivation of LED blue light to kill the harmful microbes. Photodynamic inactivation is a technique that employs photosensitizers that absorb the visible

light for its activation to form reactive oxygen species as well as singlet oxygen. The Reactive Oxygen Species (ROS) undergoes oxidation of biomolecules and lysis of cells. It has the potential to treat diseases and infections without causing any drug resistance as like as antibiotic treatment (Hamblin, 2016). Antibiotic resistance is also a major problem for eradicating microbes. It is due to the rapid evolution of microbes, which promotes the adaptation to constituents present in the media (Mania, 2017).

Besides that, the airborne micro-organisms such as bacteria, viruses, and fungi are commonly known as bio-aerosols, which have a prominent role in the environment and human health. (Prussin et al., 2015). The adverse health effects and risks are caused by indoor microbes exceeding their maximum limits, especially in the healthcare centers. There is a wide range of studies have already described the role of micro-organisms in causing diseases (Setlhare et al., 2014) and factors promoting microbial growth for inducing food spoilage (Preetha, and Narayanan, 2020). So, Photodynamic inactivation plays a pivotal role in eliminating the indoor and outdoor micro-organisms responsible for causing various diseases. So, this approach can be applied to sterilize medical equipment, healthcare environments, and also for food preservation rather than using harmful chemicals. In this review, we have summarized the evidence for the Antimicrobial Photodynamic Inactivation carried out with LED as the lighting source to eliminate the microbes such as bacteria, fungi, and virus.

Light Emitting Diode

In the olden days, people used burning wood, candles, oil lamps, kerosene lamps, and gas lamps as the lighting source, and those innovations eventually lead to the development of CFL and fluorescent lamps (Weisbuch, 2018). There have been increasing alternations and innovations in lighting technologies to create an excellent lighting system. Thus, the requirement of high grade of light and energy effective in the household and many sectors paves the way for the development of LED technology (Perdahci et al., 2018). During the last years, the furtherance of LED technology led to playing a crucial role in the day-to-day life of the human

(Lin et al., 2019). This solid-state device is highly efficient, reliable, and durable over the other forms of the lighting system, which results in the rapid growth of the LED industry (Soh et al., 2017). Producing light by LED varies from other conventional lighting systems because it is a semiconductor diode (Soni, and Devendra, 2008). The LED is the light-emitting diode that uses the terminal devices such as positive (anode) and negative (cathode), and the working is based on the PN Junction.

Doping is the process of adding the impurities such as Si, GaN, and GaAs to get the P-type as well as N-type semiconductor (Gayral, 2017). The N-type semiconductor provides free electrons, whereas the P-type semiconductor lacks free electrons, thereby creating the holes (Bourget, 2008). The emission of light happens when the electrons in the valence band move to the conduction band, thereby creating an empty state in the valence band (Gayral, 2017). The electrons started to recombine into the holes as soon as the suitable voltage is applied. The electrons fall into a lower energy state after the meeting of electrons with holes. Then, it releases energy in the form of photons, and the light is produced as the result of the emission of photons (Ahemen et al., 2014). The materials used in the semiconductor and also the energy gap of the semiconductor determines the color of the light (Yeh, and Jen-Ping Chung, 2009). The LEDs are well known for their unique properties such as temporal settings of produced light, radiant intensity, and the adjustment of spectral characteristics (Branas et al., 2013). Now, the LEDs are used in many lighting systems because it has many advantages over the conventional lighting system. It is mainly a very cost-effective approach, and it has a long life expectancy. LEDs are well known for their low emission of radiant heat (D'Souza et al., 2015). The expeditious development and extensive potential in energy efficiency and lumen output prove that LEDs can be a remarkable light source (Pattison et al., 2016) with a board range of applications.

Photodynamic Inactivation and Photosensitizers

The photodynamic inactivation is an alternative approach to kill harmful microbes such as bacteria, fungi, etc. It is also called Photodynamic Antimicrobial Therapy (PACT) or Antimicrobial

Photodynamic Inactivation (PDI) (Awad et al., 2016). Photodynamic inactivation employs photosensitizers, which absorb the visible light to activate the formation of reactive oxygen species and singlet oxygen. Harmless visible light combined with non-toxic dyes called photosensitizers is used to kill the micro-organisms (Joli et al., 2011).

Light, along with oxygen, and the non-toxic photosensitized are the essential requirements for this approach. The photon of the specific wavelength gets absorbed by the photosensitizer, and thus it gets activated from the excited state. The electrons present in the ground state level of photosensitizer promote higher energy to form the triplet state. The transformations of excited states proceed in two photochemical pathways (Ghorbani et al., 2018).

The type-1 mechanism involves the formation of Reactive Oxygen Species (ROS) by transferring the electron from the excited state. The type-2 mechanism involves the production of a single oxygen radical and then promotes the destruction of cell walls and DNA via oxidative stress (Denis et al., 2011). The reactive oxygen species causes harmful effects to the cell, so this phenomenon is employed in antibacterial treatment. The ROS can be created by visible light via absorbing porphyrins, cytochromes, which are endogenous cellular photosensitizers (Lavi et al., 2004). The endogenous photosensitizers are present in bacteria also respond to the illumination of high-intensity light, which in turn produces ROS at the highest level, and it leads to bacteria. For instance, *Propionibacterium acnes* is a bacteria, destroyed easily by visible light due to the high amount of endogenous photosensitizers (Lipovsky et al., 2009). The exposure of photosensitizers to visible light undergoes the photochemical process to produce ROS, which will ultimately damage the microbial cells. The oxidative stress is induced by hydrogen peroxide, hydroxyl radicals, and single oxygen. The fungal structures include hyphae and conidia and can be eliminated by using photosensitizers (Smijls et al., 2009). The efficacy of photosensitizers depends upon the physicochemical properties of the microbial cell wall (Usacheva et al., 2001). This method helps to inactivate the micro-organisms without disturbing the physiological flora, and it also functions very rapidly in a short period (Ryskova et al., 2010). The

antimicrobial photodynamic inactivation also plays a vital role in inactivating the various virulence factors, and it also can reduce the activity of enzymes such as lipases, proteases, and as well as secreted toxins (Fila et al., 2017). Moreover, antimicrobial photodynamic inactivation doesn't exhibit any phototoxic, cytotoxic effect, and mutagenic effect towards the eukaryotic cells (Grinholc et al., 2015).

Anti-Bacterial Effects

The visible light of 400-500 nm wavelength has also been reported for the excellent bactericidal effect against various pathogens (Feuerstein et al., 2004). The evaluation of three different wavelengths, such as 425, 525, and 625 nm corresponding to blue, green, and red LED irradiation against the *Porphyromonas gingivalis*, *Staphylococcus aureus*, and *E.coli*. The irradiation of light at the wavelength of 425nm and 525nm for 0-24hrs exhibited the reduced viability of *Porphyromonas gingivalis* and *E.coli* than *staphylococcus aureus*. The result also stated no bactericidal effect at a wavelength of 625nm (Kim et al., 2013). The irradiation of LED at the wavelength of 450, 470, and 620 nm against the planktonic *Legionella rubrilucens* showed 5 log reduction at 450nm by the dose of 300 J cm² in 470nm; the blue light of 500 J cm² exhibited comparable reduction. But there was no inactivation observed by irradiation of red light even at 500 J cm² (Schmidt et al., 2019). The illumination of the LED array at 405nm for the time interval of 5, 10, and 20 minutes showed inactivation of the monolayer biofilm of *E.coli* effectively as well as rapidly. The light exposure for 10 minutes showed a stepwise reduction of 2.52log10 in *E.coli* biofilm (McKenzie et al., 2013).

The TSA plated 18 strains of *Salmonella enterica* were subjected to the illumination of the LED at a wavelength of 405 ± five at a temperature of 4°C. The most susceptible to LED illumination was showed by *S.entericaserovarenteritidis* (ATCC 13076) whereas, *S.entericaserovarsaintpaul* ATCC 9712 was the most resistant. These studies also reported that the antimicrobial activity of the LED at the wavelength of 405 ± 5 was due to the loss of membrane function and the oxidation of DNA (Kim et al., 2017). The foodborne pathogens such

as *Lactobacillus Plantarum*, *Staphylococcus aureus*, and *Vibrio para hemolytic* were subjected to the illumination of the LED at 405, 460 and 520 nm. The results showed the inactivation ($P < 0.05$) in the *Vibrio para hemolytic* at 405 and 460 nm. But, the illumination of the LED exhibited less susceptibility for *Lactobacillus Plantarum* and *Staphylococcus aureus*. So, the excellent anti-microbial activity of LED illumination was observed at 405 nm wavelength (Kumar et al., 2016). The food borne pathogens such as *E. coli* 0157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* were suspended in trypticase soy broth. It was illuminated at 461, and 521 nm LED to evaluate the antibacterial effects at different pH values (4.5, 6.0, 7.3, 8.0, and 9.5) at 15°C. The illumination of the LED at 461 nm was more prominent in inactivating the pathogens than 521 nm LED. The result also suggested the 461 nm LED can be used to preserve food in acidic and alkaline conditions (Ghate et al., 2015). The study conducted by Endarko et al., (2012) demonstrated that 405 ± 5 nm light exhibited effective inactivation of food borne pathogen *Listeria monocytogenes* and also showed similar kinetics in other *Listeria* species. The high susceptibility of inactivation via irradiation of the 405 nm LED was showed by *E. coli*, *Salmonella enteritidis*, and *Shigella sonnei*.

The study conducted by MacLean et al., (2009) to determine the inactivation of nosocomial pathogens comprises gram-positive and negative bacteria via the irradiation of 405 nm LED array. The result reported the inactivation of both gram-positive and negative bacteria along with within Methicillin-Resistant *Staphylococcus aureus* by the illumination of a narrow spectrum of 405 nm visible light from the LED source without pre-treatment of any chemicals. The highest inactivation level was exhibited by *Staphylococcus*, *Streptococcus*, and *Clostridium* strains whereas, *Enterococcus faecalis* was appeared to inactivate after the longer exposure time. Hence, a longer exposure time was required by gram-negative bacteria to exhibit significant inactivation than the gram-positive bacteria.

The antimicrobial blue light illuminated at the wavelength of 400 nm from the LED source was effective against the 34 different nosocomial wound pathogens, including *Pseudomonas*

aeruginosa, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, and *Elizabethkingia meningoseptica*. The decrease in colony-stimulating unit by $>5\text{-log}_{10}$ in viability occurred in all bacterial strains after 15 to 30 min of exposure of LED light at 54 J/cm² - 108 J/cm² (Halstead et al., 2016).

Anti-Fungal Effects

The photodynamic inactivation studies carried out with 12 isolates of *Trichophyton rubrum*, at 630 nm LED light along with toluidine blue as a photosensitizer by Baltazar et al., (2013). The illumination of 630 nm LED light at 48 J/cm² and ten mg/L of toluidine blue have inhibited more than 98% of fungal growth (Baltazar et al., 2013). A chlorin derivative (TONS 504) and the irradiation of 660 nm LED light demonstrated the antifungal activity against the *Fusarium solani* and *Aspergillus fumigatus*. The optimal conditions for complete photodynamic inactivation for *Fusarium solani* were one mg/L of TONS 504 and 30 J/cm² of LED illumination. But, the *Aspergillus fumigatus* showed a partial inhibition with 10 mg/L of TONS 504 and 20 or 30 J/cm² of LED irradiation (Sueoka et al., 2019). The fungicidal effect of the 405 nm LED array against *Botrytis cinerea* shows the significant spore reduction in detached tomato leaves. The remarkable inhibition of mycelium growth was exhibited after irradiation of light at a wavelength at 405 nm and 415 nm (Imada et al., 2014).

Anti-Viral Effects

The photodynamic antimicrobial chemotherapy against the ACV (acyclovir) -sensitive and -resistant Herpes Simplex Virus type 1 (HSV-1) revealed that TONS 504 at the concentration of 10 mg/L and LED light illumination of specific wavelength 660 nm at 10 to 30 J/cm² have completely eradicated both viruses (Latief et al., 2015). The photodynamic inactivation carried out with curcumin as a photosensitizer and LED light reduced the titers of norovirus surrogates such as feline calicivirus and murine norovirus (Randazzo et al., 2016).

Conclusion

Hence, the above evidence has shed light on the Photodynamic inactivation of LED as a lighting source for inhibiting micro-organisms such as bacteria, fungi, and viruses. There are a wide number of antibiotics as well as chemical substances are used to eradicate harmful micro-organisms. The increased usage of antibiotics and chemical agents led to the development of resistance and resulted in numerous detrimental effects in humans. The illumination of LED light at a specific narrow wavelength employs Photodynamic Inactivation to exhibit antimicrobial activity to eradicate the disease-causing pathogens. The LEDs can also be employed as an effective source for sterilization to eliminate microbes even present in the medical equipment, food substances, healthcare environments, etc., rather than using chemicals. So, the Antimicrobial Photodynamic inactivation is a promising alternative approach to cure various life-threatening diseases via eliminating the harmful pathogens without causing the development.

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