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Effects of Ultraviolet Light-Emitting Diodes (UVA-Leds) Irradiation on Escherichia Coli for Inactivation of Microorganisms

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ABSTRACT

Ultraviolet (UV) radiation has been extensively involved in human life for years, providing wide range of disinfection applications. Recently the trend has been diverted towards UV light, as a primary source of disinfection in water treatment rather than traditional methods and same can be said for medical instrumentations. Existing methods make use of mercury based UV lamps which possess many limitations and therefore the use of UV-LEDs is becoming popular. In this study, a standard LED and UVA-LED has been compared in terms of disinfection capabilities to understand which one of two has highest microorganism inactivation properties. The results clearly demonstrated that UVA-LED was able to provide significant disinfection whereas standard LED was incapable of providing noticeable inactivation. Post-exposure of 1 h to LED and UVA-LED light on their respective samples concluded that 3.8 log inactivation was observed for latter whereas for former only 0.1 log inactivation was achieved.

Keywords: Ultraviolet, UVA-LED, Disinfection

INTRODUCTION

Disinfection and sterilization have been part of human life for years, providing important means to ensure human well-beings is kept at utmost high standards. Traditional methods, used to carry out aforesaid tasks, involve the extensive use of chemicals, heat, steam and gas etc. These methods have been used in water treatment, sterilization of medical devices and surgery related tools widely. However, these techniques pose some serious limitations such as extremely time consuming, tedious process, very expensive to maintain the practice. Moreover, these chemicals sometime cause serious skin related allergies and also capable of altering the surface structure of the devices these methods have been applied on.

Ultraviolet (UV) irradiation, proven to be an effective method, has been used widely for disinfection in range of applications such as water, food and medicine. UV light inactivates microorganisms by disrupting the DNA hence making them unable to replicate. The one UV technology that is predominantly used in the world today is the mercury based UV lamp. Monochromatic and polychromatic are two main types of UV lamps. Monochromatic, also referred as low pressure (LP), mercury lamps emit most of their light at 254 nm wavelength whereas polychromatic can provide light in various wavelengths (Bohrerova & Linden, 2006). LP mercury lamps are the most common type being used for various disinfection applications.

Unfortunately, UV lamps face unsolved challenges which significantly reduces their scope. Some of these limitations include high operation voltage and current ranging from 110 – 240V AC (Lui et al., 2014). These lamps work at whopping 100 °C for monochromatic (Yoshinobu et al., 2011). Moreover, mercury contents are extremely hazardous to human and environment and high maintenance cost associated with this technology adds another layer of distress. Generally, a warm-up time between 2 – 15 minutes is required before operation (Chatterley & Linden, 2010) and frequent replacement of UV lamp is needed due to extremely short lifecycle of 8000 – 10,000 hours (Rasoulifard, Fazli, & Eskandarian, 2015). Apart from this, the cost required to properly dispose of mercury substance after use creates further complications on the continuous use of this technology (Rasoulifard et al., 2015).

These limitations have caused the development of a new type of UV light. Ultraviolet light emitting diodes (UV-LEDs) are considered to be one of the most influential alternatives to UV lamps due to numerous advantages. UV-LED basically, is a p-n junction based semiconductor device capable of producing electroluminescence as well as able to produce narrow spectrum of light in all UV sub-bands (Yoshihiko, Masahiro, & Suguru, 2014). When compared with UV lamps, UV-LEDs undoubtedly stand out because they are able to provide highly efficient energy (Zhou, Li, Lan, Yan, & Zhu, 2017), no warm-up time is required, has extremely long lifecycle, able to produce UV light without the use of mercury contents (Wurtele et al., 2011). These LEDs are very cost effective and do not require regular maintenance as is the case with UV lamps. They are completely environmentally friendly and can be easily disposed of without any complications (Yoshinobu et al., 2011).

One striking similarity between the UV lamps and the UV-LEDs, is their mechanism of disinfection. UV light, wavelength mainly between 200 to 300 nm, is considered to be most effective in targeting DNA of the microorganisms. Generally, it is accepted that the maximum absorption wavelength through DNA is around 260 nm. However, the optimum wavelength is dependent on the type of microorganism hence can vary greatly from one microorganism to

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another. The main limitation with LP mercury lamps is that it only emits light at wavelength of 254 nm therefore it cannot efficiently target all different sort of microorganism. In contrast, the UV-LEDs can be manufactured at different peak emission wavelengths which has the potential to produce better results in inactivating microbes.

Wavelengths between 254 and 280 nm are considered to be most effective in eliminating microorganisms because they fall closely to the DNA maximum absorption rate. Damage caused by UV-C light results in the formation of pyrimidine dimers which eventually causes microorganism inability to reproduce (Chatterley & Linden, 2010; Chevremont, Farnet, Coulomb, & Boudenne, 2012; Hamamoto et al., 2007). Traditional UV lamps also use UV-C wavelength therefore the majority of the research on UV light disinfection has been limited to this region which clearly indicates the effectiveness of UV-C region in inactivating microorganisms (Chatterley & Linden, 2010; Hamamoto et al., 2007; Mary H Crawford, 2005; Oguma, Kita, Sakai, Murakami, & Takizawa, 2013; Oguma, Rattanakul, & Bolton, 2016). Unfortunately, only a handful research has been directed to study the effects of other UV regions namely UV-B and UV-A respectively.

DNA damage caused by UV-C light is likely to be repaired by the DNA repair mechanisms such as the photo-reactivation and dark repair hence making treatment with UV-C less long-lasting (Nebot Sanz, Salcedo Dávila, Andrade Balao, & Quiroga Alonso, 2007; Rodriguez et al., 2014). Since DNA repair mechanism is completely unwanted to achieve maximum and long-lasting disinfection therefore this process must be weakened if not eliminated entirely. By targeting the repair enzymes responsible for DNA repair mechanism can help in achieving lasting disinfection. Repair enzymes are sensitive to higher UV intensities (Sommer, Haider, Cabaj, Pribil, & Lhotsky, 1998) therefore using UV-A instead of UV-C could produce better results. Though disinfection through UV-A radiation is less efficient when compared to UV-C but it still has the ability to carry out disinfection as reported by various studies (Chevremont, Farnet, Sergent, Coulomb, & Boudenne, 2012; Hwang, 2013; Nakahashi et al., 2014). However, when it comes to prevent DNA repair this is where UV-A stands out. UV-A radiation has not been as widely studied as UV-C. Moreover, no literature, as per our review, was found on the comparison of UV-A and LED light sources in order to determine their efficiency. In this paper, a comparison of standard LED and UVA-LED has been studied in order to understand their behaviour in disinfection of pathogens. UVA-LED with peak wavelength of 385 nm has been selected and compared with standard LED for the purpose of inactivating Escherichia coli (E. coli) and comparing the disinfection efficiency.

MATERIALS AND METHOD

Preparation of microorganism

Escherichia coli (ATCC 11229) was used mainly in this research study. E. coli strain was added with saline solution and thereafter was streaked onto nutrient agar petri dish using an inoculation loop in order to get isolated colonies. The petri dishes were then incubated at 37 °C for approximately 24 h. Isolated colonies from petri dishes were removed using inoculation loop and about 5 – 7 colonies were further added into saline solution and mixed gently to even the concentration. the process was repeated until satisfactory level of concentration was obtained. The concentration was compared with 0.5 McFarland for turbidity until desired concentration of approximately 1.5×10^{8} was obtained. E. coli was then swabbed onto a nutrient agar petri dish with the help of sterilized cotton bud and left to dry before placing upside down and sealing with parafilm. The process was repeated for control, LED and UV-LED samples. E. coli swabbed petri dishes were then exposed to their respective light for treatment.

Design of experimental device

A standard super bright 3 mm LED (F33CC4SB-3) with 460 nm wavelength was used to provide light for LED samples. Its compact size, high brightness, low power consumption as well as higher output stability and reliability were some of the key features which made it stand out. A DC constant power supply was used to power on the LED. A resistor based voltage and current limiter circuit was designed to driver the LED, to ensure it stays working efficiently and to maintain a constant current flow (30 mA) in the circuit. The total power consumption of the LED circuit was around 0.2 W. On the other hand, a high power 385 nm wavelength UVA-LED (NVSU233A(T)-D1) from Nichia, Japan was selected to carry out disinfection tasks in this experimental setting. A constant current of 700 mA was applied to the UV-LED. The total power consumption of the circuit was 2.45 W. Irradiation dose of 57.6 J/cm2 was received by the sample during 1 h exposure to UVA-LED light. Every possible effort was made to make sure the current and voltage did not exceed the maximum limit. The UV-LED was able to provide maximum output power at 1400 mW. Both LED and UV-LED ran in continuous mode and the distance between the sample and light source was kept at 70 mm so that the light could easily cover the whole petri dish. The dimension of the LED and UV-LED can be seen in Fig. 1 (a) and Fig. 1 (b) respectively.

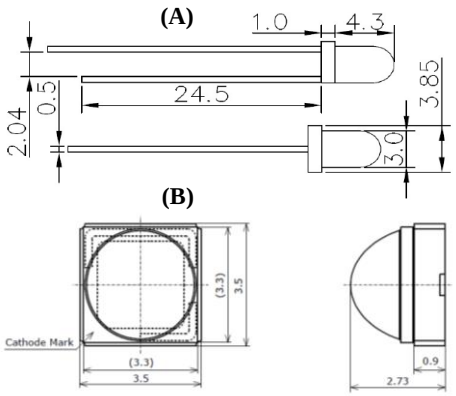

Fig. 1 (a) Dimensions of 3 mm LED and (b) 385 nm UVA-LED.

Exposure to LED and UV-LED light

Three separate cardboard boxes were used in this experiment. One box was dedicated for control sample while second and third was used for LED and UV-LED samples respectively. All experiments were conducted in well ventilated and sterilized environment with room temperature approximately at 25 °C. During the experiment, petri dishes for LED and UV-LED were exposed to their respective light for 1 h while the control sample was left untreated and was not exposed to any light as shown in Fig. 2. The boxes were covered with lid to provide dark environment as well as to prevent outside environment influence on the samples. After treatment, the petri dishes were incubated for approximately 24 h at 37 °C. The dishes were observed, post-incubation, for bacteria growth.

Fig. 2 Schematic representation of experimental setting.

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Colony forming unit inactivation level

Colony forming unit (CFU) method was used in order to clearly distinguish which of the two (LED/UVA-LED) is the most efficient one in inactivating microorganisms. The post treatment petri dishes were then swabbed with cotton bud and mixed with 1 ml of saline solution. Same 1 ml was then added into 9 ml of bacteria-free saline solution in a process called serial dilution. The process was repeated until 10^{-9} dilution was achieved. Approximately 30 µl of each of these dilutions was cultured on petri dish at 37 °C for 24 h. Following day, the petri dishes were observed for bacteria growth and number of colonies were counted for CFU. The measurement of CFU was calculated using Eq. (1):

$$c = \frac{n \times d}{v} \qquad (1)$$

where

c=CFU/ml
n=number of colonies on petri dish
d=dilution factor
v=volume transferred on the plate

Log inactivation level

Log inactivation is another way of indicating the level of disinfection occurred following UV treatment. It is sometime also referred as log survival ratio. This method provides results in log form indicating the overall disinfection achieved as a result of UV treatment. Log inactivation ratio was calculated using Eq. (2):

$$\text{Log inactivation ratio} = \log\left(\frac{Nt}{N0}\right) \qquad (2)$$

where

Nt=Number of colonies post UV treatment
N0=Number of colonies before UV treatment

RESULTS AND DISCUSSION

In order to assess the disinfection capabilities of LED and UVA-LED, the samples (control, LED and UVA-LED) were observed for disinfection after exposure of 1 h to their respective light. The results received after treatment are illustrated in Fig. 3. Almost instantly it is noticed that the control sample, Fig. 3 (a), has witnessed overgrown colonies to that extant the whole of the control petri dish was covered with bacteria and no single colonies were observed. With respect to the LED sample, similar was observed indicated no significant differences between control and LED sample as shown in Fig. 3 (b). Moreover, from mere observance it was easily concluded that no disinfection properties have been seen by the use of LED light and overgrown bacteria colonies covered the entire petri dish.

The situation with the UVA-LED sample, illustrated in Fig 3 (c), is completely different from its counterparts. The UV-LED sample clearly showed almost no bacteria colonies in the center of the petri dish marked with "X" where the light intensity was at maximum indicating high level of disinfection properties. A much wider disinfection circle is visible which is not present in the LED sample. The colonies concertation increased as moved towards the edge of the petri dish highlighting that the intensity of light reduced as move further away from the center. This is due to the fact that only one UV-LED was used in this study and the spot area was not significant enough to disinfect all the colonies. This limitation can easily be overcome with the introduction of more UV-LEDs. UVA-LED treated sample outweighed disinfection of microorganism when compared with standard LED, which did not produce meaningful disinfection.

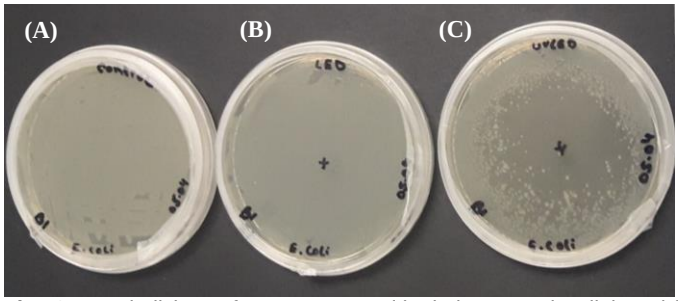

Fig. 3 Petri dishes after treatment with their respective lights. (a) untreated control sample (b) LED exposed sampled (c) UVA-LED treated petri dish.

Determination of CFU

In order to identify, quantitively, the amount of disinfection occurred as a result of 1 h light exposure to LED and UV-LED samples, the control, LED and UV-LED samples were first observed for disinfection by looking at the inhibition zone. From mere observance, it was noticed immediately that no observable difference was seen between the control and LED samples. Both samples looked identical resultantly highlighting no disinfection properties. However, the situation for UVA-LED was completed different when compared with control and LED samples. A clear microorganism-free inhibition zone was observed instantly indicating that the exposure to UVA light was successful in inactivating microorganisms. No colonies were found in the center area of the petri dish, where the intensity of the light source was at maximum, however colonies were visible towards the edge of the petri dish. This is due to the fact, that only one UVA-LED was used in this experiment, however, if multiple LEDs were used this limitation would have been easily dealt with.

The CFU was calculated using serial dilution method. Different dilution factors had different number of colonies present in them. Some colonies were so highly dense that it was impossible to count each and every one of them while other dilution factors had so little colonies that it would provide statistically unreliable results. To deal with this situation viable count standard was used which helps to identify the correct dilution factor based on the number of colonies present. Typically, a dilution factor having 30 – 300 colonies is considered accurate. The same process was repeated for control, LED and UVA-LED samples. The calculation results showed that control sample had the maximum number of CFU/ml followed by LED sample. The UVA-LED sample had the least number of colonies present, confirming that UVA-LED was able to inactivate most of the microorganisms. The CFU/ml values are illustrated in Table 1.

Table 1 Number of viable colonies present in each sampled post CFU calculation.

	Control	LED	UV-LED
CFU/ml	27×10^{8}	19×10^{8}	0.0043×10^{8}

Log Inactivation Level

It was further decided to identify the amount of inactivation using log scale. Log inactivation is a convenient way of representing the numeric or percentage value of the total amount of microorganisms inactivated through the disinfection process. Generally, 3 log inactivation value represents the 99.9% inactivation of microorganisms. Log inactivation was calculated using the equation (Eq.) 2, given previously. The results indicated that LED treated sample experienced 0.1 log inactivation. The UVA treated sample, however, showed incredible amount of disinfection at whopping 3.8

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log inactivation. The results showed the standard LED in comparison with the UVA-LED produce negligible microorganisms inactivation.

The disinfection system designed in this study was on a smaller scale and there is a possibility of having some challenges when extended to large system. This experiment used one LED and UV-LED, however, when multiple LEDs have been combined the overall inactivation efficiency could alter. The output power produced by both sources were not the same this is because the standard LEDs are not designed to withhold high current e.g. 1 A whereas UVA-LED used in this could handle up to 1.4 A. This problem can easily be solved by using LED which has either similar output power or can handle high currents. The experiment was conducted using continuous mode in which light sources remained on during the whole experiment. In future, pulsed mode could also be introduced which can provide higher current for limited period of time for inactivation of pathogen. Pulsed mode has the ability to disinfect as efficiently as continuous mode. Research studies (Li et al., 2010; Wengraitis et al., 2013) reported that pulsed mode performed better compared to continuous mode for microorganism inactivation.

UV light is an amazing alternative to traditional methods having awesome advantages unbeatable by the existing methods. This proposed device has the potential to be used in hospitals saving millions of dollars each year spent annual on buying disinfection related chemicals etc. Moreover, UV is completely environmentally friendly hence its importance and contribution in our life is beyond measure. In near future, further experiments will be conducted where larger scale applications will be tested to develop a practical device capable of disinfection. Moreover, rather than having single light source, a combination of multiple sources will be applied in near further to deliver required UV dose in limited time possible to achieve high bacteria inactivation. The overall size of the device will be reduced to accommodate portability function and safety features will also be used to protect human beings for UV exposure.

CONCLUSION

A comparison study was carried out to understand the behaviour of standard LED and UVA-LED as well as their efficiency in inactivation of pathogens. The results clearly indicate the standard LED possess very minimum to none disinfection abilities. The UVA-LED, on the other hands, is capable of providing disinfection beyond a shadow of a doubt and was able to inactivate over 99.9% of microorganisms. The research study carried out has the potential to be used in various applications for disinfection such as food, water treatment and healthcare settings.

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