EFFECTIVENESS OF MULTIVARIATE PARAMETERS ON MEDICAL DEVICE DISINFECTION USING SIMULTANEOUSLY-COUPLED TRIADIC WAVELENGTH UV-LEDS

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To my family for their constant prayers, support and encouragement
throughout my postgraduate studies.

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ABSTRACT

Ultraviolet light emitting diodes (UV-LEDs) have shown a great potential to replace traditional UV lamps for microorganisms disinfection. Most research is focused on water and food disinfection applications whereas the utilization of UV-LEDs for healthcare disinfection is not fully understood due to limited exploration on this area. This study presented a comprehensive work on UV-LEDs solitary and coupled wavelength combinations in the context of inactivation, photoreactivation and morphological characteristics which is of significance to expand UV-LEDs scope beyond water applications, specifically to be adopted in healthcare disinfection system. In this study, UV-LEDs with peak emission at 276, 311 and 364 nm were studied for the inactivation of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Under the solitary wavelength, the effectiveness of each LED was studied separately and the effects of varied exposure times as well as UV doses were investigated for the inactivation of *E. coli* and *S. aureus*. It was found that the 276 nm LED produced the highest inactivation efficiency as compared to the 311 and 364 nm LEDs which required significantly lower exposure time and UV dose to achieve maximum inactivation of both bacteria. In the focus of investigating the effects of coupled wavelengths, simultaneously, the coupled triadic wavelength (SCTW) 276|311|364 nm UV-LEDs enhanced the inactivation effects and was able to produce the highest inactivation of *E. coli* (98.42%) and *S. aureus* (99.34%). The combination of 276|311 nm achieved the second best results on *E. coli* (97.36%) and *S. aureus* (98.63%), followed by the 276, 276|364, 311, 311|364 and 364 nm, respectively. For both, the solitary and the coupled wavelengths, a relatively higher inactivation of *S. aureus* was found in comparison with *E. coli*, indicating that *S. aureus* was more sensitive to UV irradiation. Moreover, the evaluation of morphological images of *E. coli* and *S. aureus* showed that all UV treated samples caused significant damage and deterioration of cellular membranes. However, the most pronounced damages such as membrane transparency, pore formation, blebs protrusion and lysis were seen when both bacteria were treated with the SCTW 276|311|364 nm UV-LEDs. The results also showed that the SCTW 276|311|364 nm UV-LEDs was the most effective combination in providing better persistence against the photoreactivation of *E. coli* (1.75%) and *S. aureus* (4.29%) as compared to the 276 nm which projected the photoreactivation of 2.14% on *E. coli* and 8.92% on *S. aureus*. These results are of significance for future applications in healthcare.

ABSTRAK

Diod pemancar cahaya ultraviolet (UV-LED) telah menunjukkan potensi besar untuk menggantikan lampu UV tradisional dalam pembasmian kuman. Kebanyakan penyelidikan tertumpu pada aplikasi pembasmian kuman dalam air dan makanan dimana penggunaan UV-LED untuk pembasmian kuman dalam penjagaan kesihatan masih tidak difahami sepenuhnya kerana penerokaan yang terbatas dalam bidang ini. Kajian ini mengemukakan pembelajaran secara komprehensif mengenai UV-LED bersendirian dan gabungan panjang gelombang dalam kontek penyahaktifan, fotoreaktivasi dan ciri-ciri morfologi untuk memperluaskan skop UV-LED lebih daripada aplikasi air, khususnya ke sistem pembasmian kuman bagi penjagaan kesihatan. Dalam kajian ini, UV-LED dengan pelepasan puncak pada 276, 311 dan 364 nm dikaji untuk penyahaktifan bakteria jenis *Eschericia coli* (*E. coli*) dan *Staphylococcus aureus* (*S. aureus*). Di bawah panjang gelombang bersendirian, keberkesanan setiap LED telah dikaji secara berasingan dan kesan masa pendedahan yang berbeza-beza serta dos UV telah disiasat untuk penyahaktifan *E. coli* dan *S. aureus*. Didapati bahawa LED 276 nm menghasilkan kecekapan penyahaktifan tertinggi berbanding dengan LED 311 dan 364 nm yang memerlukan masa pendedahan dan dos UV yang lebih rendah bagi mencapai peningkatan kesan penyahaktifan maksimum untuk kedua-dua bakteria. Dalam fokus menyiasat kesan panjang gelombang berpasangan, secara serentak, gabungan tiga gelombang (SCTW) 276|311|364 nm UV-LED telah meningkatkan kesan penyahaktifan dan mampu menghasilkan penyahaktifan tertinggi terhadap *E. coli* (98.42%) dan *S. aureus* (99.34%). Gabungan 276|311 nm mencatatkan hasil kedua terbaik ke atas *E. coli* (97.36%) dan *S. aureus* (98.63%), diikuti oleh 276, 276|364, 311, 311|364 dan 364 nm secara berturutan. Bagi kedua-dua panjang gelombang bersendirian dan gabungan, kesan penyahaktifan *S. aureus* adalah lebih tinggi berbanding *E. coli*, menunjukkan bahawa *S. aureus* lebih sensitif terhadap sinaran UV. Tambahan, penilaian gambar morfologi *E. coli* dan *S. aureus* menunjukkan bahawa semua sampel yang dirawat dengan UV mengalami kerosakan dan kemerosotan membran sel. Namun, kerosakan yang paling ketara seperti ketelusan membran, pembentukan liang, penonjolan lebam dan pecahan sel dapat diperhatikan apabila kedua-dua bakteria dirawat dengan SCTW 276|311|364 nm LED UV. Hasil kajian juga menunjukkan bahawa SCTW 276|311|364 nm UV-LED adalah gabungan paling berkesan dalam memberikan keputusan lebih berterusan terhadap fotoreaktivasi *E. coli* (1.01%) dan *S. aureus* (4.14%) berbanding dengan 276 nm yang menunjukkan fotoreaktivasi 2.14% ke atas *E. coli* dan 8.92% ke atas *S. aureus*. Hasil kajian ini adalah signifikasi bagi aplikasi rawatan kesihatan pada masa yang akan datang.

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LIST OF ABBREVIATIONS

AC	-	Alternating current
ATCC	-	American type culture collection
BSI	-	Blood stream infection
C. difficle	-	*Clostridium difficile*
CDC	-	Centers for disease control and prevention
CFU	-	Colony-forming unit
COPD	-	Chronic obstructive pulmonary disease
CPDs	-	Cyclobutane pyrimidine dimers
CW	-	Continuous wave
DC	-	Direct current
DNA	-	Deoxyribonucleic acid
E. coli	-	*Escherichia coli*
E. faecalis	-	*Enterococcus faecalis*
EM	-	Electromagnetic
FESEM	-	Field emission scanning electron microscope
FWHM	-	Full width at half maximum
HAIs	-	Healthcare associated infections
ICU	-	Intensive care unit
IR	-	Infrared
LB	-	Luria-Bertani
LED	-	Light emitting diode
LP	-	Low pressure
MP	-	Medium pressure
MRSA	-	Methicillin-resistant *Staphylococcus aureus*
NA	-	Nutrient agar
P. aeruginosa	-	*Pseudomonas aeruginosa*
POC	-	Point of care
ROS	-	Reactive oxygen species
S. aureus	-	*Staphylococcus aureus*
SCTW	-	Simultaneously coupled triadic wavelength

SEM - Scanning electron microscope
SSI - Surgical site infection
US - United States
UTI - Urinary tract infection
UTM - University Technology Malaysia
UV - Ultraviolet
UV-LED - Ultraviolet light emitting diode
WHO - World Health Organization

LIST OF SYMBOLS

° - Degree
°C - Degree celsius
f - Frequency
Ω - Ohm
% - Percentage
λ - Wavelength
Al - Aluminium
AlGaN - Aluminium gallium nitride
cm - Centimetre
cm^2 - Square centimeter
g - Gram
GaN - Gallium nitride
h - Hour
J/m^2 - Joule per square metre
kPa - Kilopascal
kV - Kilovolt
L - Litre
m^2 - Square metre
mA - Milliampere
mg - Milligram
min - Minutes
mJ/cm^2 - Millijoules per square centimeter
mm - Millimetre
mL - Millilitre
mW - Milliwatts
mW/cm^2 - Milliwatts per square centimeter
NaCl - Sodium chloride
nm - Nanometre
sec - Second
µL - Microliter

μW - Microwatt

μW/cm^2 - Microwatts per square centimeter

V - Volt

W - Watt

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CHAPTER 1

INTRODUCTION

1.1 Research Background

In this modern age of science and technology, a lot of developments are being made on daily basis to further enhance and improve the quality and living standards of human beings. Similar pattern is also observed when it to comes to hospitals. Hospitals including the community centers have been an important part of human life for generations, playing a pivotal role in providing necessary medical care to the sick and injured. Presence of microorganisms in environment is no stranger, microbes are encountered on regular basis. Some are considered beneficial while others can cause serious problems to humans. Existence of bacteria, virus and fungi is widely spread throughout the atmosphere covering areas like water, food, environment, surfaces and even human body. Similarly, presence of these microorganisms in healthcare is a common phenomenon. Infectious diseases caused by these microorganisms can range from being minor infections to life threatening situations thereby causing morbidity and mortality.

The importance of disinfection in healthcare is undeniable and every possible effort is made to ensure highest level of disinfection. Over the years many procedures have been adopted to cope with the situation of infectious diseases caused by pathogenic microorganisms. It is evident that total elimination of pathogenic microbes in most cases is not possible but efforts have been made to reduce their number hence lowering the probability of these microorganisms to cause infections. The use of chemicals is an important element that is kept into consideration when targeting pathogenic microbes. This method has been applied frequently in disinfection of water, decontamination of food (Al-Abri et al., 2019; Goodburn & Wallace, 2013). The use of chemicals, dry heat and water is also widely present in healthcare for disinfection of medical devices, surgical tools, hospital surface and many more (Rutala & Weber, 2004). These methods have been in place for years, providing essential disinfection and sterilization in healthcare. Unfortunately, these methods have serious limitations which make their use in the current age questionable.

Traditional disinfections practise cause skin irritation, respiratory diseases and alter the surface structure of the devices (Casey et al., 2017; Mahoney & Lim, 2012; Quinn et al., 2015). A study conducted by Harvard University which took account of more than 55,185 nurses in US, confirmed that nurses who regularly used disinfectants had as much as 32 % risk of developing chronic obstructive pulmonary disease (COPD) (Slawson, 2017). Moreover, these methods are very expensive to maintain because they require frequent replacement of chemicals and raw material necessary to perform disinfection. Their tedious disinfection protocols waste very valuable time in healthcare where every second is crucial in determining life and death (Matsuyama et al., 1997). Regardless of the various limitations of these methods, they are still being used widely due to the fact that no significant alternatives have been proposed, aiming to achieve efficient disinfection.

With the introduction of electronic based medical devices and its ever growing increase in healthcare, it has become mandatory to carry out their disinfection on regular basis. Traditional disinfection methods cannot be used on electronic medical devices due to their sensitivity to these methods. Water, chemicals and heat can significantly disrupt electronic devices causing them to malfunction or become out of order completely (Allen, 2019; Nichols, 2011). These limitations often result in these devices being uncleaned for prolonged period of time hence they are frequently contaminated and become source of infections.

The use of ultraviolet (UV) light is being accepted widely as an alternative disinfection method. UV lamps have been widely used in disinfection of water as well as foodborne pathogen to preserve the quality of the food (Hijnen et al., 2006). The use of UV lamps is also being used in healthcare for disinfection of environment, surfaces and textile clothing. UV lamps have various limitation such as use of mercury vapour, short lifecycle and require proper disposal after expiry which make them highly uninteresting for disinfection (Hölz et al., 2017; Shin et al., 2016; Soloshenko et al., 2006). These limitations have caused the development of a new type of UV light called ultraviolet light emitting diodes (UV-LEDs). They 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 in a narrow spectrum of light in all UV sub-bands (Yoshihiko et al., 2014). When compared with UV lamps, UV-LEDs undoubtedly stand out because they are able to provide highly efficient energy, require no warm up time and have extremely long lifecycle (Zhou et al., 2017).

UV-LEDs have also been widely used in water treatment (X. Li et al., 2019; Nguyen et al., 2019; Nyangaresi et al., 2018; Zou et al., 2019) and studies confirm that UV-LEDs are effective alternatively to UV lamps (K. A. Sholtes et al., 2016). Most studies carried on UV-LEDs make use of ultraviolet C (UVC) light for inactivation because studies have reported that UVC is the best region for inactivation of microorganisms (K. Sholtes & Linden, 2019; Song et al., 2018). UVC LEDs have also been used in healthcare for stethoscope disinfection and found to inactivate substantial amount of microorganisms (G. Messina et al., 2014; Gabriele Messina et al., 2015). However, studies have shown that combination of UV-LEDs from various regions can resultantly produce higher inactivation as compared to UVC alone (Chevremont, Farnet, Coulomb, et al., 2012; Song, Taghipour, et al., 2019). UVC LEDs have been combined either with ultraviolet B (UVB) or ultraviolet A (UVA) in previous studies for disinfection applications. However, no study has been found investigating the effectiveness of coupled UV-LEDs involving all UV regions.

In this study, the effectiveness of solitary UV-LEDs having wavelength of 276, 311 and 364 nm has been investigated for inactivation of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The variation of exposure time and resultant UV dose has been studied in the context of solitary UV-LEDs for determination of UV dose for optimum inactivation. The morphological characteristics of *E. coli* and *S. aureus* following irradiation from solitary UV-LEDs has also been explored. In the second part, the efficiency of simultaneously coupled triadic wavelength (SCTW) UV-LEDs has been studied for inactivation of *E. coli* and *S. aureus*. Various combinations of UV-LEDs were configured to study the effects of coupled UV-LEDs as a function of varied exposure time and UV dose. The cellular damage to *E. coli* and *S. aureus* caused by SCTW UV-LEDs were also investigated. Finally, the effects of photoreactivation were also studied to prolong bacteria growth delay while increasing the inactivation efficiency of SCTW UV-LEDs.

1.2 Problem Statement

The use of electronic based medical devices is increasing at an astonishing rate. Non-critical electronic medical devices such as stethoscope, sphygmomanometer, blood glucose meter and oximeter are being used as the first line of diagnosis in healthcare sector, providing essential diagnosis for effective treatments (Maki, 2014; Sahiledengle, 2019b). However, the utilization of these contaminated devices including use in intensive care units (ICUs) can increase the rate of mortality manifold (Gialluly et al., 2006; Whittington et al., 2009).

Unfortunately, disinfection of non-critical medical is often neglected by medical staff. The absence of disinfection of these devices is so prolific that many medical staff are completely oblivious to the fact that these devices need to be disinfected on regular bases (Sahiledengle, 2019a, 2019b). Non-critical medical devices have been found to be a reservoir for bacterial colonization and up to 86% stethoscopes were found to be contaminated (Chigozie Jesse et al., 2014; D. et al., 2016; Jain et al., 2013; Sengupta et al., 2000; Shiferaw et al., 2013). Disinfection of other medical devices such as blood pressure meter, blood glucose meter and oximeters also are frequently neglected (Desai et al., 2019; Matsuo et al., 2013; Risteen et al., 2018). Weldegebreal (Weldegebreal et al., 2019) stated that 75% stethoscopes or blood pressure meters, used by medical staff in ICU were contaminated and 77% health professionals did not clean stethoscopes in between examining each patient. In addition, 33.3% medical staff were recorded to have never disinfected medical devices under their monitoring (Uneke et al., 2014). These contaminated medical devices harbor various pathogens that can cause severe illness. Some of the most frequently isolated pathogens from medical devices are *S. aureus* and *E. coli* with various frequencies of isolation as reported by previous studies (Agaba et al., 2017; H. A. Khan et al., 2015; Tolera et al., 2018; Uneke & Ijeoma, 2011; Weldegebreal et al., 2019).

Existing disinfection and sterilization practices in healthcare such as the use of chemicals, dry heat, and steam have significant limitations which make medical personnel to neglect the disinfection protocols (Peters et al., 2018). Some of the limitations are as follows: i) long and tedious procedures; ii) cause skin irritation and respiratory diseases; iii) alter surface structure of the medical device. Over the years, significant consideration has been given to UV light as a mean of combating pathogenic microorganisms (Yang et al., 2019). Ultraviolet lamps such as monochromatic and polychromatic have been extensively used in the treatment of drinking and waste water (Beber de Souza et al., 2015; Bolton & Cotton, 2011; Sisti et al., 2017). The efficiency of UV lamps in healthcare disinfection has also been reported by previous studies for decontamination of healthcare rooms and medical devices (Deverick J. Anderson et al., 2017; Gostine et al., 2016; C. Green et al., 2017; Pegues et al., 2017). Tru-D and Xenex are some of the top leading UV lamp based devices that have carried out clinical trials for determining their ultimate effectiveness and found them to be useful for room terminal decontamination.

Unfortunately, UV lamp possesses serious limitations that makes their use in healthcare highly controversial. Breakage and improper disposal of mercury based devices not only contaminates the environments but often exposes staff, patients and community at high risk of serious illness. Mercury has been linked to various birth defects, a study conducted by National Academies of Science reported that 60,000 children are born in United States (US) alone with neurological problems due to exposure to mercury (EPA, 2002). Illnesses related to nervous, digestive, immune, kidney and lungs are some of many diseases linked with exposure to mercury. To protect human and environment the use of mercury is banned in hospitals in various countries including Denmark (banned in 1994), Sweden (banned in 1991), France (banned in 1999), Norway (banned in 1999), Netherland (banned in 2000) and Malaysia (banned in 2006) (Heal, 2006; Singh, 2016) and the number of countries joining this is continuously increasing. Even mercury based thermometer as well as sphygmomanometers have also been banned (Rustagi & Singh, 2010). In this intense atmosphere, the use of mercury based UV lamps will eventually fade away hence newer alternatives are needed to continue the use of UV light in healthcare.

Unfortunately, these lamps pose many drawbacks which makes their use in the modern era highly unfavorable. The lamps are generally made of fragile quartz material hence the risk of mercury leakage is always present throughout the lifecycle of the lamps (Chevremont et al., 2013; Shin et al., 2016). The lamps require warm-up time before operation therefore instantaneous disinfection cannot be carried out (Chatterley & Linden, 2010). Moreover, they require high voltage and produce a lot of heat during operation (Yoshihiko et al., 2014). The frequent replacement of these lamps is very common due to extremely short lifecycle (Autin et al., 2013; Hölz et al., 2017). Additionally, it can only be used in continuous mode and thus, have to be remained switched on throughout entire disinfection process. These limitations do not allow these lamps to be used in point-of-care (POC) disinfection applications (Chen et al., 2017).

Due to above mentioned limitations the focus has been diverted towards alternative UV sources such as UV-LEDs. These diodes are capable in providing benefits unmatchable to traditional UV lamps. Mercury free, compact size, low power consumption, wide range of wavelengths availability, durability and long life are some of many key advantages of UV-LEDs (Chen et al., 2017; Song et al., 2016; Würtele et al., 2011). Various comparative studies between UV-LEDs and UV lamps have been conducted where UV-LEDs were proven to be as efficient as UV lamps (Beck et al., 2017; Crook et al., 2014; G.-Q. Li et al., 2017; Rattanakul & Oguma, 2018; K. A. Sholtes et al., 2016).

Though the use of solitary UVC, UVB and UVA LEDs has been used for water and food disinfection applications, it has been predominantly limited to UVC region due to the fact that it has the highest inactivation efficiency (Beck et al., 2017; S.-J. Kim et al., 2016). Unfortunately, these studies cannot simply be used as a mean to estimate the effectiveness of UV-LEDs in healthcare disinfection because of the various elements involved in water that can alter the UV transparency considerably thereby changing UV effectiveness. Type of water, turbidity, flow rate, water depth, wavelength of UV light and the presence of nanoparticles in water are some of the elements that can attenuate UV penetration in water (Huff et al., 1965; Johnson et al., 2010; Okpara et al., 2011). Similarly, in healthcare, the use of UVC LEDs has been studied for disinfection of medical devices (G. Messina et al., 2014; Gabriele Messina et al., 2015). However, these studies are very few and did not taken into consideration the effects of varied exposure time and UV doses on inactivation efficiency. Hence more studies are required to fully understand the exact scope of solitary UVC, UVB and UVA LEDs for prospective applications in healthcare disinfection.

Undoubtedly, on its own, the UVC region provides the best inactivation efficiency, however, it can be combined with other UV regions to further enhance the inactivation effectiveness. The coupling effects of UV-LEDs has been investigated in various studies for water disinfection (Beck et al., 2017; Chevremont, Farnet, Coulomb, et al., 2012; A. Green et al., 2018; Nyangaresi et al., 2018; Song, Taghipour, et al., 2019). Studies have shown that by combining UVBC irradiation, higher inactivation can be achieved as compared to UVC alone (Song, Taghipour, et al., 2019). Similarly, in other studies the combinations of UVAC irradiation has been shown to achieve enhanced inactivation than used alone (Chevremont, Farnet, Sergent, et al., 2012).

However, as per the literature review no study was found where simultaneously coupled UVABC irradiation was used to study its effects on microorganisms' inactivation. This combination can be extremely beneficial as DNA damage caused by UVC and UVB can provide rapid inactivation and with the presence of UVA the damage to photolyase can help prevent photoreactivation thereby prolonging bacterial growth delays. Similarly, investigating the effects of simultaneously coupled triadic wavelength (SCTW) UV-LEDs is also important in the context of scanning electron microscope (SEM) and field emission scanning electron microscope (FESEM) image analysis to study the morphological effects caused by coupled UVABC irradiation. As per literature review no research study was found to have investigated the morphological changes post coupled UVABC irradiation. Studying such image analysis can give deep understanding of behavior of UV irradiation and its effects on frequently isolated microorganisms responsible for healthcare acquired infections

(HAIs) which can ultimately help in combating such infectious microorganisms. Another important factor is the photoreactivation which helps in bacteria growth after it has been inactivated by UV light. Hence it is important to study the photoreactivation aspect to determine whether the coupled UVABC irradiation suppresses photoreactivation. There was no study found investigating the photoreactivation effects of coupled UVABC irradiation. Proposed irradiation from SCTW UV-LEDs will not only increase inactivation due to the presence of UVC and UVB irradiation but can also damage enzymes responsible for causing photoreactivation resultantly making the disinfection system that is capable of causing enhanced inactivation as well as reduced photoreactivation hence disinfected medical devices can be used for longer period before being required to re-disinfect.

1.3 Research Objectives

The main objectives of this research work are:

(a) To study the antibacterial activity of solitary UV-LEDs as a function of varied exposure time against Gram-negative and Gram-positive bacteria for applications beyond water treatment.

(b) To investigate the germicidal effectiveness of simultaneously coupled triadic wavelength (SCTW) UV-LEDs using multivariate combinations on microbial inactivation of *Escherichia coli* and *Staphylococcus aureus*.

(c) To analyse the morphological characteristics of *E. coli* and *S. aureus* following post UV exposure from solitary and SCTW UV-LEDs.

(d) To assess the photoreactivation characteristics of SCTW UV-LEDs for prolonged inhibition of bacterial growth.

1.4 Scope of the Research

The scope of this study covers investigation of various solitary and coupled UV-LEDs with various exposure time, UV doses as well as multivariate coupling combinations. The scope is illustrated in Figure 1.1.

(a) This study only focused on *E. coli* (ATCC 11229) and *S. aureus* (ATCC 6538) bacteria to determine the effectiveness of solitary and coupled UV-LEDs. In addition, nutrient agar was used as culture medium for this study

(b) During investigation of solitary UV-LEDs various exposure times were selected to represent all three UV-LEDs as shown in Figure 1.1.

(c) Multivariate coupling combinations were used when investigating the effectiveness of coupled UV-LEDs. The exposure time was limited to 30 and 60 sec for all combinations.

(d) UV irradiance was kept constant while the exposure time and UV dose were varied to study the effects of solitary and coupled UV-LEDs on microorganism inactivation.

(e) The morphological characteristics were studied using SEM and FESEM to observe the damage caused by various UV-LEDs.

(f) Photoreactivation was studied in the context of UVC and coupled UVABC LEDs. The photoreactivation experiments were conducted for 4.5 h at room temperature to minimize temperature effects.

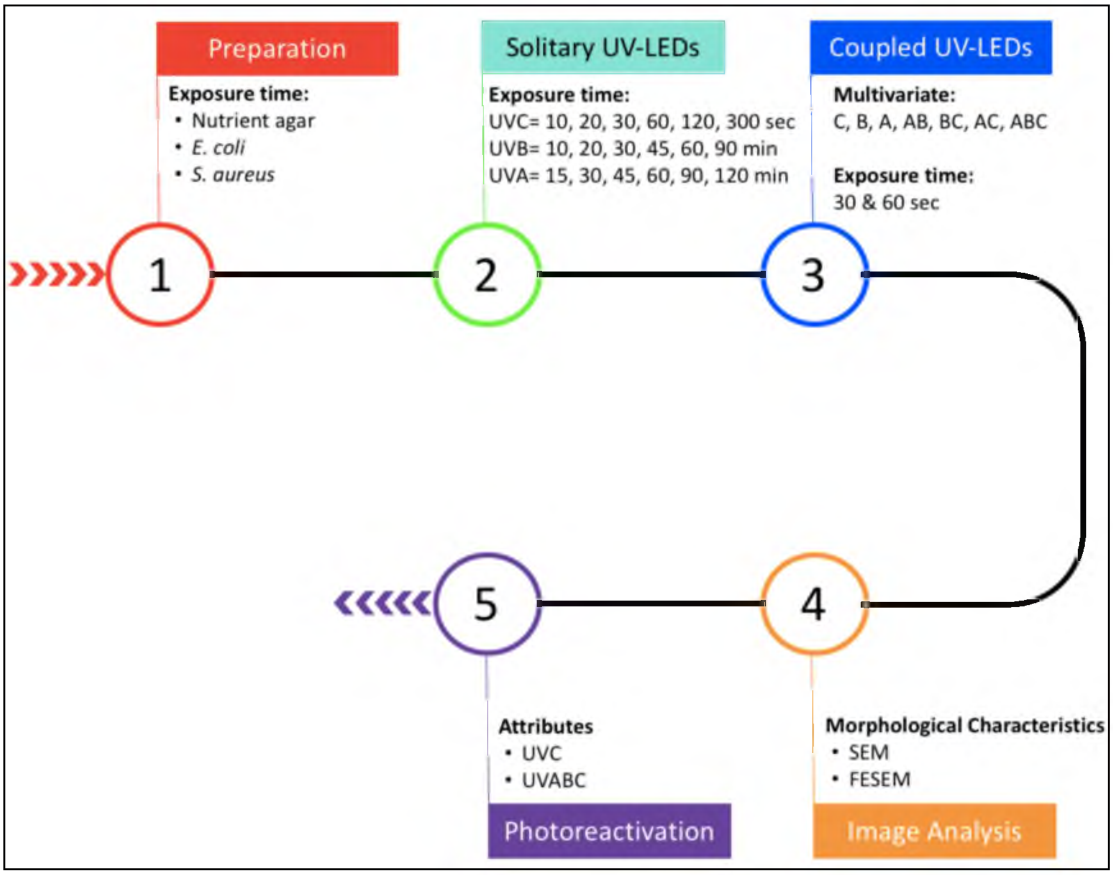

Figure 1.1 Research scope.

The limitations of the proposed system are listed below:

(a) This study only focused on non-pathogenic bacteria due to the fact that the pathogenic are far more dangerous to work it and require higher level of safe working environment.

(b) Wavelengths 276, 311 and 364 nm were used in this research to represent UVC, UVB and UVA irradiation.

(c) The distance between source and sample can affect the overall inactivation efficiency of a system hence it was kept constant at 20 mm to ensure that the entire sample area can be covered by the LED.

(d) Majority of existing UV devices make use of UVC LEDs for inactivation hence during photoreactivation experiments the SCTW UV-LEDs was compared with UVC LED only to determine whether SCTW UV-LEDs could suppress photoreactivation.

1.5 Significance of Research

Prevention of infections has long been a struggle for medical personnel in healthcare. Developing and developed countries alike, HAIs are a serious threat to patient safety in hospitals. HAIs are not only causing morbidity and mortality among patients worldwide but the burden associated with these infections is giving significant rise to excessive healthcare expenditures. Centers for Disease Control and Prevention (CDC) estimates that 1.7 million HAIs cases are reported annually in US alone costing around $ 45 billion in treatment (Klevens et al., 2007; Stone, 2009). Developing countries can have as many as 20 times the HAIs cases reported in developed countries (Padoveze & Fortaleza, 2014; Pittet et al., 2008).

UV-LEDs have been extensively used in disinfection applications. Majority of the research conducted on UV-LEDs has been mainly directed toward water treatment. The use of UV-LEDs for disinfection of medical devices has not been explored greatly. To the best of our knowledge only few studies have been conducted on UV-LEDs for the inactivation of healthcare applications (G. Messina et al., 2014; Gabriele Messina et al., 2015; Gabriele Messina et al., 2018) . These published studies focused on UVC LEDs as the main source of UV light for studying antibacterial properties on *E. coli* and *S. aureus* as target microorganism. Detailed investigation of UVA, B and C LEDs as a function of varied exposure time as well as studying the post UV exposure morphological characteristics has not been explored in the context of healthcare disinfection.

Combining UV-LEDs from various regions can increase inactivation efficiency as reported by previous studies for water treatment. The studies do exists on coupled LEDs combining UVC LEDs with UVB or UVA, however, combination of UVABC regions simultaneously has not been explored. As per the literature review carried out, no study was found on investigating the inactivation efficiency of SCTW UV-LEDs involving UV-LEDs from all UV regions.

SCTW UV-LEDs will produce increased bacterial inactivation for frequently isolated microorganisms in healthcare as compared to UVC. Moreover, simultaneous irradiation from triadic wavelength UV-LEDs will target photolyase enzymes thereby reducing the photoreactivation. Reduced photoreactivation will result in prolonged growth delays of bacteria thereby keeping the device disinfected for longer periods. Moreover, it will reduce the need to disinfect medical devices frequently especially when the medical personnel are extremely busy in dealing with urgent cases.

Use of SCTW UV-LEDs in healthcare disinfection can help reduce HAIs thereby reducing financial burden substantially. Moreover, it can also help in reducing hospital stay time resultantly improving the quality of life and preventing morbidity and mortality. This environmentally friendly disinfection can minimize the use of mercury based UV device that are being banned from hospitals due to their hazardous contents.

This methods can provide semi-automatic disinfection, where the assistance from medical staff is not needed, which could allow medical personnel to attend to more urgent mattes such as taking care of patients and giving them more attention. The scope of this method can be extended from medical device disinfection to disinfection of various healthcare related products such as laptops, keyboard, phone, medical surfaces and environment. This system can provide staff with flexibility to perform point-of-care disinfection especially in emergency rooms and in operation theatres. The method can be implemented in disinfection of various applications such as treating water and foodborne pathogens.

1.6 Thesis Organization

The thesis consists of five chapters.

Chapter 1 focuses on the background of the research as well as presenting concrete evidence to justify the purpose of studying the effectiveness of solitary and SCTW UV-LEDs. The chapter further contains objectives, scope of the study and significance of the study. Thesis organization covers a brief summary of its contents.

Chapter 2 plays a pivotal role in understanding the existing studies thereby providing comprehensive literature review on UV-LEDs and its effectiveness in various disinfection applications. An extensive review of the various configuration of individual as well as coupled UV-LEDs has been discussed in this chapter. Moreover, comparison between different types of healthcare disinfection methods such as traditional disinfection, UV lamps, UV-LEDs has been covered in this chapter. Moreover, theoretical background such as fundamentals of UV light, its mechanism of disinfection and reactivation have also been explored.

Chapter 3 discusses all the materials and methods used during the investigation of this study. The selection of UV-LEDs and their spectral responses using spectrometer are presented. The design and development of UV-LEDs driver circuits and experimental setup for UV exposure are also included in this chapter. The procedures undertook for preparation of bacterial samples such as saline, ethanol, inoculation and bacterial suspension are discussed along with the steps required to prepare samples for morphological analysis. The configuration of UV-LEDs including various combination of SCTW UV-LEDs are also presented. The selection of various UV irradiance instruments along with their spectral responses are also shown in this chapter.

Chapter 4 presents experimental results obtained by applying methods disclosed in chapter 3. The effectiveness of solitary UV-LEDs as a function of varied exposure time for the inactivation of *E. coli* and *S. aureus* has also been discussed. Moreover, under constant UV fluence, the effectiveness of solitary UV-LEDs for antibacterial activities are also shown. A multivariate approach is further presented that observe the performance of solitary and simultaneously coupled UV-LEDs under higher concentration of bacterial suspension. Various combination of SCTW UV-LEDs have also been evaluated as a function of varied exposure time to study enhanced microbial inactivation performance under coupled UV-LEDs. Physical damaged caused by multivariate UV-LEDs has been analysed by studying the morphological characteristics of microorganisms. Finally, the results obtained from photoreactivation is presented in this chapter highlighting the efficiency of UV-LEDs to prolonged growth delay.

Chapter 5 concludes the overall research work and provides limitations and recommendations for future work.

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Appendix A List of Publication

(a) Sameen Ahmed Malik, Swee, T. T., Malek, N. A. N. N., & Hou, T. J. (2019). Microorganisms Disinfection Using Ultraviolet Light Emitting Diodes. In T. T. Swee (Ed.), *BIOMEDICAL RESEARCH AND APPLICATION VOLUME II* (Vol. II, pp. 55-75). Malaysia: Penerbit UTM Press.

(b) Sameen Ahmed Malik, Tan Tian Swee, Nik Ahmad Nizam Nik Malek, Azli Yahya, Takahiro Emoto, Masatake Akutagawa, . . . Hiik, K. L. C. (2019) 'Effectiveness of visible and ultraviolet light emitting diodes for inactivation of Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli: A comparative study' *Malaysian Journal of Fundamental and Applied Sciences*, 15(4), pp. 572-576.

(c) Sameen Ahmed Malik, Tan Tian Swee, Nik Ahmad Nizam Nik Malek, Mohammed Rafiq Abdul Kadir, Leong Kah Meng, Tan Jia Hou, . . . Akutagaw, M. (2017, 6-7 September). *Effects of ultraviolet light-emitting diodes (UVA-LEDs) irradiation on Escherichia coli for inactivation of microorganisms.* Paper presented at the International Medical Device and Technology Conference (iMEDiTEC 2017), Johor Bharu, Johor.

(d) Sameen Ahmed Malik, Tan Tian Swee, Nik Ahmad Nizam Nik Malek, Mohammed Rafiq Abdul Kadir, Takahiro Emoto, Masatake Akutagawa, . . . Alang, T. A. I. T. (2017) 'Comparison of standard light-emitting diode (LED) and 385 nm ultraviolet A LED (UVA-LED) for disinfection of Escherichia coli' *Malaysian Journal of Fundamental and Applied Sciences*(Special Issue on Medical Device and Technology (IMEDITECH 2017)), pp. 430-43