Decontamination Efficacy of Ultraviolet Radiation against Biofilms of Common Nosocomial Bacteria

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Background: *Ultraviolet radiation (UV) is commonly used to destroy microorganisms in the health-care environment. However, the efficacy of UV radiation against bacteria growing within biofilms has never been studied.*
Objective: *To measure the sterilization effectiveness of UV radiation against common healthcare associated pathogens growing within biofilms.*
Material and Method: *Staphylococcus aureus, Methicillin-resistant S. aureus (MRSA), Streptococcus epidermidis, Escherichia coli, ESBL-producing E. coli, Pseudomonas aeruginosa and Acinetobacter baumannii were cultivated in the Calgary Biofilm Device. Their biofilms were placed 50 cm from the UV lamp within the Biosafety Cabinet. Viability test, crystal violet assay and a scanning electron microscope were used to evaluate the germicidal efficacy.*
Results: *Within 5 minutes, UV radiation could kill S. aureus, MRSA, S. epidermidis, A. baumannii and ESBL-producing E. coli completely while it required 20 minutes and 30 minutes respectively to kill E. coli and P. aeruginosa. However, the amounts of biomass and the ultrastructure between UV-exposed biofilms and controls were not significantly different.*
Conclusion: *UV radiation is effective in inactivating nosocomial pathogens grown within biofilms, but not removing biofilms and EPS. The biofilm of P. aeruginosa was the most durable.*

Keywords: *Biofilms, Nosocomial infections, Ultraviolet radiation, Sterilization*

The growth of bacteria in nature is usually in the form of sessile microcolonies called "biofilms". This growth pattern is created when microorganisms attach to surfaces and aggregate in a self-produced extracellular polymeric substance (EPS)[1], offering protection from various environmental challenges ranging from heavy metal toxicity to host immune response and antimicrobial agents[2]. Bacteria growing within biofilms were found to be more resistant to treatment with antimicrobial agents than planktonic cells of the same species[3].

Biofilms are ubiquitous and have several undesirable impacts in a number of areas. In the body, bacteria growing on biofilms have been recognized as an important cause of several conditions such as catheter-associated infections, infections of prostheses and heart valves, bacterial endocarditis, and infections in people with cystic fibrosis[4]. In healthcare facilities, bacteria can colonize and form biofilms on various areas such as water taps, hand-wash basins and plumbing systems as well as respiratory ventilators and medical devices[5]. With nosocomial infections being a current global problem, an accumulation of data is beginning to point to the role that contaminated surfaces play in environment-to-patient transmission[6]. Several causative agents of nosocomial infections have been found to be associated with biofilms formation. These agents include *Legionella pneumophilia*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Aeromonas* spp.[7,8]. Other common bacteria such as *Staphylococcus aureus* and coagulase-negative staphylococci have been found colonizing indwelling catheters and medical devices[9]. Such biofilms serve as a possible source of transmission, contributing to the increasing incidence of hospital-acquired infections.

As infection rates in healthcare facilities are a major patient safety concern, several methods have been suggested for minimizing environmental infection, and one of these is Ultraviolet (UV) radiation[10]. With its germicidal activity, UV radiation has been used for the control of microorganisms in operating rooms, patient isolation rooms and biosafety cabinets. Its application is usually for the destruction of airborne organisms or microorganisms on surfaces;

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however, its germicidal effectiveness can be hindered by organic matter such as soil and, perhaps, biofilms[11].

While hospitals generally have sanitation protocols regarding surface bio-decontamination, they are not created specifically to deal with biofilms. This study is thus conducted to measure the efficacy of UV radiation against common pathogens associated with health-care infections when they grow within the biofilms.

Material and Method
Bacterial isolates and cultivation

Tested bacterial isolates included five standard strains: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 15305, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and two clinical isolates: methicillin-resistant *S. aureus* (MRSA), and extended spectrum beta-lactamase (ESBL)-producing *E. coli*. All standard strains were purchased from Department of Medical Sciences Thailand (DMST), Thailand, and the clinical isolates were from Thammasat Hospital. All isolates except *P. aeruginosa* were grown in tryptic soy broth supplemented with 2% glucose. *P. aeruginosa* isolate was grown in cation-adjusted Muller Hinton broth.

Biofilm cultivation

Bacterial biofilms were cultivated using the Calgary Biofilm Device (CBD) as previously described[12]. In brief, 200 uL of each tested bacterial inoculum were suspended in a 96-well plate that was covered by a lid that had 96 pegs. Plates were incubated without shaking for one hour to allow bacterial cells to attach to the pegs' surface. They were then incubated at 37°C with shaking at 40 rpm for 24 hours. Pegs were washed with 0.9% saline solution to remove unattached cells prior to each experiment.

Assessment of UV efficacy
Viability plate count

Biofilms growing on pegs were placed in the Biosafety cabinet class II with UV lamp (SafeFast™) 50 centimeters away from the UV light source. Biofilms were tested against UV radiation at different time points including 1, 5, 10, 15, 20, 30 minutes and 1, 2 and 3 hours. After each time point, the viability of biofilms on each peg was assessed. Briefly, pegs were placed in a 96-well plate containing 0.9% saline solution, followed by 5-minute ultrasonication two times and shaking at 600 rpm for 5 minutes. Bacterial solution was serially diluted and plated on nutrient agars. Parallel pegs with biofilms that were covered with aluminum foil were used as UV-non-exposed control.

Crystal violet assay

The biomass (both living and dead cells and extracellular polymeric matrix) of biofilms was assessed using a crystal violet (CV) assay. After being treated with UV for 30 minutes, pegs with biofilms were immersed in CV for 5 hours. After this, the unbound CV was removed by washing, and the biofilm-bound dye was released in acetone-ethanol solution. The absorbance was measured at OD 595 nm. UV-non-exposed biofilms were used as control. Tests were done in triplicate.

Scanning electron microscopy (SEM)

The ultra-structure of biofilms of *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* were visualized under SEM. After being exposed to UV for 30 minutes, each peg was fixed in 0.2M cocadylate buffer containing 3% glutaraldehyde and 0.15% alcian blue for 3 hours. Pegs were then washed out with buffer followed by dehydration in serial-dilution alcohol, and then immersed in hexamethyldisilazane (HMDS) for 5 minutes before being dried overnight in a desiccator. Each peg was then coated with gold film using a sputter coater (SC7640, Polaron-Fisons) before visualization under a SEM (JEOL, model JSM-5410LV).

Statistical analysis

A standard t-test was applied to analyze the amount of biomass as stained by CV between UV-exposed and non-exposed samples. The *p*-value of <0.05 were considered to be significant.

Results
Biofilm cultivation

The CBD created conditions favorable to the growth of bacteria in the biofilms mode. Pegs provided a surface for bacterial cells to attach to while surrounded by broth that continuously flowed around it. Within 24 hours, biofilms were observed as shown in Fig. 1.

UV efficacy against viability of biofilms

The germicidal efficacy of UV radiation against bacterial biofilms was measured using viability count. The average viable bacteria per peg before UV exposure was 6.5×10^5 cells. *P. aeruginosa* had the highest number of cells attaching to the peg surface
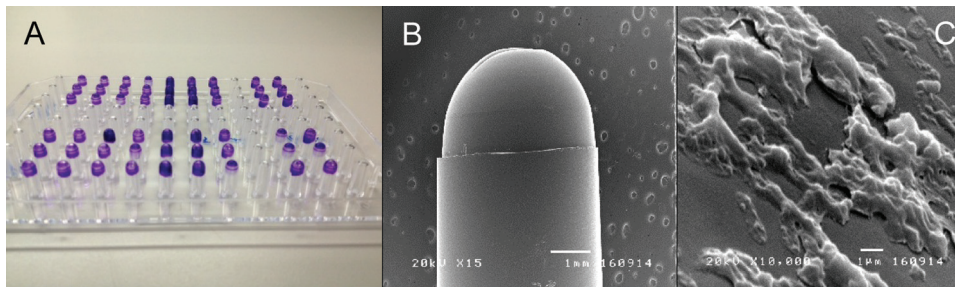
Fig. 1 Calgary Biofilm Device. (A) Biofilms grown on pegs and stained by crystal violet, (B) Scanning electron micrograph of a peg (original magnification x15), (C) Scanning electron micrograph of *P. aeruginosa* biofilms (original magnification x10,000). Note cells attached to surface and were covered by EPS.

(2.1x10^6 cells). Biofilms of *A. baumannii* showed no growth after 1 minute of UV exposure while biofilms of *S. aureus*, *S. epidermidis*, MRSA and ESBL-producing *E. coli* took 5 minutes and biofilms of *E. coli* took 20 minutes (Fig. 2). *P. aeruginosa* had the highest number of cells recovered from peg-attached biofilms, which required up to 30 minutes of UV exposure to inhibit the growth completely of this isolate (Fig. 2).

Biomass quantification using CV assay

The total biomass, which included bacterial cells (both living and dead) and extracellular polymeric matrix, was measured from the peg samples obtained after 30 minutes of UV exposure. There was no significant difference between biomass of biofilms of each isolate at 0 minute and biofilms both exposed and not exposed to the UV light (Fig. 3).

Scanning electron microscopy

The detailed structure of biofilms was revealed under SEM (Fig. 4). All isolates showed biofilms in which cells attached to the surface. EPS appeared as a matrix supporting cellular attachment in *S. aureus* (Fig. 4A) and *S. epidermidis* (Fig. 4C) or covering bacterial cells as found in *E. coli* (Fig. 4E) and *P. aeruginosa* (Fig. 4G). *P. aeruginosa* appeared to produce more EPS than other isolates. Overall, the cellular structure of UV-treated and control samples appeared to be similar, but the amount of EPS seemed to be lesser in the UV-treated samples (especially in *S. epidermidis* and *E. coli*) than the control samples.

Discussion

An increasing body of evidence indicates that contamination of the environment contributes to hospital-associated infections[6]. This environment contamination can exist in air-borne form, be
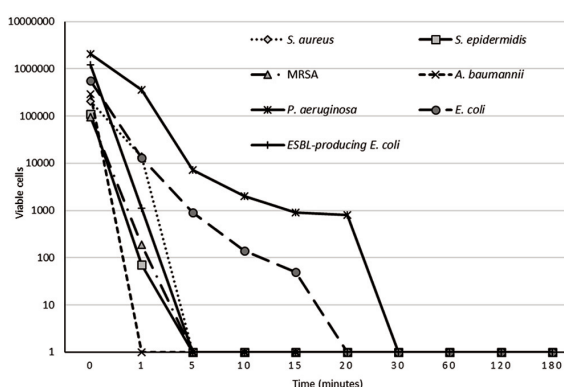

Fig. 2 UV efficacy against the viability of biofilms. Biofilms of *A. baumannii* showed no growth after 1 minute of UV exposure. *S. aureus*, *S. epidermidis*, MRSA, ESBL-producing *E. coli* showed no growth after 5 minutes of UV exposure. *E. coli* and *P. aeruginosa* showed no growth after 20 and 30 minutes of UV exposure, respectively. Each experiment was performed in duplicate on two separate occasions.
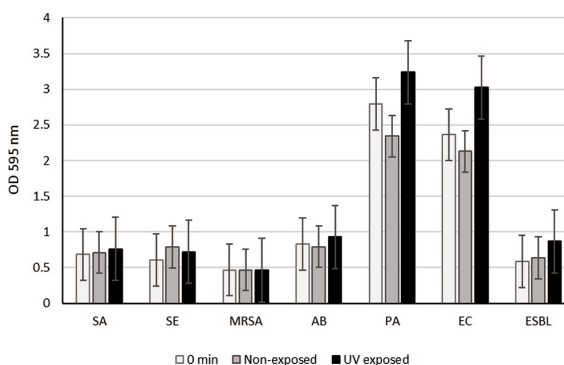

Fig. 3 Crystal-violet stained biomasses of UV-exposed and UV-non-exposed samples. Within each isolate, there was no significant difference among the total biomasses of biofilms measured at 0 minute and 30 minutes after UV exposure and non-exposure.
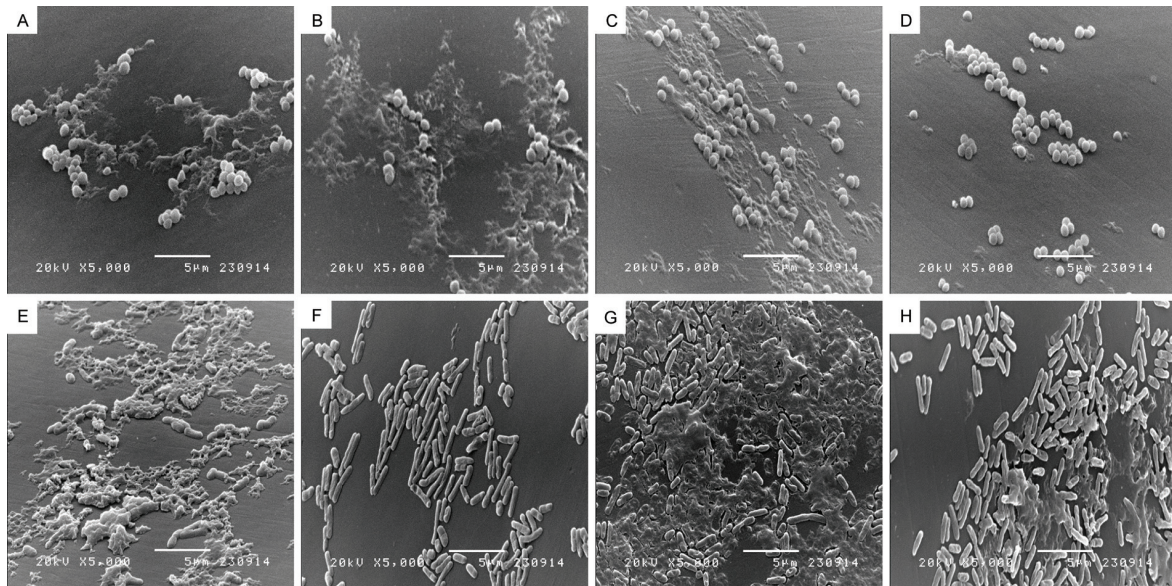
Fig. 4 Scanning electron micrographs of (A) *S. aureus* no-UV-exposure, (B) *S. aureus* with 30-minutes UV exposure, (C) *S. epidermidis* no-UV-exposure, (D) *S. epidermidis* with 30-minutes UV exposure, (E) *E. coli* no-UV-exposure, (F) *E. coli* with 30-minutes UV exposure, (G) *P. aeruginosa* no-UV-exposure, (H) *P. aeruginosa* with 30-minutes UV exposure. All images were x5,000 magnification. Biofilms can be seen as surface-attached cells which are surrounded by an EPS matrix. Overall, the cellular structure of UV-treated and control samples appeared to be similar, but the amount of EPS seemed to be less in the UV-treated samples (especially in *S. epidermidis* and *E. coli*) than in the control samples.

water-borne or manifest itself as contamination of the inanimate objects around patients. It has been suggested that surface contamination is likely to exist in the form of biofilms[13]. Indeed, a number of hospital items from one intensive care unit (including curtain, door, washbasin rubber and reagent bucket) were found to be colonized by biofilms that still contained viable pathogens[13]. Various pathogens such as *Legionella* spp., *P. aeruginosa*, *Acinetobacter* spp., and *Aeromonas* spp., have also been shown to be associated with biofilms in the hospital environments. Biofilms from one of the most common pathogens associated with healthcare infections, *P. aeruginosa*, found in water taps in hospitals, was recognized as an ideal reservoir for environment-to-patient transmission[14].

Infection rates in healthcare facilities are a major patient safety concern. As biofilms contribute to hospital-associated infections, efforts to improve environmental hygiene should be encouraged, at the very least, effective cleaning and disinfecting surfaces in healthcare facilities. Currently, sterilants used for room decontamination include formaldehyde and hydrogen peroxide vapor[11]. UV radiation has also

been suggested as an alternative method for the control of microorganisms[10,11]. It has several potential applications in the healthcare environments, including being used for controlling contamination within operation rooms and isolation rooms[15]. However, there are limited available data concerning its true efficacy against biofilms.

In the present study, the CBD was chosen for growing bacteria in the biofilm mode. The pegs (part of the CBD) were shown to support the biofilm proliferation. As seen in Fig. 1, *P. aeruginosa* cells irreversibly attached to the surface and were encased by EPS. The UV radiation was shown to be able to kill bacterial cells completely with a maximum exposure time of 30 minutes (Fig. 2). *P. aeruginosa* and *E. coli* were found to require longer periods of exposure (30 minutes and 20 minutes, respectively) compared to *S. aureus*, *S. epidermidis*, MRSA, *A. baumannii*, and ESBL-producing *E. coli*. This correlates with the amount of biofilms they produced: *P. aeruginosa* and *E. coli* produced the highest amount of biofilms compared to other isolates (Fig. 3). These findings suggest that biofilms may play role as a protection from destruction by UV.

The CV assay showed that there was no significant difference in the amount of biomass between UV-exposed or non-exposed samples. This is because the biomass contained both living and dead cells as well as EPS. As expected, although UV radiation can completely kill cells within the biofilms, it cannot remove the biofilms from surfaces. We further looked into the ultra-structure of biofilms after UV exposure, using SEM. Cellular structures of both samples were unchanged, although it could be noticed that in *S. epidermidis* and *E. coli*, the amount of EPS in UV-exposed samples was less than for UV-non-exposures. However, this SEM finding did not correlate with the amount of biomasses as stained by CV. The biomass of UV-exposed *E. coli* was lower (not statistically significant) than in the UV-non-exposed sample (Fig. 3). It is possible that EPS became dry and exfoliated during the sample preparation process for SEM.

The biofilm mode of growth is normally found in nature-in both the environment and the human body. In this study, we selected common bacterial pathogens associated with healthcare infections and grew them in biofilms, in order to represent the real contamination burden found in the hospital environment. Overall, the present study demonstrates that UV radiation is effective in destroying bacteria growing in the form of biofilms. It should be noted that all experiments were conducted within the biosafety cabinet, instead of in hospital rooms, as it is not possible to expose these notorious pathogens to the environment according to National Biosafety Regulation.

The application of UV radiation systems in a hospital setting has been previously reported[16]. Rutala et al showed that UV radiation could decontaminate more than 99.9% of MRSA within isolation rooms after they were occupied by an infected patient[16]. A study using a simulated health-care room also showed that UV radiation reduced up to 98% of aerosolized *Mycobacterium* spp. and up to 80% of *Bacillus subtilis* spores[17]. Moreover, in the water industry, UV disinfection technology has long been used to control water quality. It is effective against waterborne pathogens including bacteria (such as *E. coli*, *Salmonella* Typhi, *Vibrio cholerae*, *Campylobacter jejuni* and *L. pneumophila*), viruses (such as Hepatitis A virus, Calicivirus, Rotavirus, and Poliovirus) and protozoa (such as *Cryptosporidium parvum*, *Giardia lamblia*, and *Acanthamoeba* spp.)[18]. Findings from this study emphasize the efficacy of UV radiation against common pathogens, which are the leading causes of healthcare-associated infections. Although the present study and those mentioned in this paper show the benefit of UV radiation, the current view on its applicability indicates that UV germicidal irradiation cannot be applied as a primary intervention for infection control. However, it can be considered for use in conjunction with other well-established methods, such as appropriate heating, ventilating, and air-conditioning (HAVC) systems for air cleaning[19] or the use of liquid chemical disinfectants for surface disinfection[20].

Conclusion

The present study shows that UV radiation is effective in destroying common nosocomial bacterial pathogens grown within biofilms, but not in removing biofilms from surfaces. Bacteria with greater biofilm-formation capacity (*P. aeruginosa* and *E. coli*) require longer periods of UV-exposure time. UV germicidal irradiation may provide an enhanced method of surface disinfection especially when used in combination with conventional cleaning methods.

What is already known on this topic?

Biofilms contaminating hospital environments are known as a potential source of transmission to patients. UV radiation has been used as one of the methods for control of hospital infections. However, there are limited available data concerning the true efficacy of this method against biofilms especially those of common pathogens causing healthcare-associated infections.

What this study adds?

This study shows that UV radiation can kill common nosocomial bacteria growing within biofilms, but not remove biofilms on surfaces. Up to 30 minutes were required to kill viable cells of bacteria (*P. aeruginosa*) completely, which produced the highest amount of biofilms.

Potential conflict of interest

None.

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ประสิทธิภาพของรังสีอัลตราไวโอเลตต่อการทำลายเชื้อแบคทีเรียก่อโรคที่พบบ่อยในโรงพยาบาลซึ่งเจริญในรูปแบบไบโอฟิล์ม

พลวัฒน์ ติ่งเพ็ชร, รัตนา เตียงทิพย์, สุมาลี คอนโด

ภูมิหลัง: รังสีอัลตราไวโอเลตถูกนำมาใช้ในการกำจัดเชื้อก่อโรคที่ปนเปื้อนในสิ่งแวดล้อมภายในโรงพยาบาล อย่างไรก็ตามยังไม่มีข้อมูลเกี่ยวกับประสิทธิภาพของรังสีอัลตราไวโอเลตต่อการทำลายเชื้อที่เจริญในไบโอฟิล์ม ซึ่งเป็นรูปแบบการเจริญของเชื้อที่พบได้ในสิ่งแวดล้อม

วัตถุประสงค์: เพื่อประเมินประสิทธิภาพของการใช้รังสีอัลตราไวโอเลตในการกำจัดเชื้อที่เจริญในรูปแบบไบโอฟิล์ม โดยทดสอบในเชื้อแบคทีเรียที่เป็นสาเหตุของภาวะการติดเชื้อในโรงพยาบาล

วัสดุและวิธีการ: เพาะเลี้ยงเชื้อ *Staphylococcus aureus, Methicillin-resistant S. aureus (MRSA), Streptococcus epidermidis, Escherichia coli, ESBL-producing E. coli, Pseudomonas aeruginosa* และ *Acinetobacter baumannii* ให้เจริญในรูปแบบไบโอฟิล์มใน *Calgary Biofilm Device* แล้วทำการทดสอบไบโอฟิล์มกับรังสีอัลตราไวโอเลตที่เวลาต่างๆ กัน เพื่อวัดปริมาณเชื้อที่มีชีวิต นอกจากนั้นไบโอฟิล์มที่ผ่านการฉายรังสีเป็นเวลา 30 นาที ได้ถูกนำมาวัดปริมาณโดยการย้อมด้วย *crystal violet* และตรวจดูโครงสร้างภายใต้กล้องจุลทรรศน์อิเล็กตรอน เปรียบเทียบกับไบโอฟิล์มที่ไม่ได้รับการฉายรังสี

ผลการศึกษา: เชื้อ *A. baumannii* ถูกทำลายทั้งหมดที่ 1 นาที และเชื้อ *S. aureus, MRSA, S. epidermidis, ESBL-producing E. coli* ถูกทำลายทั้งหมดที่ 5 นาที ส่วนเชื้อ *E. coli* และ *P. aeruginosa* ต้องใช้เวลาถึง 20 และ 30 นาที จึงจะถูกทำลายทั้งหมด อย่างไรก็ตามปริมาณไบโอฟิล์มจากการวัดด้วย *crystal violet* และลักษณะของไบโอฟิล์มภายใต้กล้องจุลทรรศน์อิเล็กตรอนระหว่างตัวอย่างที่ได้รับการฉายรังสีไม่แตกต่างกับตัวอย่างที่ไม่ได้รับการฉายรังสี

สรุป: รังสีอัลตราไวโอเลตมีประสิทธิภาพในการทำลายเชื้อในไบโอฟิล์ม แต่ไม่ได้กำจัดไบโอฟิล์มให้หมดไป ไบโอฟิล์มของ *P. aeruginosa* มีความทนทานต่อการทำลายมากที่สุด