Report

Germicidal effect of 222-nm UVC on *Staphylococcus aureus* and *Bacillus cereus* endospores adhered to fabrics

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Abstract: We investigated the bactericidal effect of 222-nm UVC light on methicillin-resistant *Staphylococcus aureus* and *Bacillus cereus* endospores adhered to cotton cloths, cotton polyester cloths, and cotton woven towels used in hospital linen. Irradiation at 210 mJ/cm^2 of the 222-nm UVC light inactivated both bacteria on the cotton cloths, but not on the cotton polyester cloths.

Keywords: *Staphylococcus aureus, Bacillus cereus* endospores, fabric disinfection, ultraviolet light

1. Introduction

Healthcare-associated infection (HAI) leads to longer hospitalization and higher mortality rates in hospitalized patients[1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a worldwide major problem in HAI[2]. A main route of its transmission is contact infection through linens. Various types of fabrics such as cotton and cotton polyester are used for clothing in hospitalized patients, and towels and bed sheets in hospital facilities. Linens are easily contaminated with droplets, blood, and excrement of patients with infectious diseases[3,4]. Therefore, disinfection of both linens and hospital environment are crucial.

Various disinfecting techniques have been developed and widely used for disinfection of materials and environment. Irradiation with ultraviolet light, especially 254-nm ultraviolet C (UVC) light is often used for disinfection in healthcare settings. The germicidal effect of 254-nm UVC is mainly related to the absorption of UV by nucleic acid components. This wavelength is considered to be harmful to the dermis and cornea and is limited to unattended use. In contrast, 222-nm UVC has a germicidal effect equal to or greater than 254-nm UVC against microbial pathogens[5], and less toxic to mammalian cells[6]. Especially, the ability of 222-nm UVC to inactivate bacterial endospores is superior to that of 254-nm UVC[5].

The bactericidal effect of 222-nm UVC light on MRSA and *Bacillus cereus* endospores, which are resistant to most disinfectants and cause bloodstream infection, was investigated on different types of cloths.

2. Materials and methods

2.1 Fabrics

Commercially available 100% cotton cloth, polyester cotton cloth (65% cotton-35% polyester blend), and woven towels (100% cotton) were used. They are commonly used for hospital clothing and sheet. All fabrics were cut to 5 x 5 cm and sterilized with a steam iron before use.

2.2 Bacterial strains and culture conditions

S. aureus MRSA strain USA300 (ATCC BAA1516) was grown in tryptic soy broth (BD diagnosis Systems, Sparks, MD, USA) at 37°C for 16 h. A clinical isolate of *B. cereus* was cultured in tryptic soy agar (TSA; BD Diagnosis Systems) at 37°C for 14 h, left at room temperature for at least 7 days, and then colonies were scraped, suspended in sterile phosphate-buffered saline (PBS). The suspension was treated at 80°C for 20 min in a water bath to kill vegetative cells. They were resuspended in sterile PBS and adjusted to 1 x 10^7 CFU/mL for MRSA and 1 x 10^6 CFU/mL for *B. cereus* endospores.

2.3 UVC light source

The SafeZone UVC device (Ushio Inc. Tokyo, Japan), which emits 222 nm, combines a Crypton-Chloride (Kr-Cl) excimer lamp with an optical filter, limiting the spectrum of light emitted in the 200-230 nm range. It emits 35 mJ/cm^2 of 222-nm UVC with irradiation for 5 seconds at a distance of 10 mm from the radiation window.

2.4 UVC light irradiation on fabrics

MRSA or *B. cereus* endospores suspension (0.25 mL each) was dropped onto the center of fabric. Fabrics were dried in a safety cabinet at room temperature for 30 min. Both sides of fabrics were irradiated with 222-nm UVC at total doses of 0, 70

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and 210 mJ/cm^2. After irradiation, adhering bacteria on fabric pieces were detached with 10 mL of PBS containing 0.1% Tween 20 by using a mixer (CM-1000, EYELA, Tokyo, Japan) at 2000 rpm for 3 min. The wash-off solution was diluted 10-fold with PBS and inoculated into TSA. Colonies were counted after culturing at 37°C for 24 h.

2.5 Statistical analysis

Data were expressed as mean ± standard deviation. Statistical analysis was performed by Student's t-test. P<0.05 was considered to indicate a significant difference.

3. Results

Both sides of MRSA-adhering cotton cloth, cotton polyester cloth, and woven towel were irradiated with 0, 70 and 210 mJ/cm^2. The number of colonies decreased in proportion to the irradiation dose of 222-nm UVC. Colonies were reduced to undetectable levels by irradiation at 70 mJ/cm^2 for cotton cloth and woven towel. In contrast, colonies were detected on cotton polyester cloth even after irradiation at 210 mJ/cm^2.

When *B. cereus* endospores were used, colonies on cotton cloth decreased at 70 mJ/cm^2 and to an undetectable level at 210 mJ/cm^2. In contrast, cotton polyester cloth and woven towels showed no reduction in colonies even at 210 mJ/cm^2 (Fig. 1).

4. Discussions

HAI causative agents, such as MRSA and *B. cereus* endospores, are adhered to hospital linen[2,7]. Contamination of linen causes contact infection, and spread of pathogens in the hospital is a difficult problem[8].

Our results demonstrated that the effect of 222-nm UVC differs depending on the bacterial species and types of the fabrics. Irradiation of 222-nm UVC is effective in killing MRSA as reported previously[6]. However, MRSA on the cotton polyester cloth was failed to be killed completely even at 210 mJ/cm^2, different from the cloth and cotton woven towel (Fig. 1). The result indicates that efficacy of 222-nm UVC irradiation is varied according to types of fabrics.

We have previously demonstrated that the 222-nm UVC has a higher germicidal effect on bacterial endospores than the conventional 254-nm UVC[5]. In this study, the germicidal effect of 222-nm UVC irradiation on *B. cereus* endospores was demonstrated at 70 mJ/cm^2 and completed at 210 mJ/cm^2 on cotton cloth. However, the irradiation failed to reduce endospores in cotton polyester cloth and woven towel even at 210 mJ/cm^2 (Fig. 1). Although both cloth and woven towel are made of cotton, the 222-nm UVC transmittance was 25.4% for cotton, 2.4% for woven towel and 2.0% for cotton polyester cloth, respectively, suggesting that the germicidal effect of the 222-nm UVC depends on the weave of fabrics.

Safety of the 222-nm UVC light to human body has been confirmed because this wavelength shows no DNA-damaging effect on human tissues such as skin, different from 254-nm UVC light[6,9]. The recent study demonstrated that the 222-nm UVC light is available for decontamination of environment[10]. Therefore, the light may be applicable to disinfection of hospital linens. However, it should be cautious about types and weave of fabrics and targeted pathogens.

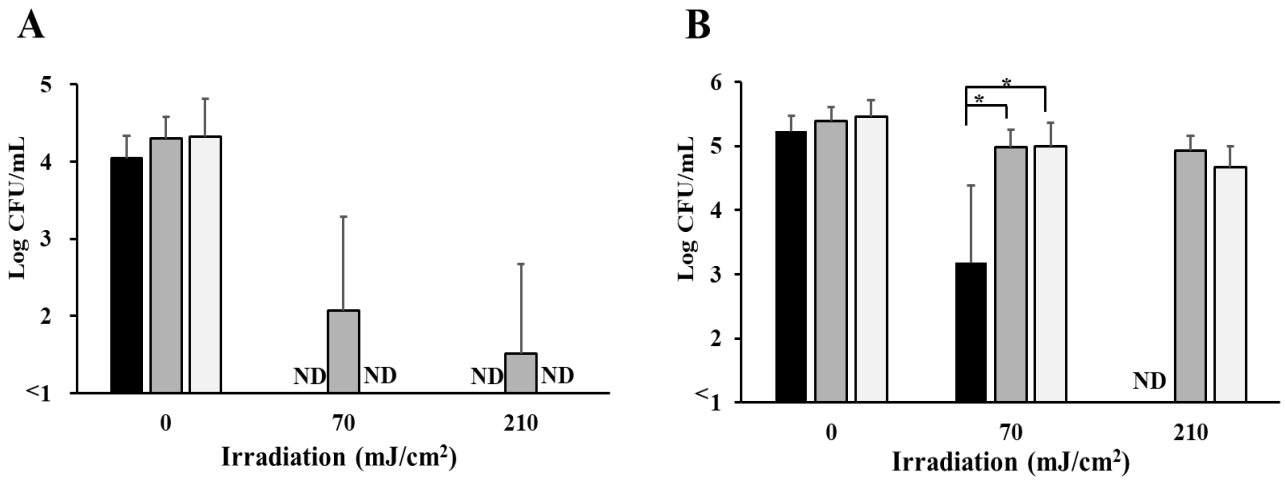

Fig. 1 Effect of 222-nm UVC single-sided irradiation on fabrics adhered with MRSA (A) and *B. cereus* endospores (B).
MRSA (2.5 x 10^6 CFU) or *B. cereus* endospores (2.5 x 10^5 CFU) was adhered to each fabric and irradiated with different doses of UVC 222-nm. The numbers of remained viable cells were determined by colony formation. The black bar is cotton fabric, the gray bar is cotton polyester fabric, and the white bar is woven towel. *P < 0.01, ND; not detected.

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【報告】

布地に付着した黄色ブドウ球菌およびセレウス菌芽胞に対する 222-nm UVC の殺菌効果

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要旨：病院用リネンに使用される綿布，綿ポリエステル布，綿織りタオルに付着したメチシリン耐性黄色ブドウ球菌およびセレウス菌芽胞に対する 222-nm UVC の殺菌効果を検討した． 210 mJ/cm2 の 222-nm UVC 照射により，どちらの細菌についても綿布上では不活化されたが，綿ポリエステル布では不活化されなかった．

キーワード：黄色ブドウ球菌，セレウス菌芽胞，布の殺菌，紫外線

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