Journal of Hospital Infection 105 (2020) 459–467

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Ultraviolet C light with wavelength of 222 nm inactivates a wide spectrum of microbial pathogens

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ARTICLE INFO

Article history:
Received 11 December 2019
Accepted 23 March 2020
Available online 31 March 2020

Keywords:
222-nm UVC light
Germicidal effect
Microbial pathogens

SUMMARY:

Background: Ultraviolet C (UVC) light has been used to inactivate several pathogens. Unlike conventional 254-nm UVC, 222-nm UVC is harmless to mammalian cells.
Aim: To investigate the disinfection efficacy of 222-nm UVC against human pathogens which are commonly found in the environment and healthcare facilities.
Methodology: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica* subsp. serovar Typhimurium, *Campylobacter jejuni*, *Bacillus cereus* (vegetative cells and endospores), *Clostridium sporogenes* (vegetative cells and endospores), *Clostoridioides difficile* (endospores), *Candida albicans* (yeast), *Aspergillus niger* (hyphae and spores), *Trichophyton rubrum* (hyphae and spores), feline calicivirus and influenza A virus were irradiated with 222-nm UVC at various doses. The remaining live bacterial and fungal cells, and viral infectivity were evaluated. The germicidal effect of 222-nm UVC was compared with that of 254-nm UVC.
Results: UVC with a wavelength of 222 nm had a potent germicidal effect on vegetative bacterial cells, yeast and viruses, and was as efficient as 245-nm UVC. In addition, 222-nm UVC had a more potent germicidal effect on bacterial endospores compared with 254-nm UVC. The fungicidal effect of 222-nm UVC against fungal spores and hyphae was weaker than that of 254-nm UVC.
Conclusions: UVC with a wavelength of 222 nm was able to inactivate a wide spectrum of microbial pathogens. In comparison with 254-nm UVC, the germicidal effect of 222-nm UVC for fungal hyphae and spores was low, but 222-nm UVC had a strong germicidal effect on bacterial endospores.

Introduction

Several disinfectant techniques have been developed and are widely used to kill and/or inactivate pathogenic microorganisms in the environment [1]. Irradiation with ultraviolet (UV) light is one of the most common techniques, and elicits a

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https://doi.org/10.1016/j.jhin.2020.03.030

highly germicidal effect [2]. The mechanism is mainly related to the absorption of UV by nucleic acid components. DNA/RNA damage and dimers formed between adjacent thymines in the polynucleotide chains of DNA are the primary photoproducts of UV-exposed DNA [3]. It has also been reported that the risk of infection in operating rooms at healthcare facilities is reduced by 254-nm UVC irradiation [4,5]. However, 254-nm UVC is able to induce cyclobutyl pyrimidine dimers in mammalian cells and cause epidermal hyperplasia [6,7]. Therefore, 254-nm UVC is considered to be hazardous to human health as it is a potential cause of dermatitis and skin cancer.

To reduce the hazardous effect of conventional 254-nm UVC, a krypton-chloride (Kr–Cl) excimer lamp was developed to generate UVC with a shorter wavelength [8]. It has been reported that 222-nm UVC is harmless to mammalian cells [9–12]. When mice were chronically irradiated with 450 mJ/cm^2 of 254-nm UVC, sunburn and desquamation were shown on the dorsal skin. Histological analysis demonstrated that 254-nm UVC also induced parakeratosis, epidermal hyperplasia, intracellular oedema and mitotic figures in the stratum spinosum. In contrast, these macroscopic appearances and histological abnormalities were not found in the dorsal skin of mice irradiated with 222-nm UVC [11]. In addition to the germicidal mechanism of UVC which is mainly related to absorption of the light by nucleic acid components, 222-nm UVC is also well absorbed by peptide bonds and the amino acids, tryptophan and tyrosine [13,14]. Thus, 222-nm UVC is expected to be absorbed by proteins and/or other biomolecules in the stratum corneum layer before reaching nuclei within the epidermal cells of mouse skin. This suggests that the Kr–Cl excimer lamp is more suitable than the conventional mercury lamp for use in several applications, especially in the residential setting.

It has been reported that 222-nm UVC has a germicidal effect against some pathogenic bacteria [12,15,16]. However, more evidence regarding the effect of 222-nm UVC on several other pathogens is required, especially endospore-forming bacteria and fungi which are highly stable and spread widely in the environment. In order to apply 222-nm UVC as a tool to control the spread of infectious diseases, this study investigated the disinfection efficacy of 222-nm UVC against various important human pathogens commonly found in the environment and healthcare facilities. Unlike previous studies, the germicidal effect of 222-nm UVC on bacterial and fungal spores was also examined in this study. The efficiency of the germicidal effect of 222-nm UVC against each pathogen was demonstrated by comparison with 254-nm UVC.

Materials and methods

UVC source

Two types of lamps were used: a 5 mW/cm^2 222-nm UVC lamp at 10 mm from the emission window and a 3 mW/cm^2 254-nm lamp at 20 nm from the window. Detailed specification was described elsewhere [11,12].

Bacterial strains and culture conditions

Clinical isolates of *Staphylococcus aureus* [meticillin-resistant *S. aureus* (MRSA)], *Pseudomonas aeruginosa*, *Escherichia coli* (enterohaemorrhagic *E. coli*), *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Bacillus cereus* were grown in tryptic soy broth (BD Diagnosis Systems, Sparks, MD, USA) at 37°C for 14 h. *Campylobacter jejuni*, a clinical isolate, was grown in brain heart infusion broth (BHI; BD Diagnosis Systems) at 37°C in humidified CO_2-enriched atmospheric conditions using an AnaeroPack-Microaero Gas System (Mitsubishi Gas Chemicals, Tokyo, Japan) for 48 h. For preparation of *B. cereus* endospores, the bacterial cells were grown on tryptic soy agar (TSA; BD Diagnosis Systems) at 37°C for 14 h, and the plate was then placed at room temperature for at least 7 days. Thereafter, the colonies were collected, washed and resuspended in sterile phosphate-buffered saline (PBS), and then treated in a water bath at 80°C for 20 min to kill vegetative cells. *Clostridium sporogenes* strain JCM 1416 was grown in cooked meat medium (BD Diagnosis Systems) at 37°C for 18 h in an AnaeroPack Gas System. For preparation of *C. sporogenes* endospores, the bacteria were grown in BHI at 37°C for 24 h and then the culture was placed at room temperature in an AnaeroPack Gas System for at least 7 days. The bacterial cells and cell-free endospores were collected, washed and resuspended in sterile PBS, and then treated in a water bath at 80°C for 20 min to kill vegetative cells. *Clostoridioides difficile* JCM1296 was pre-incubated in GAM bouillon (Nissui Pharmaceutical Co., Tokyo, Japan) at 37°C for 24 h in an AnaeroPack Gas System, and then cultures were placed in BHI agar (Nissui) at 37°C for more than 14 days to form endospores in an AnaeroPack Gas System. The bacterial cells and cell-free endospores were collected, washed and suspended in sterile PBS, and then treated in a water bath at 65°C for 30 min to kill vegetative cells. The bacterial cells and endospores were harvested by centrifugation, washed and resuspended with sterile PBS. Stock solutions were adjusted at an optical density of 550 nm for 3.0. *C. sporogenes* and *C. difficile* were purchased from RIKEN BioResource Centre, Tsukuba, Ibaraki, Japan.

Fungal strains and culture conditions

Candida albicans strain NBRC1385 was pre-incubated on Sabouraud agar (Nissui) plates at 37°C for 24 h, and then a single colony was inoculated in YPD medium containing 10 g/L yeast extract (BD Diagnosis Systems), 20 g/L peptone (BD Diagnosis Systems) and 20 g/L D(+)-glucose (Wako Pure Chemical Industries, Co., Ltd, Osaka, Japan), and cultured at 37°C for 16 h. The fungal cells were harvested by centrifugation, washed and resuspended in sterile PBS. *Aspergillus niger* strain IFM 63883 and *Trichophyton rubrum* strain IFM 64661 were cultured on potato dextrose agar slant (Nissui) at 25°C for 7 days to form spores. Spores were resuspended in PBS and stored at -80°C until use. For hyphal preparation, diluted spore suspensions of *A. niger* and *T. rubrum* were spread on Sabouraud agar plates and incubated to induce germination at 25°C for 24 h. Germination was confirmed microscopically. Fungal strains used in this study were purchased from the Medical Mycology Research Centre, Chiba University, Chiba, Japan.

Viral strains and culture conditions

Feline calicivirus (FCV) strain F4 and Crandell feline kidney (CRFK) cells were kindly provided by Dr Yukinobu Tohya from the Department of Veterinary Medicine, Nihon University

Table I
Bacterial and fungal numbers

Bacteria and fungi	Number (colony-forming units/mL)
Staphylococcus aureus (MRSA)	2×10^7
Pseudomonas aeruginosa	1×10^7
Escherichia coli (EHEC)	1×10^7
Salmonella enterica subsp. *enterica* serovar Typhimurium	2×10^7
Campylobacter jejuni	7.5×10^5
Bacillus cereus vegetative cells	1.7×10^4
Clostridium sporogenes vegetative cells	3×10^6
Bacillus cereus endospores	4×10^5
Clostridium sporogenes endospores	1.7×10^6
Clostridioides difficile endospores	1×10^6
Candida albicans	4×10^5
Aspergillus niger hyphae	2×10^4[a]
Aspergillus niger spores	2×10^4
Trichophyton rubrum hyphae	1×10^5[a]
Trichophyton rubrum spores	1×10^5

MRSA, meticillin-resistant *S. aureus*; EHEC, enterohaemorrhagic *E. coli*.
[a] On a culture plate.

College of Bioresource Sciences. CRFK cells were grown at 37°C, 5% CO_2 in Eagle's minimum essential medium (EMEM; Nissui) supplemented with 10% fetal calf serum (FCS; JRH Biosciences, Lenexa, KS, USA), 1% L-glutamine (Wako) and 1×non-essential amino acids (Thermo Fisher Scientific, Waltham, MA, USA). FCV was absorbed into CRFK cells for 1 h and incubated at 37°C, 5% CO_2 for 18–24 h. After a cytopathic effect was found, the supernatant was collected and freeze-thawed once. Cell debris was removed by centrifugation at 8000×*g* at 4°C for 20 min. The resulting supernatant was kept at -80°C and used as stock virus. The stock virus was titrated by calculating the 50% tissue culture infectious dose ($TCID_{50}$) after infecting CRFK cells grown in 96-well plates. The stock viral solution contained 1.8×10^7 $TCID_{50}$/mL.

Influenza virus H1N1/pdm09 strain A/Michigan/45/2015 was kindly provided by the Influenza Virus Research Centre, National Institute of Infectious Diseases, Musashimurayama, Tokyo, Japan. This virus was propagated in Madin-Darby canine kidney (MDCK) cells purchased from DS Pharma Biomedical, Osaka, Japan. A monolayer of MDCK cells was prepared by cultivating the cells in Dulbecco's Modified Eagle Medium (DMEM; Nissui) supplemented with 10% FCS and 1% L-glutamine. Influenza virus was absorbed into MDCK monolayer cells for 1 h and incubated at 34°C, 5% CO_2 in DMEM supplemented with 0.1% bovine serum albumin (BSA; Wako), 1% antibiotic-antimycotic (Abx; ×100 stock, Thermo Fisher Scientific) and 0.6 μg/mL of N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (Thermo Fisher Scientific). The cytopathic effect was monitored for 4–6 days. After a cytopathic effect was observed, the culture supernatant was collected and centrifuged at 8000×*g* for 20 min. The supernatant was kept at -80°C as stock virus until use. The titre of the stock virus was evaluated by $TCID_{50}$. The stock viral solution contained 1.0×10^5 $TCID_{50}$/mL.

UVC irradiation of micro-organisms

Stock suspensions of bacteria were 10-fold diluted with sterile PBS just before irradiation. Bacterial numbers are shown in Table I.

S. aureus, *P. aeruginosa*, *E. coli*, *S. enterica* subsp. *enterica* serovar Typhimurium, *C. jejuni*, *B. cereus* vegetative cells and endospores, *C. sporogenes* vegetative cells and endospores, and *C. difficile* endospores were irradiated with 222- or 254-nm UVC light at 0–72 mJ/cm^2. Bacterial numbers in the irradiated suspensions were enumerated by plating 10-fold serial dilutions of the suspensions on TSA. Colonies were counted 24–48 h after incubation at 37°C and expressed as colony-forming units (cfu). *C. jejuni* was inoculated on BHI agar plates and cultivated in humidified CO_2-enriched atmospheric conditions using an AneroPack-Microaero Gas System for 48 h. The irradiated vegetative cells and endospores of *C. sporogenes* were grown on BHI agar plates at 37°C for 24 h in an AnaeroPack Gas System. The vegetative cells of *C. sporogenes* were inoculated within 30 min after irradiation. The irradiated *C. difficile* endospores were inoculated on 'KBM'CCMA medium (Kohjin Bio, Sakado, Saitama, Japan) and incubated for 48 h in an AnaeroPack Gas System.

Yeast cell suspension of *C. albicans* was irradiated with 222- or 254-nm UVC light at 0–72 mJ/cm^2. The irradiated cells were incubated on Sabouraud agar plates at 37°C for 24–48 h and colonies were counted. *A. niger* spore suspension was irradiated with 222- or 254-nm UVC light at 0–500 mJ/cm^2. *T. rubrum* spore suspension was irradiated with 222- or 254-nm UVC light at 0–72 mJ/cm^2. The irradiated fungal suspensions were incubated on Sabouraud agar plates at 25°C for 72 h (*A. niger*) or 96 h (*T. rubrum*), and colonies were counted. *A. niger* germinated hyphae inoculated on Sabouraud agar plates were irradiated with 222- or 254-nm UVC light at 0–1000 mJ/cm^2. *T. rubrum* germinated hyphae inoculated on Sabouraud agar plates were irradiated with 222- or 254-nm UVC light at 0–250 mJ/cm^2.

FCV and influenza virus were irradiated with 222- or 254-nm UVC light at 0–36 mJ/cm^2. CRFK cells grown in 96-well microplates were inoculated with irradiated FCV after preparing 10-fold serial dilutions in EMEM supplemented with 5% FCS, 1% L-glutamine and 1% non-essential amino acid. MDCK cells grown in 96-well microplates were inoculated with irradiated influenza virus after preparing 10-fold serial dilutions in DMEM supplemented with 1% BSA, 1% Abx and 0.6 μg/mL of TPCK-treated trypsin. CFRK cells and MDCK cells were stained with Gentian violet after 1 day and 4 days of incubation, respectively, and $TCID_{50}$ values were calculated.

Disinfection by ethanol

One hundred microlitres of suspensions of bacterial vegetative cells, bacterial endospores and fungal spores were mixed with 900 μL of ethanol disinfectant (Et; ethanol 76.9–81.4%, Nipro Pharma Co., Osaka, Japan). The bacterial vegetative cells were incubated with Et at room temperature for 10 s, whereas the bacterial endospores and fungal spores

were incubated for 30 s. Thereafter, the bacterial and fungal suspensions were immediately diluted 10-fold with PBS, inoculated into appropriate media and incubated under appropriate conditions. For fungal hyphae, Et was poured on to Sabouraud agar plates of germinated hyphae. The poured plates were kept at room temperature for 10 s, and then the plate surface was washed gently three times with sterile PBS. It was confirmed that Et with 10-fold dilution showed no microbicidal activity. Stock solutions of FCV and influenza virus (100 μL) were mixed with 900 μL of Et at room temperature for 60 s, and then immediately diluted 10-fold with PBS and inoculated into CRFK cells and MDCK cells, respectively. It was confirmed that Et with 10-fold dilution showed no cytotoxic activity against these cells.

Results

Effect of 222-nm UVC irradiation on bacterial vegetative cells

Suspensions of *S. aureus*, *P. aeruginosa*, *E. coli*, *S. enterica* subsp. *enterica* serovar Typhimurium, *C. jejuni*, vegetative *B. cereus* and vegetative *C. sporogenes* were irradiated with 222- or 254-nm UVC at various irradiation doses, and the numbers of viable bacteria were counted by colony formation. In the case of *S. aureus*, irradiation with 6 mJ/cm^2 reduced the bacterial number 10^4-fold, and the bacterial number was reduced to an undetectable level when irradiated with 12 mJ/cm^2 of both UVC wavelengths (Figure 1A). In the case of *P. aeruginosa* and *E. coli*, irradiation with 6 mJ/cm^2 reduced the bacterial number 10^2- to 10^3-fold, and the bacterial number was reduced to an undetectable level when irradiated with 24 mJ/cm^2 of 222-nm UVC or 6 mJ/cm^2 of 254-nm UVC (Figure 1B,C). Although the bacterial number of *S. enterica* subsp. *enterica* serovar Typhimurium reduced 10^2–10^3-fold at 12 mJ/cm^2 of both UVC wavelengths, reduction to an undetectable level required 24 mJ/cm^{2}and 36 mJ/cm^2 of 254- and 222-nm UVC, respectively (Figure 1D). *C. jejuni* was highly sensitive to irradiation at even 6 mJ/cm^2 of either UVC wavelength (Figure 1E). The sensitivity of *B. cereus* vegetative cells to UVC was similar to *S. enterica* subsp. *enterica* serovar Typhimurium (Figure 1F). For *C. sporogenes*, reduction of the bacterial number to an undetectable level required 36 mJ/cm^2 of 222-nm UVC and 72 mJ/cm^2 of 254-nm UVC (Figure 1G).

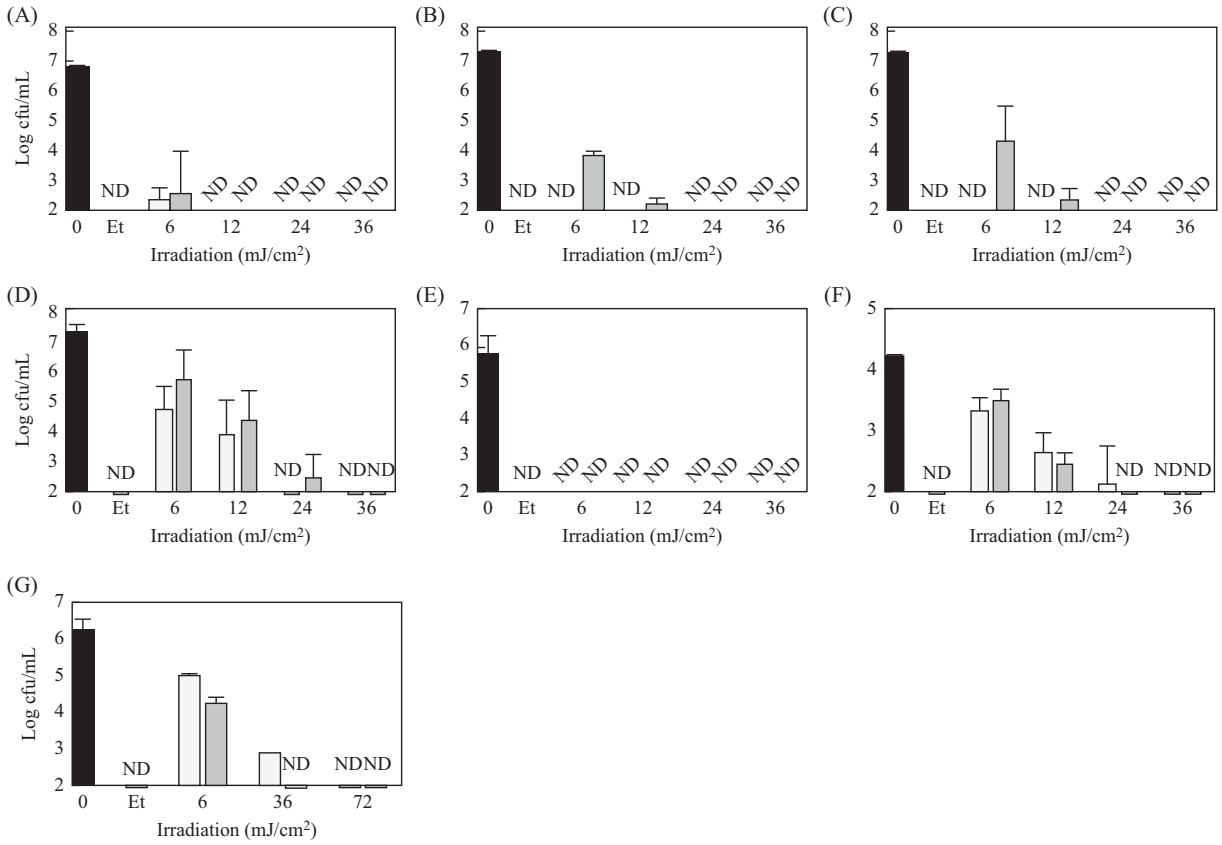

Figure 1. Effect of 222-nm ultraviolet C (UVC) light on bacterial vegetative cells. Vegetative cell suspensions (0.5 mL each) of *Staphylococcus aureus* (A), *Pseudomonas aeruginosa* (B), *Escherichia coli* (C), *Salmonella enterica* subsp. serovar Typhimurium (D), *Campylobacter jejuni* (E), *Bacillus cereus* (F) and *Clostridium sporogenes* (G) were overlaid on 35-mm culture dishes and irradiated with 254-nm UVC (white bars) or 222-nm UVC (dark bars). Non-irradiated controls are shown as black bars. In each experiment, bacteria were incubated with ethanol (Et) at room temperature for 10 s. Bacterial numbers in the irradiated suspensions were enumerated by plating 10-fold serial dilutions of the suspensions on tryptic soy agar, with colonies counted 24–48 h after incubation at 37°C. Bacterial numbers are expressed as colony-forming units (cfu). Data are expressed as mean ± standard deviation of two independent experiments. ND, not detected.

These results indicate that both 222-nm UVC and 245-nm UVC have a potent bactericidal effect. On the other hand, the bacterial numbers of all bacteria used in this study were reduced to undetectable levels by contact with Et for 10 s (Figure 1).

Effect of 222-nm UVC irradiation on bacterial endospores

Spore suspensions of *B. cereus*, *C. sporogenes* and *C. difficile* were irradiated with 222- and 254-nm UVC at various irradiation doses, and the numbers of remaining live endospores were counted by colony formation. Live endospores of *B. cereus* and *C. sporogenes* reduced dependent on irradiation doses of both 222-nm UVC and 254-nm UVC. Live endospores of both bacteria reduced to an undetectable level at 96 mJ/cm^2 of 222-nm UVC, while live endospores could still be detected at 96 mJ/cm^2 of 254-nm UVC (Figure 2A,B). On the other hand, live endospores of *C. difficile* reduced dependent on irradiation doses of 222-nm UVC, and reduced to an undetectable level at 50 mJ/cm^2; the reduction of live endospores plateaued at 30 mJ/cm^2 of 254-nm UVC (Figure 2C). These results indicate that 222-nm UVC has more potent germicidal effects on bacterial endospores than 254-nm UVC. Et was almost ineffective for inactivation of bacterial endospores used in this study (Figure 2A,B).

Effect of 222-nm UVC irradiation on fungi

Yeast cell suspensions of *C. albicans* were irradiated with 222- or 254-nm UVC at various irradiation doses, and the numbers of viable cells were counted by colony formation. Irradiation with 222- or 254-nm UVC reduced the viable number of yeast cells at 24 mJ/cm^2 and reduced the number to an undetectable level at 72 mJ/cm^2, although both UVC wavelengths failed to kill the yeast cells at 6 mJ/cm^2 (Figure 3A). Next, the effect of UVC on fungal spores and hypha was investigated. *A. niger* spores reduced to an undetectable level after irradiation with 254- and 222-nm UVC at 250 mJ/cm^2 and 500 mJ/cm^2, respectively (Figure 3B). Irradiation with 254- and 222-nm UVC reduced *T. rubrum* spores to an undetectable level at 36 mJ/cm^2 and 72 mJ/cm^2, respectively (Figure 3C). Extension of *A. niger* and *T. rubrum* hyphae was strongly inhibited by 254-nm UVC irradiation at 250 mJ/cm^2 and 72 mJ/cm^2, respectively, and by exposure to Et (Figure 3D,E). In contrast, 222-nm UVC failed to inhibit hyphae extension of both fungi at either dose. These results indicate that the fungicidal effect of 222-nm UVC to yeast forms of fungi was similar to that of 254-nm UVC, but the effect of 222-nm UVC against fungal spores and hyphae was weaker compared with 254-nm UVC. Moreover, irradiation at up to 1000 mJ/cm^2 of 222-nm UVC was not able to disinfect hyphae of *A. niger*.

Effect of 222-nm UVC irradiation on viruses

Stocks of FCV and influenza A virus were irradiated with 222- and 254-nm UVC at various irradiation doses, and the remaining viral infectivity was estimated by $TCID_{50}$. $TCID_{50}$ of FCV was decreased depending on the increased irradiation dose, but infective FCV could be detected even after irradiation at 36 mJ/cm^2 of 222- and 254-nm UVC. The effect of 222-nm UVC on FCV was comparable with that of 254-nm UVC (Figure 4A). Et was ineffective to inactivate FCV. $TCID_{50}$ of influenza A virus reduced to an undetectable level at 6 mJ/cm^2 of lights (Figure 4B). Similarly, Et treatment was able to inactivate influenza A virus effectively. These results suggest that 222-nm UVC and 254-nm UVC were unable to inactivate FCV, but both UVC wavelengths were able to inactivate influenza A virus at 6 mJ/cm^2.

Discussion

Microbiota can cause opportunistic infections under certain conditions, especially in elderly, debilitated or immunocompromised persons [17,18]. In particular, antibiotic resistance mechanisms can originate in non-pathogenic micro-organisms, and the genes then transfer to human pathogens causing major problems in clinical treatment [19,20]. In order to reduce the risk of infections and emergence of antibiotic-resistant pathogens in healthcare facilities, efficient disinfectant techniques that are safe for healthcare workers are required. Unlike 254-nm UVC, 222-nm UVC has been shown to be harmless to mammalian cells [9–11]. However, its germicidal effect against various pathogens remains to be clarified. This study

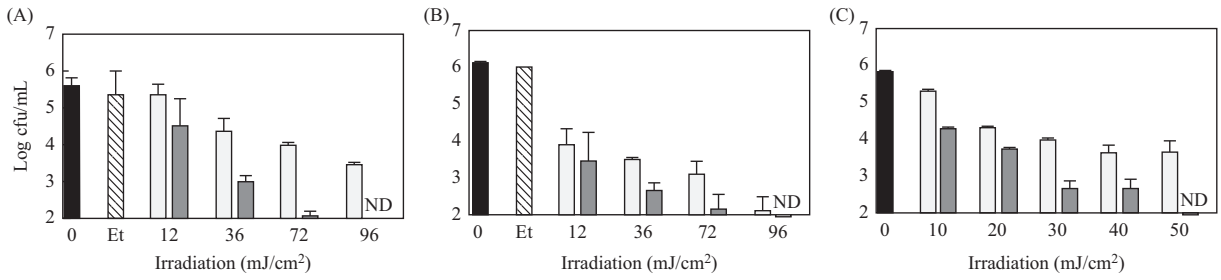

Figure 2. Effect of 222-nm ultraviolet C (UVC) light on bacterial endospores. Spore suspensions (0.5 mL each) of *Bacillus cereus* (A), *Clostridium sporogenes* (B) and *Clostridiodes difficile* (C) were overlaid on 35-mm culture dishes and irradiated with 254-nm UVC (white bars) or 222-nm UVC (dark bars). Non-irradiated controls are shown as black bars. In each experiment, bacteria were incubated with ethanol (Et) at room temperature for 30 s (striped bars). Bacterial numbers in the irradiated suspensions were enumerated by plating 10-fold serial dilutions of the suspensions on tryptic soy agar, with colonies counted 24–48 h after incubation at 37°C. Bacterial numbers are expressed as colony-forming units (cfu). Data are expressed as mean ± standard deviation of two independent experiments. ND, not detected.

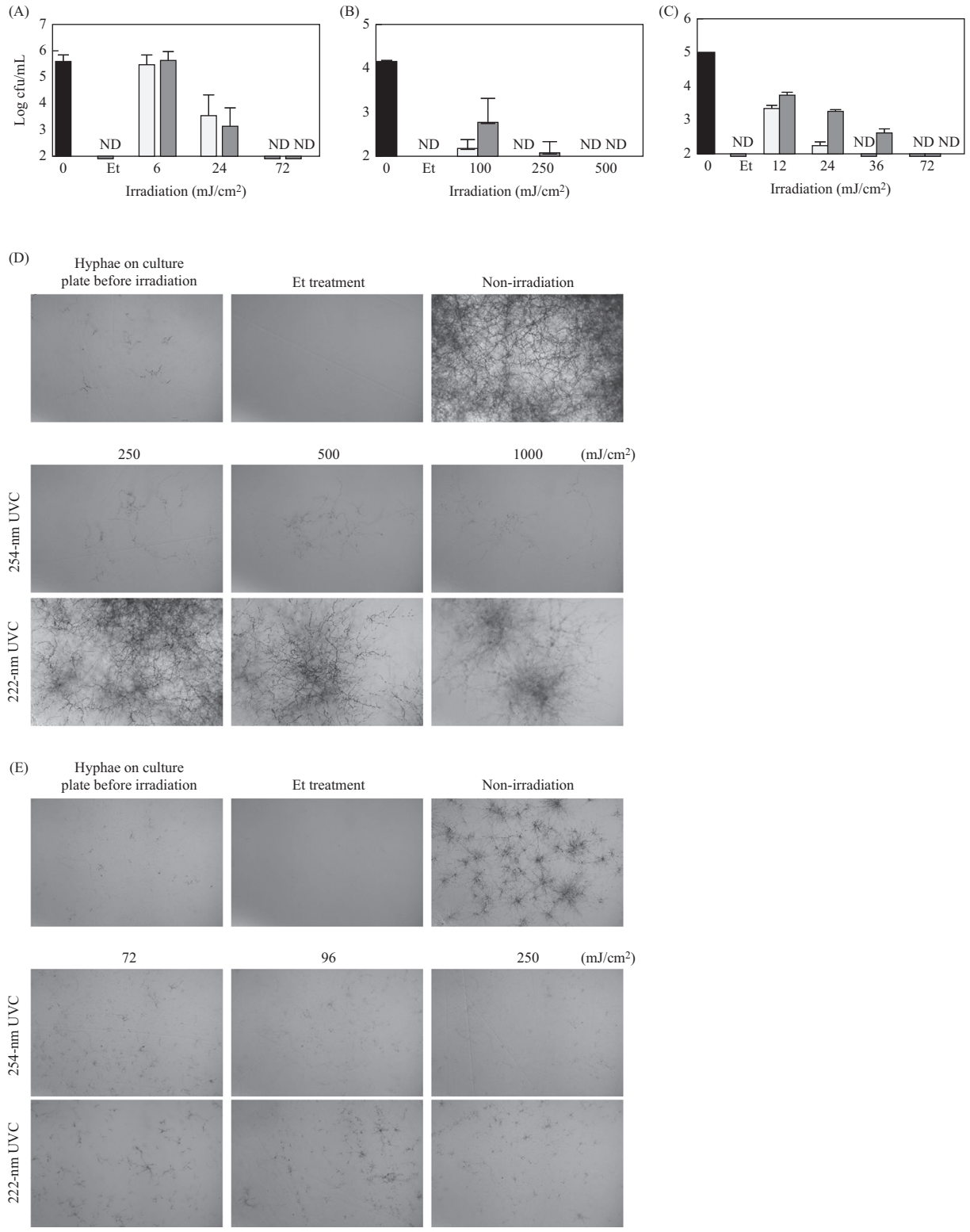

Figure 3. Effect of 222-nm ultraviolet C (UVC) light on fungal hyphae and spores. Suspensions (0.5 mL each) of *Candida albicans* yeast (A), *Aspergillus niger* spores (B) and *Trichophyton rubrum* spores (C) were overlaid on 35-mm culture dishes and irradiated with 254-nm UVC (white bars) or 222-nm UVC (dark bars). Non-irradiated controls are shown as black bars. The irradiated fungal yeast and spore suspensions were incubated on Sabouraud agar plates at 25°C for 24–48 h (*C. albicans* yeast), 72 h (*A. niger* spores) or 96 h (*T. rubrum* spores), and colonies were counted. Live cell numbers were expressed as colony-forming units (cfu). *A. niger* hyphae (D) and *T. rubrum* hyphae (E) inoculated on Sabouraud agar plates were irradiated directly with 254-nm UVC or 222-nm UVC, incubated at 25°C for 48 h, and hyphal extension was observed microscopically (×40). In each experiment, fungal yeast cells and spores were incubated with ethanol (Et) at room temperature for 30 s. Fungal hyphae were incubated with Et at room temperature for 10 s. Data are expressed as mean ± standard deviation of two independent experiments. ND, not detected.

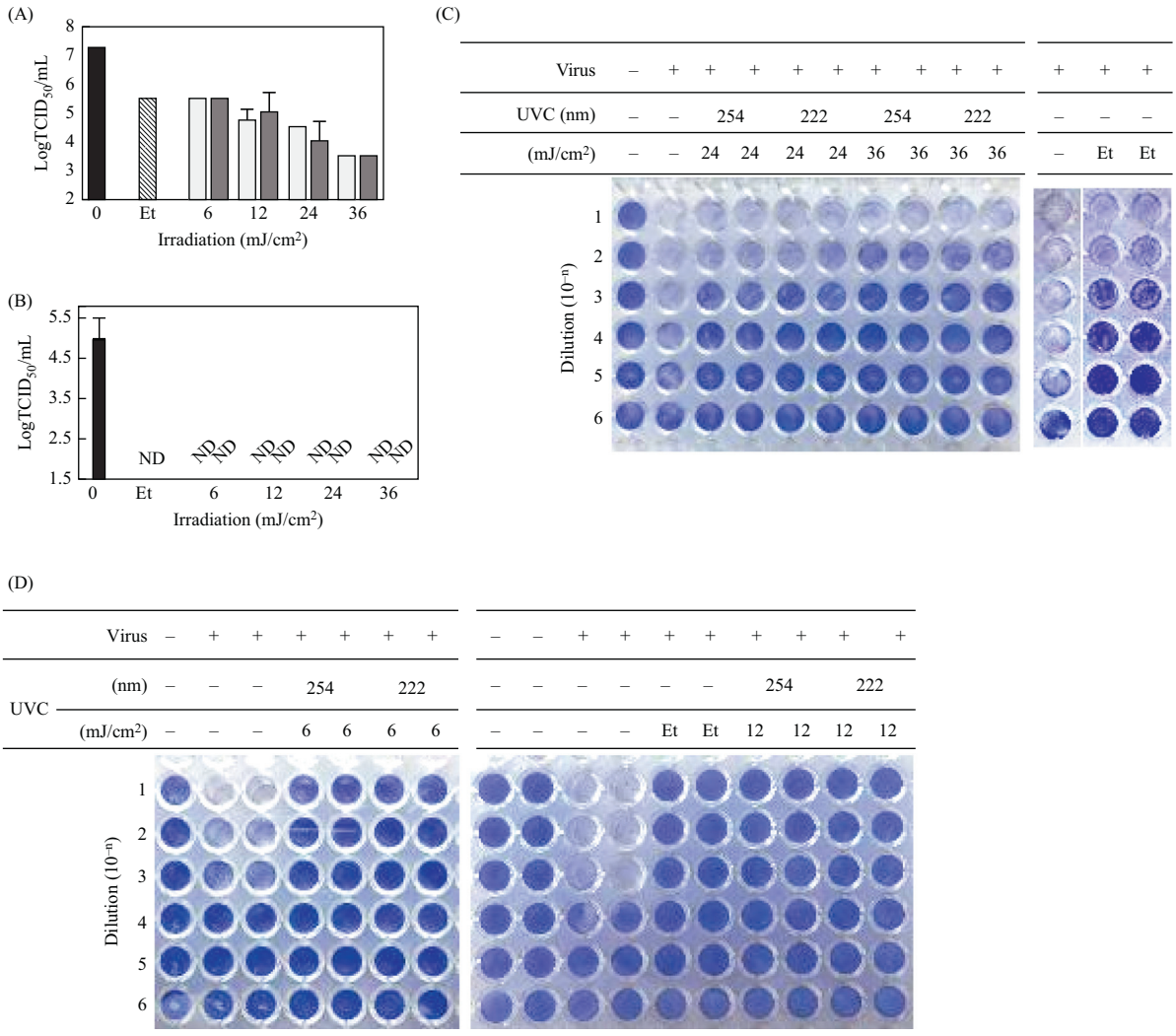

Figure 4. Effect of 222-nm ultraviolet C (UVC) light on viruses. Stocks (0.2 mL each) of feline calicivirus (FVC) (A) and influenza A virus (B) were overlaid on 35-mm culture dishes and irradiated with 254-nm UVC (white bars) or 222-nm UVC (dark bars). Non-irradiated controls are shown as black bars. In each experiment, viruses were incubated with ethanol (Et) at room temperature for 60 s (striped bars). The irradiated FCV and influenza A virus were inoculated into Crandell feline kidney cells and Madin-Darby canine kidney cells grown in 96-well microplates, respectively. Infected cells were stained with Gentian violet after 24 h of incubation for FCV (C) and 4 days of incubation for influenza A virus (D), and then 50% tissue culture infectious dose ($TCID_{50}$) values were calculated. In each experiment, viruses were incubated with Et at room temperature for 30 s (striped bars). Data are expressed as mean ± standard deviation of two independent experiments. ND, not detected.

investigated the germicidal effect of 222-nm UVC against several bacteria (vegetative cells and endospores), fungi (unicellular yeast, multi-cellular mould hyphae and spores) and viruses (enveloped and non-enveloped virions). Most are commonly found in the environment and healthcare facilities and are associated with opportunistic infections. *S. aureus* is a normal human flora that causes foodborne poisoning, toxic shock syndrome and a wide range of infections, especially surgical site infections in immunocompromised patients [21]. *P. aeruginosa*, commonly found in the environment, can cause refractory respiratory tract infections, especially in patients with cystic fibrosis [22]. *E. coli* is a normal bacteria found in human and animal intestines and faeces, but the pathogenic strains cause diarrhoeal-related syndrome, septicaemia and meningitis [23]. *S. aureus*, *E. coli* and *P. aeruginosa* have developed multi-drug resistance, which increases mortality in immunocompromised patients [24–26]. *S. enterica* subsp. *enterica* serovar Typhimurium and *C. jejuni* are foodborne pathogens commonly associated with poultry. Serious systemic illness caused by these bacteria is rare, but can lead to sepsis and death [27,28]. *B. cereus* is an environmental microorganism that can cause opportunistic infections by insufficient cleaning of hospital laundry and devices [29]. *C. sporogenes* and *C. difficile* are commonly found in mammalian intestinal tracts, and their spores are widely spread in soil and marine sediment [30,31]. The frequency and severity of *C. difficile* infection have increased and it is now one of the most common hospital-acquired infections. The important risk factors of *C. difficile* infection include antibiotic therapy, old age, and hospital or nursing home stay [31]. *C. albicans* is one

of very few fungal species that cause disease in humans. Changes in the host immune response in immunocompromised individuals and healthy people with implanted medical devices can enable *C. albicans* to cause serious infection [32]. *A. niger* is a representative of *Aspergillus* spp., which are the most common moulds recovered from respiratory specimens of patients with cystic fibrosis [33]. *T. rubrum* is a dermatophyte which is a common skin pathogen throughout the world [34]. Influenza A virus is an airborne infectious particle [35], and FCV is a surrogate of norovirus which is a common cause of food-borne gastroenteritis in children aged <5 years in developing countries [36].

Among the pathogens tested in this study, viruses have the simplest structure. Influenza A virus has a membrane envelope in the outermost layer, whereas FCV is a naked, non-enveloped virus. It was expected that UVC would be absorbed efficiently by nucleic acid components and proteins in both viral particles, resulting in impaired infectivity. However, FCV was found to be more resistant to both 222-nm and 254-nm UVC than influenza A virus. Therefore, the virucidal effect of both UVC wavelengths seems to be strongly influenced by phospholipids and glycoproteins on the envelope of viral particles. In addition, the nucleic acid repair system for the viral genome in CRFK cells may be more efficient than that in MDCK cells. Although the virucidal mechanism of UVC is unclear, the effect of 222-nm UVC on both enveloped and non-enveloped viruses was not significantly different from that of 254-nm UVC. Moreover, both UVC wavelengths at 36 mJ/cm^2 had a stronger germicidal effect on FCV than Et.

In comparison with bacteria and viruses, the structure of fungal cells is much larger and more complex. In addition to the plasma membrane, fungal cells are covered with sugars on their cell wall. Fungi can produce hydrophobic spores with a thick coat of sugar and heat-shock proteins. Spores of these organisms not only guard genetic information during unfavourable conditions, but also adapt to wind dispersal and may remain airborne for long periods [37]. The present study demonstrated that 222-nm UVC could inactivate *C. albicans*, a unicellular yeast form of fungi, as efficiently as 254-nm UVC. On the other hand, 254-nm UVC had a greater germicidal effect on multi-cellular fungal spores and hyphae than 222-nm UVC. In particular, irradiation with 222-nm UVC at 1000 mJ/cm^2 could not completely inactivate the hyphae of *A. niger*. The low efficiency of 222-nm UVC to inactivate multi-cellular fungal cells, especially hyphae, may be similar to the effect found in mammalian cells. UVC with a wavelength of 222 nm might be absorbed by proteins and other biomolecules on the fungal surface before reaching fungal nuclei. Although 222-nm UVC had a low germicidal effect on fungal hyphae and spores, Et can be used as an alternative disinfectant. It was not surprising that *T. rubrum* in both spore and hyphae forms was more susceptible to both UVC wavelengths than *A. niger*. This result correlates with a previous study which demonstrated that *Trichophyton microconidia* is more susceptible to 405-nm violet-blue light than *A. niger* [38]. The presence of aspergillin pigment in *A. niger* may explain the higher resistance of this fungus to UVC.

For vegetative forms of bacteria, the germicidal effect of 222-nm UVC was not significantly different to that of 254-nm UVC. Among the bacteria used in this study, *C. jejuni* was most susceptible to both UVC wavelengths. MRSA, *P. aeruginosa* and *E. coli* were also very sensitive to 222-nm UVC. It was expected that 222-nm UVC would also have an efficient germicidal effect on various antibiotic-resistant bacteria. However, *S. enterica* subsp. *enterica* serovar Typhimurium, *B. cereus* and *C. sporogenes* were found to be more resistant to both UVC wavelengths. Although the reasons for this resistance remain unknown, it is thought that these bacterial cells may produce substances or repairing enzymes which can protect them from UVC damage. Endospores of bacteria exhibit striking resistance to a wide range of environmental stresses, such as heat, desiccation and UV radiation. Their abilities to survive harsh conditions result from multi-layered surface structures and extremely low water content [37]. As expected, the endospores of *B. cereus* and *C. sporogenes* exhibited higher resistance to Et and UVC than their bacterial vegetative counterparts. Surprisingly, 222-nm UVC had a stronger germicidal effect on bacterial endospores than 254-nm UVC, possibly due to the strong detrimental effect of 222-nm UVC on peptide bonds and amino acids in the cell walls of bacterial endospores.

In conclusion, 222-nm UVC is able to inactivate a wide spectrum of microbial pathogens. Although its germicidal effect on fungal hyphae and spores is low, 222-nm UVC exhibited a stronger germicidal effect on bacterial endospores than 254-nm UVC. Given its germicidal results and the fact that 222-nm UVC is harmless to mammalian cells, the Kr—Cl excimer lamp has high potential for use as a tool to inactivate various types of pathogens in the residential setting. In future, microbial samples obtained from various carrier surfaces will be used to examine the disinfection efficacy of 222-nm UVC.

Conflict of interest statement
None declared.

Funding source
This study was supported by a research grant from Ushio Inc.

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