Photochemistry and Photobiology, 20**, **: *–*

Research Article

Improved Spectral Purity of 222-nm Irradiation Eliminates Detectable Cyclobutylpyrimidine Dimers Formation in Skin Reconstructs even at High and Repetitive Disinfecting Doses

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Received 29 September 2021, revised 14 December 2021, accepted 20 December 2021, DOI: 10.1111/php.13594

ABSTRACT

UVC222 nm has germicidal effects with potential clinical applications. However, UVC irradiation is capable of inducing DNA damage like cyclobutylpyrimidine dimers (CPD). Although new devices have emission peaks in the short-wavelength region of UVC (~222 nm), the remaining "collateral" radiation at longer wavelengths could be harmful to human health. We investigated the DNA damage caused by far-UVC 222 nm KrCl exciplex radiation on human skin reconstructs after additional filtering using silica filters. The skin reconstructs were irradiated with 100 mJ cm^{-2}, 500 mJ cm^{-2}, and 3 × 500 mJ cm^{-2} unfiltered and filtered (230–270 nm suppressed) far-UVC or UVB (308 nm) radiation. UVB and non-filtered UVC irradiation induced a significant amount of CPDs, compared with the background. Filtered far-UVC lowered the CPD amount compared with unfiltered UVC and UVB treatments. Repetitive UVC irradiation did not result in the accumulation of CPDs compared with UVB treatment. Reduction in excess of 99.9% of *E. coli*, *S. aureus* and *C. albicans* was detected after applying far-UVC radiation. This identifies a therapeutic window in which microorganisms are killed but tissue is still alive and not damaged, which could give rise to new clinical applications.

INTRODUCTION

Surgical site infections are the most frequently occurring hospital-acquired infections and can not only prolong the postoperative recovery time but also result in increased medical and financial burden both for the patient and the medical staff (1,2). In some cases, such infections may lead to limb amputation or even have a lethal outcome (3,4). With the large amount of antibiotics applied in human healthcare and animal farming, the number of multidrug-resistant pathogens is on the rise (5,6), which can potentially further aggravate the outcome of surgical site infection. The need to combat such drug-resistant infections increases the demand for alternative methods of effective, cheap and safe pathogen neutralization.

Irradiation with UVC is a promising strategy to neutralize bacteria (7–9) and viruses like the coronavirus (10). It is a simple and relatively easy to apply method that could be implemented even outside of the surgical field as a means to disinfect hospital rooms, appliances or even in hand sanitization. Nevertheless, the application of UVC as a disinfection method is under scrutiny due to its ability to induce protein and DNA damage in human cells leading to proliferation arrest, even cell death or mutagenicity (11–14). Especially in cases where UVC is used as a disinfectant at times when patients and medical personnel are present during irradiation, it must be a safe application.

UVC irradiation is known to induce cyclobutylpyrimidine dimers (CPD), which have the potential to block transcription and could give rise to error-prone replication and cancer (15,16). Although there seem to be little risk for cancer induction in the skin due to the shallow penetration depth of UVC (17), we do believe that there is still insufficient data on the damaging potential of UVC. Therefore, it is imperative to assure that any devices intended for hand sanitization or room sanitization in the presence of unprotected personnel are sufficiently safe and have reduced capacity of CPD induction. Since DNA has an absorption peak between 253 nm and 262 nm (18), using UVC in that wavelength range might be potentially harmful. However, shorter wavelengths that fall near the absorption minimum of DNA, such as 222 nm, have been suggested as safe to apply on human tissues (18). So far, neither in DNA repair deficient mice (XPA knockout mice) nor in wild-type mice, low-dose UVC 222 nm treatment (100 mJ cm^{-2}) could induce erythema (19). However, CPDs were detected in the upper layer of the epidermis, which usually consists of dead cells (19). This could indicate that a large amount of UVC 222 nm irradiation is absorbed by the upper layers of the skin, like the stratum corneum, and cannot reach the deeper layers of the epidermis and dermis that contain living cells (7). In support of this theory, it has been shown that UVC irradiation with a short wavelength (<253 nm) could induce less damage in the deeper layers of the skin than UVC irradiation with a longer wavelength (7,18).

Although low doses of UVC 222 nm irradiation seem to have low damage potential, higher doses and repetitive irradiations

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applied for disinfection purposes could still elicit damage. This could mean that the presence and quantity of UVC222 nm irradiation in clinics or in other buildings in everyday life and the exposure of clinic staff or normal people could be higher than the doses that have previously been tested. Therefore, to exclude potential danger to human health, the damaging effect of higher doses and repetitive irradiations have to be tested in addition to the minimal UVC 222 nm doses that are needed for successful inactivation of pathogens (7,8).

Despite improvements in the field of UV radiation sources, it is still difficult to produce single-wavelength or even extremely narrow-band emission devices like 222 nm lasers (20,21). Therefore, although far-UVC devices (like KrCl exciplex lamps) have emission peaks in the short-wavelength region of UVC (~222 nm), there is still residual "collateral" radiation at wavelengths that could be potentially harmful to human health (>230 nm). For these reasons, the use of additional filters to block UVC above 230 nm is necessary to ensure additional safety of the application on patients. The filters used here consisted of a coated fused silica window with a blocking range of 230 nm–270 nm. One filter reduced the fraction of 230 nm–240 nm radiation from 8.5% to 1.0% and the fraction of 240 nm–260 nm radiation from 11.4% to 1.18%. Doubling the number of filters further reduced the percentage to 0.45% (230 nm–240 nm) and 0.116% (240 nm–260 nm).

The aim of this study was to evaluate both the safety of high and repetitive doses of UVC 222 nm irradiation *in vitro* on 3D human full-thickness skin reconstructs (including intact stratum corneum) and the associated antimicrobial effect against *E. coli, S. aureus* and *Candida albicans*. Skin reconstructs are a good model to investigate UVC-induced DNA damage since it can closely mimic the situation in human skin. Furthermore, this study investigated whether the use of appropriate filters has an influence on the emergence of CPD in the 3D skin model, thus minimizing the damaging potential of the 222 nm UVC irradiation device, while in parallel, an antimicrobial effect takes place under the conditions used here.

MATERIALS AND METHODS

Culturing of skin reconstructs. The human skin reconstructs (day 18) from (Episkin Lyon, France) were cultured in a specialized T-Skin culture medium provided with the reconstructs. Immediately after delivery, the skin reconstructs were transferred to 6-well plates and incubated with 2 ml culture medium overnight at 37°C, 5% CO_2. After the overnight incubation, the reconstructs were used in irradiation experiments and subsequently incubated again overnight in 2 ml fresh medium before sample collection.

UV irradiation. Irradiation was carried out with a prototype lamp MED-UV from GME, Germany, with two applicators: (1) UVC 222 nm and (2) UVB 308 nm. The 222 nm radiation was provided by a KrCl Exciplex with Kryptone and Chloride excited complex. This irradiation devise has an excitation maximum at 222 nm with trace amounts radiation in the range 230–270 nm. The UVB radiation (308 nm) was provided by a XeCl Exciplex with Xenon and Chloride excited complex. Irradiation doses applied on the skin reconstructs were 100 mJ cm^{-2}, 500 mJ cm^{-2}, and repetitive irradiation of 3 × 500 mJ cm^{-2} (with 4 h between each irradiation). For the UVC irradiation, two additional conditions were investigated – UVC with 1-Filter (UVC-1F) or 2-Filter (UVC-2F). The filter consisted of a coated fused silica window with a blocking range of 230 nm–270 nm. Taking the relevant 200 nm–300 nm part of the lamp emission spectrum, one filter reduced the fraction of 230 nm–240 nm radiation from 8.5% to 1.0% and the fraction of 240 nm–260 nm radiation from 11.4% to 1.18%. Adding a second filter further reduced the percentage to 0.45% (230 nm–240 nm) and 0.116% (240 nm–260 nm).

We have used a calibrated radiometer (Opsytec Dr. Göbel GmbH, Radiometer RMD) with 222 nm calibration performed by the accredited calibration lab at Opsytec to measure the absolute power density of the lamp. A spectrometer (Lasertack GmbH, LR2) was then used to measure the relative spectral distribution of the light.

Sample preparation for DNA isolation and vitality test. After an overnight incubation after the last irradiation, 8 mm biopsies were taken from each skin reconstruct and shock-frozen in liquid nitrogen to be subsequently used for vitality staining. The remainder of the skin reconstruct was digested for DNA isolation using the manufacturer's protocol for QIAamp DNA Mini Kit (QIAGEN). After the lysis, the DNA was eluted in 100 µl buffer AE (provided in QIAamp DNA Mini Kit) and stored at −20°C for further analysis.

CPD detection ELISA. The DNA harvested from the skin reconstructs was equilibrated to an uniform concentration of 4 ng μl^{-1} for all samples with TE buffer (10 mM Tris, 1 mM EDTA, pH 8), converted to single-strand DNA by incubating the samples for 10 min at 95°C and subsequently chilling on ice for 10 min. The detection of the CPD was performed with an OxiSelect UV-Induced DNA Damage ELISA Kit (Cell Biolabs Inc.) in accordance with the manufacturer's protocol.

CPD detection IHC. To detect the penetration depth of 222 nm UVC into the skin, a CPD staining of cryosections obtained from skin reconstructs were performed. 8 mm thick sections were fixated in pre-chilled (−20°C) acetone for 10 min at −20°C. The slides were immediately washed 3x in PBS and a 15 min digestion with Proteinase K (Dako) at room temperature was performed. Subsequently, the samples were incubated for 5 min at 4°C in a 0.05 M HCl in PBS solution. After a brief wash in 70% ethanol, the slides were incubated for 2 min at room temperature in a 0.15 M NaOH in PBS. After the incubation, the samples were again washed briefly in 70% ethanol and blocked for 30 min at room temperature using Assay Diluent from the CPD-ELISA Kit (Cell Biolabs Inc.). After the blocking, primary CPD-antibody (CPD-ELISA Kit (Cell Biolabs Inc.), diluted 1:250 in Assay Diluent) was applied on the samples and incubated for 1 h at room temperature. Subsequently, the slides were washed 3 × 5 min with PBS and a secondary antibody was applied (CPD-ELISA Kit (Cell Biolabs Inc.), diluted 1:250 in Assay Diluent) and incubated for 1 h at room temperature. Following a 3 × 5 min PBS wash, the samples were stained with AEC (Dako) for 4 min and the reaction was stopped with H_2O. Cell nuclei were counterstained blue with Hematoxylin and the slides were mounted using Roti-Mount Aqua mounting medium (Carl Roth GmbH+Co.KG, Germany).

The staining was visualized using Axiostar Plus Microscope. Images were taken using AxioCam camera coupled with Axiovision software (Zeiss, Germany).

Vitality staining. A vitality staining with nitroblue tetrazolium chloride (NBTC) was performed on the shock-frozen biopsy samples. NBTC is reduced by cell-bound NADH-diaphorase that has activity only in viable cells. This leads to the production of blue granular precipitate in living cells that can be microscopically distinguished from unstained dead cells. The NBTC staining was performed as follows:

The samples were embedded in Tissue-Tek (Sacura Finetec, Netherlands) and cut via cryotome in 8 µm thick slices. To perform the vitality staining, a master mix containing 1 ml NADH (stock 2.5 mg ml^{-1}, Sigma Aldrich, Germany), 2.5 ml NBTC (stock 2 mg ml^{-1}, Sigma Aldrich, Germany), 1 ml PBS (pH 7.4) (Gibco, Great Britain) and 0.5 ml Ringer Solution (Fresenius Kabi, France). From the master mix, 60 µl were pipetted on each cryosection and the samples were incubated for 15 min at room temperature. Afterward, the reaction was stopped in PBS and the samples were covered using Roti-Mount Aqua mounting medium (Carl Roth GmbH+Co.KG, Germany). The resulting staining was evaluated microscopically via Axiostar Plus Microscope and images were taken using AxioCam camera coupled with Axiovision software (Zeiss, Germany). As a negative control, one cryosection was heat-treated at 70°C for 1 h and 15 min and subsequently treated with formalin for 15 min, resulting in a complete lack of coloration when NBTC staining was performed.

Bacterial culture and treatment. In this work, two species of bacteria – *E. coli* 25922 and *S. aureus* 25923 (ATCC, USA), and one species of fungus – *C. albicans* MYA-273 (ATCC, USA), were used. Both *E. coli* and *S. aureus* were propagated in Mueller-Hinton broth (Merck) and the further tests were performed on Mueller-Hinton agar plates at 37°C. For *C. albicans*, the fungal propagation was done in Sabouraud-Bouillon (Carl Roth GmbH+Co.KG), and further tests were performed on CASO agar plates.

After overnight propagation of *E. coli* and *S. aureus* at 37°C, the bacteria were centrifuged for 5 min at 5000 rpm and resuspended in PBS (Gibco, Great Britain). The optical density (OD) of the samples was measured with SPECORD 50 Plus Photometer (Analytik Jena GmbH, Germany) at 600 nm and the OD was adjusted with PBS to OD 0.2. After the adjustment, 1 ml from the bacterial suspension was pipetted on 3.5 mm Primaria Dishes (Corning Inc.) and irradiated with prototype lamp MED-UV (GME, Germany) with two filters, and 0/12.5/50/100/500 mJ cm^{-2} UVC 222 nm were applied to the samples. The irradiated samples and untreated controls were serially diluted to 10^{-8} and a colony-forming unit (CFU) assay was done based on the method by Miles, Misra and Irvin to evaluate the surviving number of CFU (22). After incubation over night at 37°C, the surviving colonies were counted and the CFU per irradiation were determined.

After overnight propagation at 37°C, the *C. albicans* was centrifuged for 5 min at 5000 rpm and resuspended in PBS (Gibco, Great Britain). The number of viable cells was measured with LUNA FL cell counter and Yeast Viability Kit (Logos Biosystems, South Korea) and the concentration was adjusted to 10^6 per ml viable cells. After the adjustment, the cells were treated in the same manner as the bacterial samples described above. Subsequently, the irradiated samples and untreated controls were as well serially diluted to 10^{-7} and dilutions were plated on CASO agar plates and incubated over night at 37°C. The next day, the resulting colonies were counted and colony-forming units per irradiation were determined.

Statistical analysis. Data are shown as the mean with standard deviation of at least three independent experiments and statistical significance was tested with GraphPad Prism 9.2.0 software (GraphPad Software, San Diego). A two-way ANOVA with Bonferroni multiple comparisons posttest analysis was performed and a P-value < 0.05 was considered significant.

RESULTS

The use of filters during UVC 222 nm irradiation show protective tendencies against DNA damage

For the following experiments, human full-thickness skin reconstructs including intact stratum corneum (age: day 18) from (Episkin Lyon, France) were treated with different doses of far-UVC irradiation (100 mJ cm^{-2} and 500mJ cm^{-2} single exposure and 3×500 mJ cm^{-2} repetitive exposure with 4 h intervals between treatments) with and without different filter-inserts (blocking range of 230 nm–270 nm) or with 308 nm UVB only.

Firstly, the vitality of the full-thickness skin models was determined using Nitroblue tetrazolium chloride (NBTC) staining. Doses around 100 mJ cm^{-2} UVC are usually applied to effectively inactivate bacteria (8). The dose of 500 mJ cm^{-2} UVC is higher than the minimal dose that would be usually applied for disinfection purposes but was implemented as an important safety parameter. Repetitive irradiation with 3×500 mJ cm^{-2} UVC was performed with filter-inserts (one or two filters) to test the device safety in cases of chronic UVC exposure expected for prolonged applications of the lamp, for example, during surgical procedures. As seen from the results of the performed NBTC staining shown in Fig. 1 and Figures S1 and S2 in the Supporting Information, neither of the used irradiation doses had negative effects on overall tissue viability. All irradiated samples, as well as the untreated controls, showed strong blue staining, equivalent to vitality, as an enzymatic conversion of NBTC took place. This blue coloration could be detected both after direct singular UVB irradiation and after singular UVC-222nm irradiation with and without filters, that is, the full-thickness skin constructs are vital and are enzymatically active.

The resulting DNA damage after UVC exposure was assessed by measuring the resulting CPD formation in the skin reconstructs. The data are summarized in Fig. 2. The DNA damage after a single UVC irradiation with 100 mJ cm^{-2} showed a tendency for one- and two-filters-inserts to reduce the number of detected CPD in ng ml^{-1} compared with UVC irradiation without the use of filters (Fig. 2A). However, the number of CPD (ng ml^{-1}) of UVC-treated samples in combination with one or two blocking filters were not significantly raised above the background of untreated controls. There was a tendency of an increased number of CPDs in samples treated with UVC without filter-inserts. Compared with them, the samples treated with 100 mJ cm^{-2} UVB (positive control, 308 nm) induced CPDs in a significantly higher number compared with the background signal of untreated controls. Statistical analyses are shown in Table S1 in the Supporting Information.

In the case of a single UVC irradiation with 500 mJ cm^{-2}, the use of one or two blocking filters significantly reduced the number of detected CPD in ng ml^{-1} compared with unfiltered far-UVC 222-nm KrCl exciplex radiation (Fig. 2B and Table S2 in the Supporting Information). After a single UVB irradiation (positive control, 308 nm) with 500 mJ cm^{-2}, a significantly higher number of CPD products was measured compared with the unfiltered UVC source, as expected. The corresponding evaluation is shown in Table S2 in the Supporting Information. The individual statistical evaluation showed that the number of CPD did not increase significantly after a single UVC irradiation with 500 mJ cm^{-2} using one or two filters compared with the background signal of untreated controls. That is, the skin constructs were protected by the use of the filters and these samples did not show a detectable increase in CPD. On the other hand, UVC irradiation alone (without any filters) showed a significant increase in CPD compared with the background signal of untreated controls. As expected, the UVB irradiation of the skin reconstructs also showed a significant increase in CPD (Fig. 2B).

As seen in Fig. 2C, in the case of repetitive UVC irradiations of the full-skin constructs with 500 mJ cm^{-2} the number of detected CPD in ng ml^{-1} could be significantly reduced by the use of one or two filters compared with UVC irradiation alone. A repetitive UVB irradiation (positive control, 308 nm) with 3×500 mJ cm^{-2} also showed an expected significantly higher number of measured CPD products compared with UVC irradiation. The corresponding statical evaluation is shown in Table S3 in the Supporting Information. Overall, the use of one or two filters did not show significant increase in CPD in the skin samples compared with the background signal of untreated controls and thus protected the skin samples from additional DNA damage. In contrast, there was a significant increase in CPD (ng mL^{-1}) after UVC irradiation alone compared with the number of detected CPDs in the un-irradiated control.

Visualizing UVC 222 nm penetration in human skin reconstructs and the protective effects of 1/2-Filters

To visualize the penetration depth of UV radiation into the skin, an immunohistochemistry (IHC) staining was performed on cryosections of skin reconstructs using an anti-CPD-antibody (CPD-ELISA Kit; Cell Biolabs Inc.). As shown in Fig. 3, the penetration and amount of CPD detected by IHC staining correspond to the data obtained from CPD-ELISA in Fig. 2. Again, the UVB

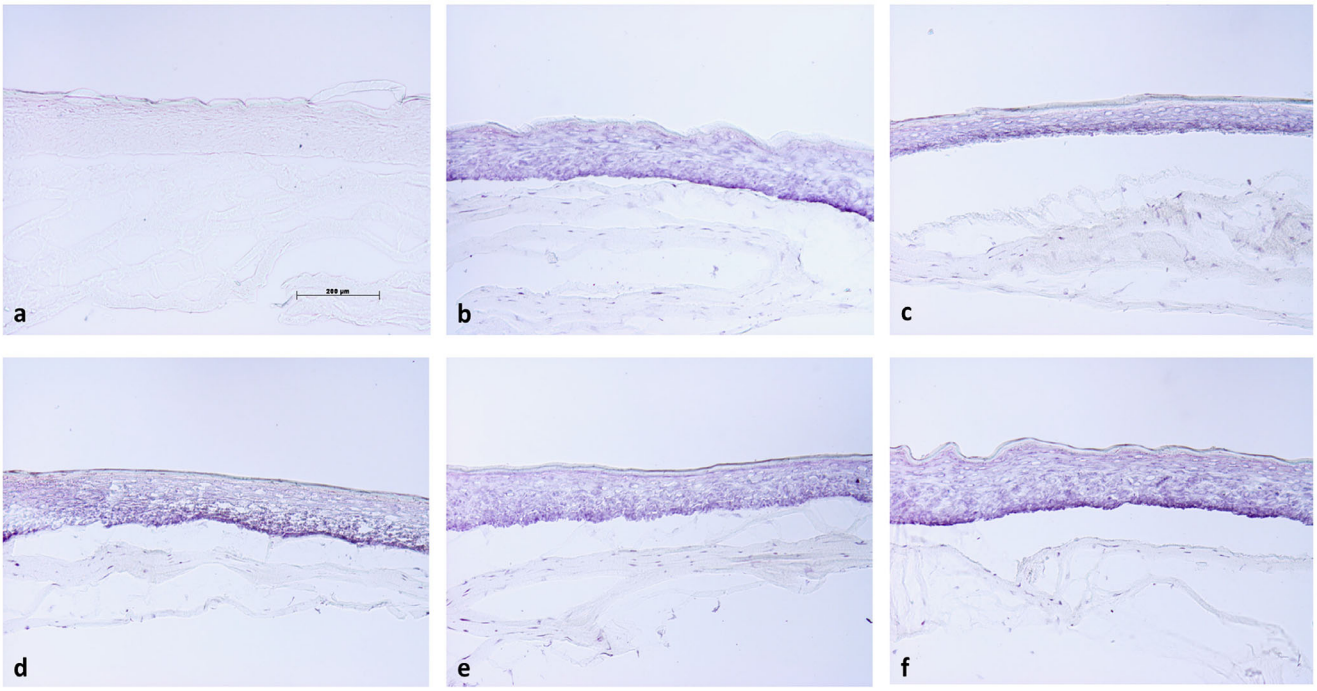

Figure 1. The viability upon 3 × 500 mJ cm^{-2} UVC 222 nm or UVB treatment was measured with NBTC staining. NBTC vitality staining of human skin reconstructs with blue staining indicating viable cells. (A) Negative control treated with 70°C heat for 1 h and 15 min and afterwards with formalin for 15 min. (B) Un-irradiated control. (C) Skin reconstruct irradiated with 3 × 500 mJ cm^{-2} UVB (308 nm). (D) Skin reconstruct irradiated with 3 × 500 mJ cm^{-2} far-UVC (222 nm). (E) Skin reconstruct irradiated with 3 × 500 mJ cm^{-2} far-UVC (222 nm) and 1 Filter. (F) Skin reconstruct irradiated with 3 × 500 mJ cm^{-2} far-UVC (222 nm) and 2 Filter. All irradiations and the un-irradiated control show a strong reaction to the NBTC reagent, which indicates viable tissue. There is a clear difference between the vital tissue (B–F) and the formalin-fixed negative control (A). The images are representative of $n = 3$ replicates per condition and were taken with a Zeiss microscope (10× magnification).

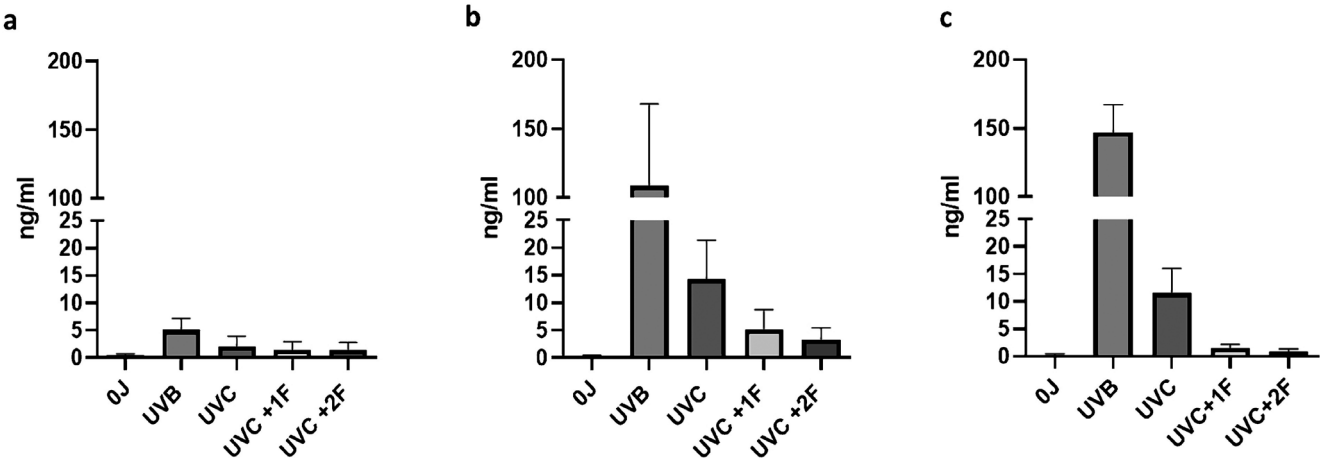

Figure 2. The amount of DNA damage induced by UVC 222 nm irradiation in human skin reconstructs can be reduced when filters are used. CPD detection after irradiation of skin reconstructs measured by a colorimetric ELISA. (A) The human skin reconstructs were exposed to a single dose of UVC irradiation (100 mJ cm^{-2}) with and without different filters or UVB (308 nm) irradiation (100 mJ cm^{-2}). The amount of damage induced with UVB irradiation is higher than the amount of damage induced by non-filtered far-UVC irradiation and the amount of DNA damage in filtered far-UVC irradiation is significantly lower than in non-filtered samples. Data are presented as mean value with standard deviation of at least three independent experiments. (B) The human skin reconstructs were exposed to a single dose of far-UVC irradiation (500 mJ cm^{-2}) with and without different filters or UVB (308 nm) irradiation (500 mJ cm^{-2}). The amount of damage induced with UVB irradiation is higher than the amount of damage induced by non-filtered UVC irradiation and the amount of DNA damage in filtered UVC irradiation is significantly lower than in non-filtered samples. Data are presented as mean value with standard deviation of at least three independent experiments. (C) The human skin reconstructs were treated with far-UVC (222 nm) repetitively (500 mJ cm^{-2}) three times with and without different filters or UVB (308 nm) irradiation (500 mJ cm^{-2}). The amount of damage induced with UVB irradiation is higher than the amount of damage induced by non-filtered far-UVC irradiation and the amount of DNA damage in filtered far-UVC irradiation is significantly lower than in non-filtered samples. Data are presented as mean value with standard deviation of at least three independent experiments.

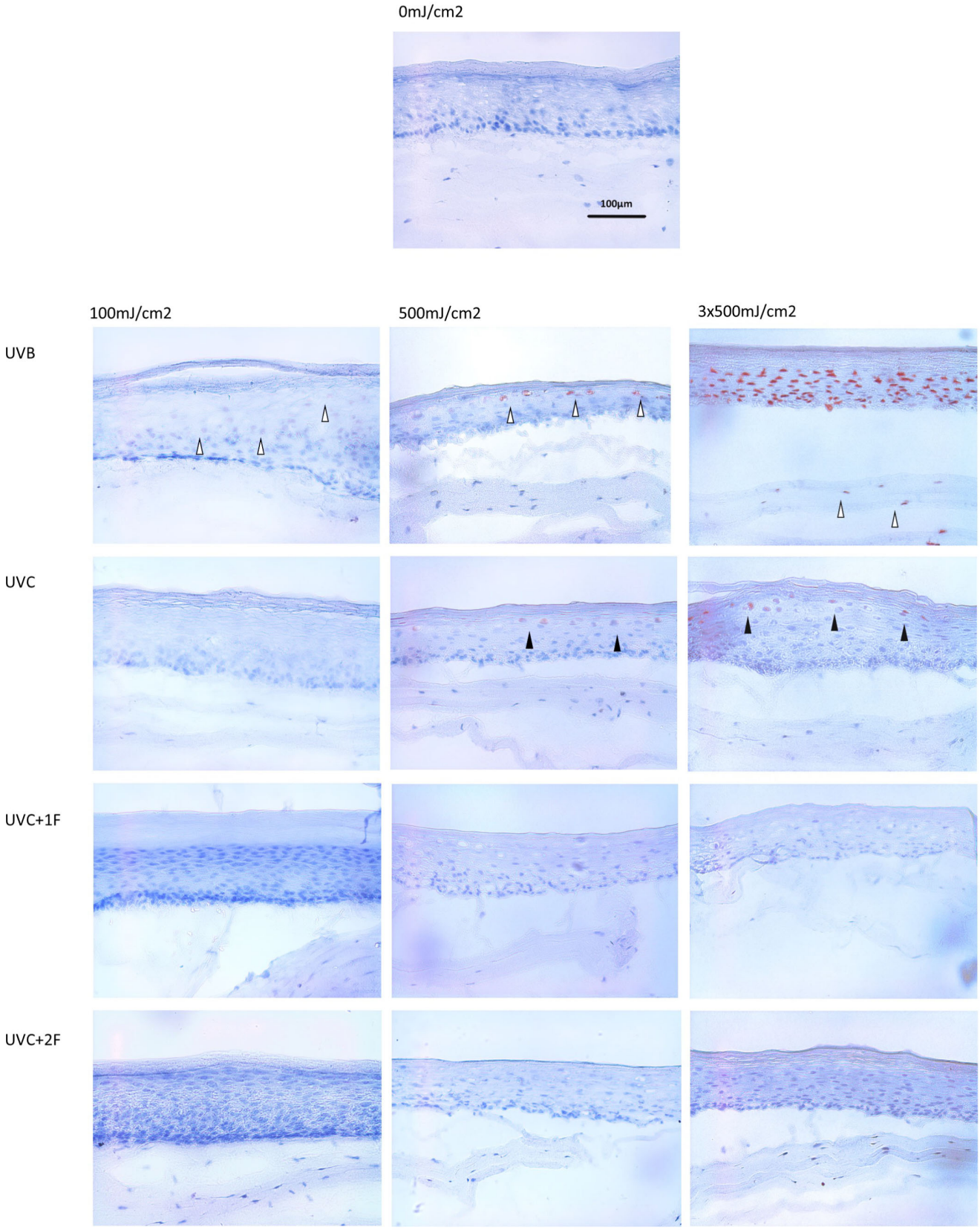

Figure 3. IHC staining of frozen skin reconstructs to visualize CPD-penetration depth. The IHC staining of skin reconstruct cryosections support the CPD-ELISA findings described above. UVB, as expected, induced highest amounts of CPD with increasing irradiation doses leading to increased DNA damage and damage-penetration depth (white arrowheads). Unfiltered far-UVC induced detectable levels of CPD for the first time at a single 500 mJ cm^{-2} irradiation treatment with DNA damage increasing after repetitive treatments (black arrowheads). The use of one or two filters during 222 nm UVC irradiation resulted in no detectable DNA damage independent of the applied irradiation dose.

treatment caused the highest amount of CPD within the epidermis (Fig. 3, UVB image panel) combined with the deepest penetration depth, where positive CPD signals could be detected. At the highest UVB irradiation intensity (3 × 500 mJ cm^{-2}), the detected DNA damage spread beyond the epidermis and into the dermis of the skin reconstruction. Irradiating of the skin reconstructs with unfiltered far-UVC radiation also resulted with gradual increase in CPDs with higher irradiation doses. Compared with UVB-treated samples, however, the damage was localized only in the upper layers of the epidermis (Fig. 3, UVC image panel). Compared with that, the irradiation with UVC 222nm in combination with one/two filters (1F/2F) resulted in no detectable CPD formation even at the highest irradiation doses of 3 × 500 mJ cm^{-2} (Fig. 3, UVC+1F or UVC+2F image panel). These results additionally support the safety of filtered far-UVC radiation.

Filtered far-UVC radiation is still able to attenuate bacterial and fungal growth despite the protective effects shown by the filters itself

Since the application of one or two filters during UVC irradiation greatly reduced the formation of CPDs, it was important to

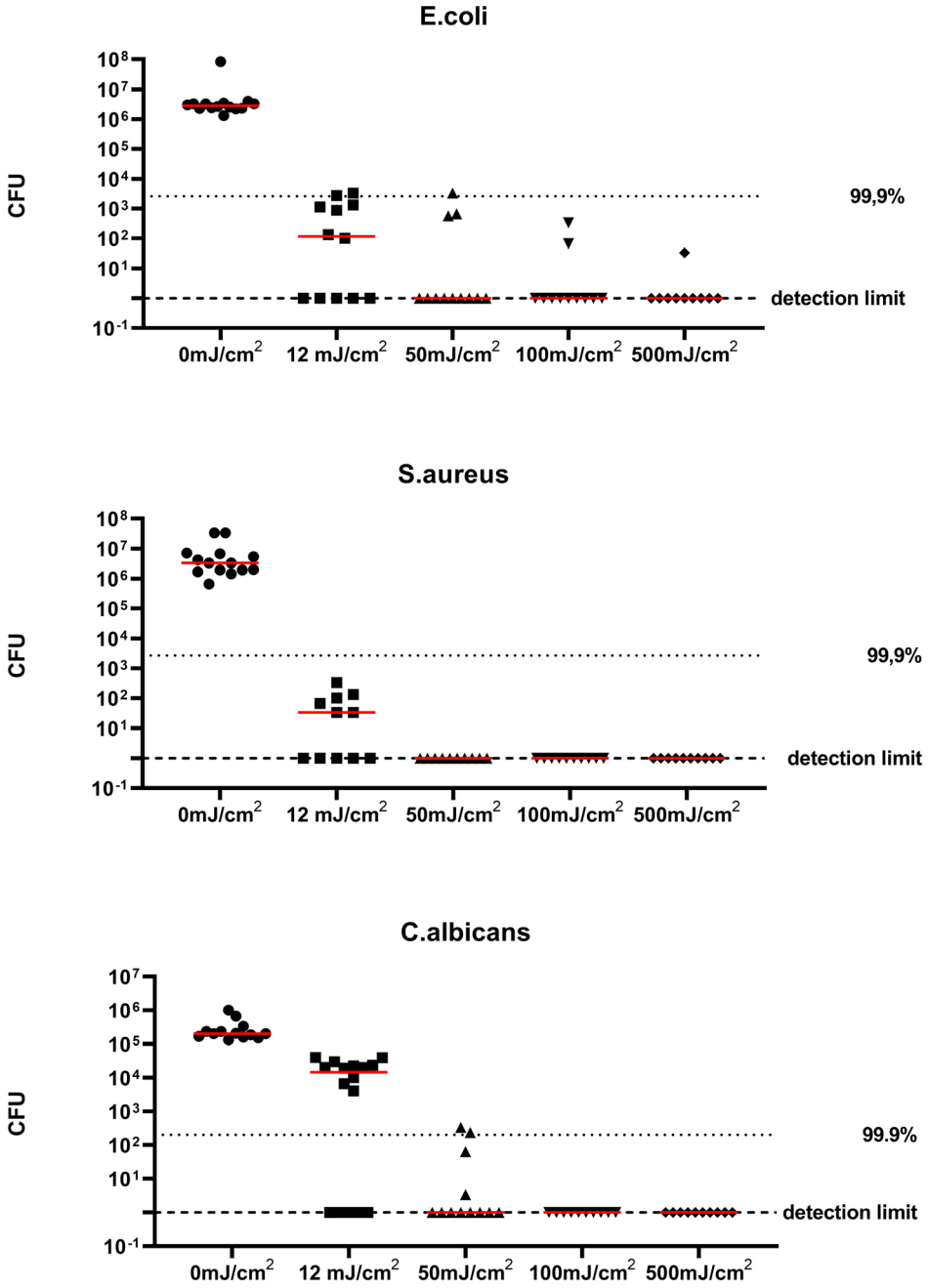

Figure 4. Filtered far-UVC retained its antibacterial properties. In the case of *E. coli* and *S. aureus* irradiation dose of 12.5 mJ cm^{-2} proved sufficiently effective to kill 99.9% of bacteria. For *C. albicans*, the antifungal dose at which colony formation was reduced by 99.9% was 50 mJ cm^{-2}. All results are depicted as medians (red line) on a $\log_{10}$-scaled ordinate. Median of CFUs on or below the black dotted line shows an antimicrobial effect of ≥99.9% (n = 5 independent experiments).

investigate whether the use of such filters in combination with UV 222nm has antimicrobial effects against bacteria and fungi. Two species of bacteria (*E. coli* and *S. aureus*) and one fungus (*C. albicans*) were used to test filtered far-UVC as an alternative means for disinfection. For the following tests, the irradiations were performed with 222 nm UVC in the presence of two filters (2F), because the use of these filters had the best protective function.

As shown in Fig. 4, there is steady reduction of colony formation for both *E. coli* and *S. aureus,* as well as for *C. albicans* with increasing irradiation doses. A reduction of 99.9% (antimicrobial effect) could be achieved after the application of 12 mJ cm^{-2} for *E. coli* and *S. aureus* and after 50 mJ cm^{-2} for *C. albicans*. Even doses below 100 mJ cm^{-2}, which proved to be on the low end of CPD induction in the human skin reconstruct (as shown in Fig. 2), resulted in significant antimicrobial reduction of 99.999% (disinfection) in bacterial and fungal growth.

DISCUSSION

This study shows that improved spectral purity of far-UVC 222 nm irradiation by using silica filters largely prevents induction of damaging CPD lesions human skin reconstructs even at high and repetitive dosages. The doses for this study, namely, 100 mJ cm^{-2}, 500 mJ cm^{-2} and 3 × 500 mJ cm^{-2} were chosen for their clinical relevance in bacterial disinfection (100 mJ cm^{-2} (7,8)) and fungal neutralization (500 mJ cm^{-2} (8)), or to test the equipment safety (3 × 500 mJ cm^{-2}).

The treatment with UVC (222 nm) irradiation with a dose of 100 mJ cm^{-2} caused no significant amount of CPD compared with untreated control in both filtered and unfiltered applications. This is in accordance with the already published data by Buonanno *et al*. (7), showing that irradiation with 222 nm UVC in the range of 50–150 mJ cm^{-2} does not induce a significant increase in CPDs. However, unlike Narita *et al*. and Yamano *et al*., we were able to observe a significant increase in CPDs after irradiation with a single UVC dose of 500 mJ cm^{-2}, and after repetitive treatments with 3 × 500 mJ cm^{-2}, in samples without filter protection (8,19). These observations are more in line with the data published by Fukui *et al*. (23). We see this discrepancy in safety assessment of UVC 222 nm as the result of the more sensitive ELISA method used in this work to quantify the amount of CPDs formed after irradiation. Using the described experimental setup, we are, to our knowledge, the first who could describe the quantitative CPD amount (ng mL^{-1}) upon UVC-treatment (222 nm) of human skin reconstructs.

When comparing the results of UVB irradiation with doses of 100 mJ cm^{-2}, 500 mJ cm^{-2} and 3 × 500 mJ cm^{-2} to the ones after UVC 222 nm treatment, we observed a higher amount of CPD in UVB-treated samples compared with UVC-treated samples both with and without filters. This could be due to the higher penetration of UVB than UVC in human skin (24,25). While most of the UVC irradiation is absorbed by the keratin layers of the upper epidermis, a large amount of UVB irradiation can pass the upper epidermal layer and penetrate the deeper epidermal layer and the basal layer of the skin (26), which is of relevance to the formation of skin tumors like SCC and BCC (27,28). This was supported by our own IHC staining for CPD and is in accordance with the findings of Yamano *et al*. and Barnard *et al*. (29,30). Considering that the amount of CPDs induced by UVC is approximately 10 times lower than the one after UVB irradiation, our findings support the relative safety of 222 nm UVC as a disinfection source. Compared with the already published data (Buonano, Narita, etc.), we could show for the first time that repetitive UVC irradiation both with and without filters did not lead to accumulation of CPDs compared with the UVB treatment. The application of filters further reduced any potentially damaging effects on the skin.

In all irradiation setups (100, 500 and 3 × 500 mJ cm^{-2}), the filtered samples had lower CPD levels than the skin reconstructs irradiated without a filter. Furthermore, the filtered samples showed no significant increase in CPDs compared with control samples, showing the protective effects of the silica filters against UVC-induced DNA damage in human skin reconstructs. This was in accordance with previous findings by Buonanno *et al*. and Hickerson *et al*. (31,32). When UVC irradiation is filtered, the amount of CPD decreases in correspondence with the number of filters used, indicating that the decrease in residual radiation over 222 nm is crucial for ensuring the safe application of UVC in the clinical environment. In addition, we showed that the use of filters did not hinder the antimicrobial properties of 222 nm UVC. Filtered far-UVC showed an antimicrobial effect (bacterial reduction in excess of 99.9%) against *E. coli*, *S. aureus* and *C. albicans*. Furthermore, a therapeutic window was identified, where microorganisms were successful inactivated, but skin is still alive and intact without any clinically observable damage.

In conclusion, this study demonstrated that filtered far-UVC radiation retains its antimicrobial effect (bacterial reduction in excess of 99.9%) against *E. coli*, *S. aureus* and *C. albicans*. Furthermore, a therapeutic window was identified, where filtered far-UVC doses with this antimicrobial effect showed no significant harmful effect on human skin, which remained intact without any clinically observable damage. Furthermore, we have shown that repetitive irradiation with filtered far-UVC do not lead to accumulation of CPDs compared with UVB treatments. Overall, these findings are important for clinical applications of UVC 222 nm radiation.

Acknowledgements—This work has been partially funded by GME GmbH (Erlangen, Germany). Open access funding enabled and organized by ProjektDEAL.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. NBTC vitality staining of human skin reconstructs with blue staining indicating viable cells (1 × 100 mJ cm^{-2}).

Figure S2. NBTC vitality staining of human skin reconstructs with blue staining indicating viable cells (1 × 500 mJ cm^{-2}).

Table S1. Statistical analysis of CPD data from 1 × 100 mJ irradiation of skin reconstructs.

Table S2. Statistical analysis of CPD data from 1 × 500 mJ irradiation of skin reconstructs.

Table S3. Statistical analysis of CPD data from 3 × 500 mJ irradiation of skin reconstructs.

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