Research article

Irradiation of UVC LED at 277 nm inactivates coronaviruses in association to photodegradation of spike protein

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ABSTRACT

To interrupt SARS-CoV-2 transmission chains, Ultraviolet-C (UVC) irradiation has emerged as a potential disinfection tool to aid in blocking the spread of coronaviruses. While conventional 254-nm UVC mercury lamps have been used for disinfection purposes, other UVC wavelengths have emerged as attractive alternatives but a direct comparison of these tools is lacking with the inherent mechanistic properties unclear. Our results using human coronaviruses, hCoV-229E and hCoV-OC43, have indicated that 277-nm UVC LED is most effective in viral inactivation, followed by 222-nm far UVC and 254-nm UVC mercury lamp. While UVC mercury lamp is more effective in degrading viral genomic content compared to 277-nm UVC LED, the latter results in a pronounced photo-degradation of spike proteins which potentially contributed to the higher efficacy of coronavirus inactivation. Hence, inactivation of coronaviruses by 277-nm UVC LED irradiation constitutes a more promising method for disinfection.

1. Introduction

The novel coronavirus SARS-CoV-2 has precipitated into the COVID-19 pandemic, and at the time of writing, resulted in more than millions of infections and deaths. The actual numbers should be much higher than reported, given the high incidence of asymptomatic cases escaping the capture by traditional diagnostic methods. Vaccination, masking, rigorous testing and thorough public disinfection strategies become vital prongs in combating virus spread within communities. Amongst the latter, ultraviolet irradiation presents as an attractive strategy, given its use being well established in inactivating viruses and killing other microbes (Lin et al., 2020; Raeiszadeh and Adeli, 2020). Consequently, UVC mercury lamps have been increasingly deployed in hospital settings.

UVC has been well known to possess germicidal properties and inactivate pathogenic microbes by damaging nucleic acids and proteins, thereby eliminating their ability to reproduce (Rauth, 1965; Sehgal, 1973; Setlow and Carrier, 1966; Werbin et al., 1966). The mechanism at which UV inactivates microbes depends highly on the specific wavelengths. 277nm UVC LEDs (Beck et al., 2017; Kim and Kang, 2018; Nguyen et al., 2019; Nunayon et al., 2020) and far UVC sources (Welch et al., 2018a, 2018b) have recently emerged as attractive alternatives to UVC mercury lamps. The former does not require mercury, which is banned by the Minamata Convention, has very short turn-on time, and is generally more reliable and has a longer lifetime (Song et al., 2016). The far UVC, on the other hand, is shown to be effective in inactivation of bacteria and human coronaviruses, and potentially poses less safety concerns for deployment (Barnard et al., 2020; Buonanno et al., 2020).

Numerous studies have studied the sensitivity of different microbes to UVC wavelengths, including human coronaviruses (Buonanno et al., 2020; Gerchman et al., 2020) and SARS-CoV-2 (Inagaki et al., 2020; Kitagawa et al., 2020; Storm et al., 2020). However, no study to date has performed a direct comparative study on the efficacy of different UVC wavelengths on inactivation of coronaviruses. In addition, mechanistic insight into how different UVC wavelengths inactivate coronaviruses is severely lacking, and greater understanding in this area would facilitate their deployment in future pandemics. Here, we utilized human coronaviruses, HCoV-229E and OC43, for efficacy studies where 277nm UVC LED consistently outperforms the other UVC wavelengths in inactivating...
Coronaviruses. Mechanistic studies suggest that this is achieved via a combination of photo-degradation of spike proteins and RNA molecules.

2. Results

2.1. Utilizing human coronaviruses for UVC-induced inactivation studies

Coronaviruses are split into the four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus (Fehr and Perlman, 2015; Woo et al., 2010). Among the different genera, alphacoronaviruses and betacoronaviruses have been known to infect mammals, and pose a significant risk to the human population. The betacoronaviruses, MERS-CoV, SARS-CoV and SARS-CoV-2, may produce severe symptoms in patients while hCoV-OC43 and hCoV-229E cause about 15% of common colds (Figure 1A). In terms of genomic organization, coronaviruses are the largest enveloped RNA viruses with positive single-stranded RNA molecules from 27 to 32 kilobases. The genome comprises of the replicase gene that encodes for the non-structural proteins of the genomes at about 20 kilobases, while similar structural proteins in the form of spike, envelop, membrane and nucleocapsid proteins are interspersed at the 3’ end of the genome (Figure 1B).

It has been established that RNA chains are directly disrupted by UVC by formation of pyrimidine dimers (Merriam and Gordon, 1967). The dimerization reaction occurs from adjacent pyrimidine bases in the form of uracil and cytosine (Beukers et al., 2010; Brown and Johns, 1968). We analyzed the genomic content of the coronaviruses based on these sequences as indicated their accession numbers from the NCBI Nucleotide: hCoV-OC43 (MW532119.1), hCoV-229e (KU291448.1) and SARS-CoV-2 (MW403500.1). We found that their overall base composition (Figure 1C) and adjacent base arrangements (Figure 1D) to be similar. We therefore hypothesize that the kinetics of UVC inactivation of coronaviruses are interspersed at the 3’ end of the genome (Figure 1B).

To examine the inactivation efficacy of UVC on hCoV-OC43 and hCoV-229E, virus was placed on plastic petri dishes and expose to various UVC wavelengths of 73 μW/cm² for different timings ranging from 30 to 300 s (Figures S1–S2). The reduction in infectivity of hCoV-OC43 (Figure 2A, Table S1) and hCoV-229E (Figure 2B, Table S2) can be observed after exposure to different UVC irradiation. The 277-nm UVC LED was most effective in carrying out the inactivation and achieved 3-log inactivation at 22 mJ/cm² for both human coronavirus strains, whereas 254-nm UV lamp achieved only 2-log inactivation for hCoV-OC43 and 1-log inactivation for hCoV-229E with the same dosage. Two-way ANOVA analyses of both sets of data reveal significant differences (Table S1-2) between the three UVC wavelengths in carrying out coronavirus inactivation (p = 0.0001 and p < 0.0001 for hCoV-OC43 and hCoV-229E respectively).

We next investigated the integration of hCoV-OC43 in human lung host cells after exposure to 300 s of UVC sources. Figure 2C shows the representative images of human lung cells HCT-8 with hCoV-OC43 illuminated at different UVC wavelengths. We assessed the human cell lines for expression of the viral spike protein and found that 277-nm UVC outperforms the other UVC wavelengths in inactivation of coronaviruses.

2.2. Inactivation of human coronaviruses after exposure to different UVC wavelengths

To the end, we subjects hCoV-OC43 to different duration of 254-nm and 277-nm UVC irradiation, and observed through Western blot that the spike protein is degraded under 277-nm UVC LED and not under 254-nm UVC lamp. Silver staining of the viral lysate indicates the overall amount of protein loaded in each lane (Figure 3B). To further confirm that spike protein is indeed degraded by 277-nm UVC LED, we performed UVC illumination on purified hCoV-OC43 spike protein in vitro. Silver staining as depicted in Figure 3C shows that hCoV-OC43 spike protein presents at a lower intensity under 277-nm UVC LED and not under 254-nm UV lamp. This is further corroborated by absorbance spectroscopy in Figure 3C which demonstrates a shift in absorbance upon 277-nm UVC LED illumination but not 254-nm UV lamp.

2.3. Examining rates of nucleotide degradation under different UVC wavelengths

To understand if the inactivation efficacy comes from RNA damage, we performed quantitative RT-PCR to examine the copy number of hCoV-OC43 after UVC irradiation. We observed that the copy number of hCoV-OC43 to be unperturbed after 300 s of 222-nm far UVC irradiation, while copy number of hCoV-OC43 after 254-nm UV lamp exerted the largest decrease in copy number followed by 277-nm UVC LED (Figure 3A).

2.4. Photodegradation of hCoV-OC43 spike protein under 277-nm UVC LED

We next hypothesized that molecular components other than nucleic acids could be implicated, and the spike protein is an especially attractive target to pursue given that it facilitates viral transmission by binding to the host receptors (Shang et al., 2020). To this end, we subjected hCoV-OC43 to different duration of 254-nm and 277-nm UVC irradiation, and observed through Western blot that the spike protein is degraded under 277-nm UVC LED and not under 254-nm UVC lamp. Silver staining of the viral lysate indicates the overall amount of protein loaded in each lane (Figure 3B). To further confirm that spike protein is indeed degraded by 277-nm UVC LED, we performed UVC illumination on purified hCoV-OC43 spike protein in vitro. Silver staining as depicted in Figure 3C shows that hCoV-OC43 spike protein presents at a lower intensity under 277-nm UVC LED and not under 254-nm UV lamp. This is further corroborated by absorbance spectroscopy in Figure 3C which demonstrates a shift in absorbance upon 277-nm UVC LED illumination but not 254-nm UV lamp.

Figure 1. Utilizing human coronaviruses for UVC-induced inactivation studies (a) The different genera of the coronavirus family. Alpha and beta-coronaviruses with the various highlighted viruses, hCoV-229e, hCoV-OC43 and SARS-CoV-2. (b) Genome organizations of SARS-CoV-2, hCoV-OC43 and hCoV-229E. (c) Overall base composition of SARS-CoV-2, hCoV-OC43 and hCoV-229E. (d) Adjacent base composition of SARS-CoV-2, hCoV-OC43 and hCoV-229E.
2.5. Photodegradation of SARS-CoV-2 spike protein under 277-nm UVC LED

First, we exposed SARS-CoV-2 spike protein S1 subunit to varied durations of 254-nm UVC lamp and 277-nm UVC LED and observed the reduction of the full-length spike protein band under 277-nm UVC LED and not under 254-nm UVC LED. This happens on both glycosylated and non-glycosylated forms of the spike protein (Figure 4A). Absorbance spectroscopy (Figure 4B) analysis showed the UV absorbance profile of 254-nm lit proteins to be relatively unchanged while there is an increase of absorbance profile in the 250–300 nm region for 277-nm lit proteins. Western blot (Figure 4C) analysis further revealed a reduction in the SARS-CoV-2 spike S1 proteins under low loading of protein samples, but under higher loadings, aggregates of spike protein in the form of dimers and trimers could be seen in a dose-dependent manner.

In the search for potential mechanisms that drive the absorption of 277-nm wavelengths and degradation/aggregation of the proteins, we studied the structure of the SARS-CoV-2 S glycoprotein (PDB: 6VXX) (Walls et al., 2020) and looked for aromatic amino acids in proximity to disulfide bonds as possible active regions upon 277-nm UVC irradiation. In particular, tryptophan has high molar absorptivity at the 280-nm wavelength and has been known to mediate energy transfer and neighboring disulfide bond breakage (Beaven and Holiday, 1952; Chan et al., 2006; Kerwin and Remmele, 2007; Wu et al., 2008). We identified the Trp 436 as a key antenna of 277-nm absorption, and conducted studies on W436R receptor binding domain (RBD) mutant alongside wild type, F377L and Y453F...
mutants, that are in close proximity but unlinked to the W436–C336–C361 transfer chain (Figure 4D). We illuminated the RBD samples with 0, 2, 5, 10 min of 277-nm UVC LED and studied the rate of oligomerization for each sample by probing with SARS-CoV-2 Spike antibody (Figure 4E). It is observed that W436R mutant has a lower rate of aggregation compared to the other species and this is quantified by comparing the intensity of the monomer fraction (at 35kDa) with respect to the rest of the lane for samples illuminated with 5 min of 277-nm UVC LED (Figure 4F). Absorbance spectroscopy further verified that after 10 min of UVC LED illumination, the W436R mutant did not exhibit as significant changes in absorbance compared to the wild-type and Y453F mutant.

3. Discussion

In this study, we focused on human coronaviruses hCoV-OC43 and hCoV-229E, which belong to the genus beta-coronavirus and alpha-coronavirus respectively. In particular, hCoV-OC43 could be considered as a surrogate for SARS-CoV-2 and the conclusions drawn here on UVC efficacy can thus be extrapolated to SARS-CoV-2. On the other hand, hCoV-229E resembles the viruses that causes the common cold. The viral efficacy tests performed here not only targeted the current COVID-19 pandemic, but also applies to future coronaviral pandemics in general.

While many studies have individually tested the 222-nm far UVC lamp, 254-nm UVC lamp and broad ranges of UVC LEDs for their efficacy towards viruses, this is the first study to report the mechanisms through which viral inactivation occurs. In summary, we find that 277-nm UVC LED outperforms the other UVC wavelengths in inactivation of human coronaviruses, and this could be aided by the contribution from spike protein degradation with absorption of UV wavelengths at Trp 436. We also find that 222-nm UVC LED does not affect the genomic material of beta-coronavirus, an observation that is congruent with a previous report (Kitagawa et al., 2020).
It has been widely believed that the efficacy of UV germicidal irradiation (UVGI) is dependent largely on the absorption by the target nucleic acids. While the mechanism holds significant merit, it is important to examine the other molecular mechanisms at which UV tools could exert their germicidal properties. It is thus important to consider the viral components individually as we characterize the multitude of UVGI solutions available to combat the current and future pandemics.

4. Limitations of study

Due to the lack of access to BSL-3 facilities, we are unable to perform the viral infectivity tests on SARS-CoV-2 or the relevant variants. However, this limitation is mitigated by our studies on the beta coronavirus hCoV-OC43, which provides a close approximation towards SARS-CoV-2 with the relevant structures of the spike proteins being relatively similar.

Declarations

Author contribution statement

Qunxiang Ong, Ph. D: Conceived and designed the research; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

J.W. Ronnie Teo: Conceived and designed the research; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Joshua Dela Cruz and Winson Wee: Performed the experiments; Analyzed and interpreted the data.

Elijah Wee: Performed the experiments.

Weiping Han: Conceived and designed the research; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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Materials and Methods

Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Anti-Coronavirus OC43 Spike Protein antibody | Cusabio | CSB-PA336163EA01H1Y |
| SARS-CoV-2 Spike RBD antibody | Sinobiological | 40592-T62 |
| Bacterial and virus strains |        |            |
| HCoV-229E           | ATCC   | VR-740     |
| HCoV-OC43           | ATCC   | VR-1588    |
| Chemicals, peptides, and recombinant proteins |        |            |
| EMEM                | ATCC   | 30–2003    |
| RPMI-1640           | ATCC   | 30–2011    |
| Purified recombinant spike proteins | Sinobiological | 40592-V08H180, 40591-V08H13, 40592-V08H9, 40592-V08H27, 40591-V08H, 40607-V08H1 |
| Carboxymethylcellulose | Sigma Aldrich | C4888 |
| Critical commercial assays |        |            |
| QIAmp Viral RNA mini kit | Qiagen |        |
| TaqPath 1-step RT-qPCR master mix CG | Applied Biosystems |        |
| Pierce™ Silver Stain Kit | Thermofisher |        |
| Deposited data      |        |            |
| SARS-CoV-2 Spike Protein | PDB | 6VXX |
| hCoV-OC43           | NCBI Nucleotide database | MWS32119.1 |
| hCoV-229e           | NCBI Nucleotide database | KU291448.1 |
| SARS-CoV-2           | NCBI Nucleotide database | MW403500.1 |

(continued on next page)
Viral infectivity experiments

The virus was propagated as previously described and stored in virus infection medium at 10^8 PFU/ml. In one sitting, we have cultured 10 plates of MRC-5 or HCT-8 cells at 10 million cells per T175 flask, and the viruses hCoV-229e or hCoV-OC43 are propagated for 60 h and clarified by centrifugation. The initial PFU was then derived via the following experimental steps. MRC-5 cells or HCT-8 cells were seeded in 12-well plates and incubated at 37°C, 5% CO2 for 3 days. Fixation with 4% paraformaldehyde was performed by centrifugation. The initial PFU was then derived via the following experimental steps. MRC-5 cells or HCT-8 cells were seeded in 12-well plates and incubated at 37°C, 5% CO2 until 80–90% confluent. Cells were inoculated with serial 10-fold dilutions of each virus sample in triplicate. The inoculum was adsorbed for 1 h at 37°C, 5% CO2 with periodic shaking every 10 min. The inoculum was then removed and 1% methylcellulose (Millipore Sigma) in DMEM with 2% heat-inactivated FBS was added. The plates were incubated at 37°C, 5% CO2 for 3 days. Fixation with 4% paraformaldehyde was performed at room temperature for an hour. Staining with 0.5% crystal violet was then conducted and the plaques are then quantified. Plates were air-dried, plaques enumerated and titre calculated. For each irradiation, 100 µl of virus suspension was placed on a 3-cm Petri dish. After each irradiation, the virus was subjected to serial dilution of 5 times, and 50 µl of each condition is diluted with 450 µl of virus infection medium into 24-hour old and 80–90% confluent MRC-5 or HCT-8 cells in 6-well plates. The cells were incubated with the virus for 1 h in a humidified incubator with 5% CO2 before addition of a liquid overlay medium, 3% carboxymethylcellulose, is applied to the cells to restrict virus growth to the originally infected loci of cells. The cells are incubated at 37°C in a 5% CO2 incubator for 3 days before the liquid overlay medium is aspirated and fixed with 4% paraformaldehyde at room temperature for 15 min and washed with PBS before being labelled with anti-Coronavirus OC43 Spike Protein antibody (CusaBio Technology LLC, Houston, TX, USA) 1:500 in PBS containing 2% FBS or horse serum for hCoV-229E and hCoV-OC43 respectively.

Immunofluorescence experiments

To assess whether 5 min of various UVC illumination reduces the number of infected cells, immunostaining was performed to detect the presence of OC43 viral particles in the host human cells. Briefly, 2 × 10^5 HCT-8 cells were plated in each Petri dish one day before the experiment. The virus suspension after their respective treatments was overlaid on the monolayer of host cells. After 1 h of incubation, the cells were washed with PBS and incubated for two days in fresh medium. The cells were then fixed with 4% paraformaldehyde at room temperature for 15 min and washed with PBS before being labelled with anti-Coronavirus OC43 Spike Protein antibody (CusaBio Technology LLC, Houston, TX, USA) 1:500 in PBS containing 2% FBS or horse serum for hCoV-229E and hCoV-OC43 respectively.

UV sources and irradiance measurement

To understand the effect of UVC wavelength on human coronaviruses, three different UVC light sources – 222-nm far UVC lamp (Ushio), 254-nm UV mercury lamp (Sankyo Denki G8T5) and 277-nm UVC LED (Lextar PU35CM1) were used in this study. These UVC light sources are measured using a calibrated spectroradiometer (GL Spectis 4.0) with an absolute measurement uncertainty of less than 6%. To provide a comparative UVGI efficacy study between these UVC light sources, the radiant intensity of the far UVC and mercury lamp is measured at different distances while the UVC LEDs are driven at different constant drive currents to obtain a common UV intensity of 73 μW/cm². The UVC LED, with a beam angle of 120°, is assembled into a 5 × 5 array at a working distance of 12 cm to ensure uniform UV intensity across the surface of the Petri dish. The relative wavelength spectra of the light sources are shown in Figure S1. Based on the UV intensity readings, an enclosure is fabricated for each light source and the radiant intensity of the enclosure is further validated with the spectroradiometer as shown in Figure S2.

Viral strains and viral propagation

hCoV-229E (ATCC VR-740) and hCoV-OC43 (ATCC VR-1558) were propagated in human lung fibroblasts MRC-5 (ATCC CCL-171) and colon adenocarcinoma cells HCT-8 (ATCC CCL-244) respectively (all from ATCC, Manassas, VA). The MRC-5 fibroblasts were grown in EMEM (ATCC 30–2003) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO). The HCT-8 epithelial cells were cultured in RPMI-1640 supplemented with 10% horse serum, 100 U/ml penicillin and 100 μg/ml streptomycin. The virus infection medium is made up of EMEM or RPMI-1640 with 2% FBS or horse serum for hCoV-229E and hCoV-OC43 respectively.

Software and algorithms

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| ImageJ              | Schneider et al., 2012 | https://imagej.nih.gov/ij/ |
| GraphPad Prism Software 8.4.3 | GraphPad Inc. | |
| Quantstudio 5 software | Applied Biosystems | |
| Other               |        |            |
| 222-nm far UVC lamp | Ushio   | N.A.       |
| 254-nm UVC mercury lamp | Sankyo Denki | G8T5 |
| 277-nm UVC LED      | Lextar  | PU35CM1    |
| Spectroradiometer   | GL Optic | GL Spectis 4.0 |
bovine serum albumin (BSA) and 0.1 % TBS-T. Cells were then washed with PBS and labelled with goat anti-rabbit Alexa Fluor-488 (Life Technologies, Grand Island, NY) in PBS containing 2 % BSA at room temperature for an hour with gentle shaking. Following washing with PBS, the cells were stained with DAPI and observed with the 40x objective of Nikon Ti-2 TIRF microscope.

Reverse Transcriptase Experiments

Beta-coronavirus RNA was extracted from the viral samples on each Petri dish using the QIamp Viral RNA mini kit (Qiagen) following the manufacturer’s instructions. 5 μl of each sample was used for qRT-PCR analysis utilizing the TaqPath 1-step RT-qPCR master mix CG (Applied Biosystems) and the primer set spanning a target region of 68bp for HCoV-OC43 as reported (Vijgen et al., 2005). The forward primer is 5’-ATGTTAGGCCGA-TAATTGAGGACCAT-3 and the reverse primer is 5’-AATGTAAGATGGCGGTATT-3’. The analysis was then performed using the QuantoStudio 5 software.

Protein degradation experiments

The antibodies that were utilized for western blots in this paper includes anti-Coronavirus OC43 Spike Protein antibody (CusaBio Technology LLC, Houston, TX, USA) and SARS-CoV-2 Spike RBD antibody (SinoBiological). All coronavirus purified spike proteins were obtained from SinoBiological. All samples for Western blot were lysed in 2x Lamelli buffer and incubated in room temperature for 20 min before loading. Approximately 200 ng of protein sample is loaded for silver staining experiments and 1ug of protein sample is loaded for Western blot experiments. The lysates were then subjected to SDS gel electrophoresis. For silver staining, we utilized the Pierce Silver Stain Kit (Thermofisher). For Western blot, the samples were transferred to nitrocellulose membranes using iBlot2 (Life Technologies), blocked with 5% BSA in TBST and incubated with primary antibodies in 5% BSA. Membranes were then incubated with rabbit-IRDye 800 CW secondary antibodies and imaged on an Odyssey Gx (LI-COR). Absorption spectroscopy was performed on Nanodrop (Thermofisher).

Quantification and Statistical Analyses

The number of replicates (n) are indicated in the respective figure legends. For all statistical tests, significance was measured against p < 0.05. For comparisons of infectivity curves, 2-way analyses of variance (ANOVA) were carried out with the different UVC wavelengths and exposure time as the factors. All statistical analyses were performed with the GraphPad Prism Software 8.4.3 (GraphPad Software Inc., La Jolla, CA, USA) and all values are expressed as means ±/− standard deviation.

Protein structure studies

The Desktop PyMOL 2.4 was used to visualize the protein structure of SARS-CoV-2 S glycoprotein (Protein Data Bank 6VXX) and calculate the distances between each indicated residue.

References

Barnard, L.R.M., Eadie, E., Wood, K., 2020. Further evidence that far-UVC for disinfection is unlikely to cause erythema or pre-mutagenic DNA lesions in skin. Photodermatol. Photoimmunol. Photomed. 36, 476–477.
Beaven, G.H., Holiday, E.R., 1952. Ultraviolet absorption spectra of proteins and amino acids. Adv. Protein Chem. 7, 319–386.
Beck, S.E., Ryu, H., Boczek, L.A., Cashdollar, J.L., Jeanis, K.M., Rosenblum, J.S., Lowel, O.R., Linden, K.G., 2017. Evaluating UV-C LED disinfection performance and investigating potential dual-wavelength synergy. Water Res. 109, 207–216.
Beukers, R., Ylstra, J., Berends, W., 2010. The effect of ultraviolet light on some components of the nucleic acids: II in rapidly frozen solutions. Recl. Des Trav. Chim. Des Pays-Bas 77, 729–732.
Brown, L.H., Johns, H.E., 1968. Photochemistry of uracil, intersystem crossing and dimerization in aqueous solution. Photochem. Photobiol. 8, 273–286.
Buonanno, M., Welch, D., Shuryak, I., Brenner, D.J., 2020. Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses. Sci. Rep. 10, 1–8.
Chan, L.-H., Gaffney, P.R., Waterfield, M.D., Anderle, H., Peter Matthiessen, H., Schwartz, H.-P., Turecek, P.L., Timms, J.F., 2006. Proteomic analysis of UVC irradiation-induced damage of plasma protein: serum amyloid P component as a major target of photodamage. FEBS Lett. 580, 3220–3226.
Fehr, A.R., Perlman, S., 2015. Coronavirus: an overview of their replication and pathogenesis. In: Coronavirus: Methods and Protocols. Springer, New York, pp. 1–23.
Gerchman, Y., Mamane, H., Friedman, N., Mandelboim, O., 2020. UV-LED disinfection of Coronavirus: wavelength effect. J. Photochem. Photobiol. Photophys. B. 212, 121044.
Inagaki, H., Saito, A., Sugiyama, H., Okabayashi, T., Fujimoto, S., 2020. Rapid inactivation of SARS-CoV-2 with deep-UV LED irradiation. Emerg. Microb. Infect. 9, 1744–1747.
Kervin, B.A., Remmel, R.L., 2007. Protect from light: Photodestruction and protein biologics. J. Pharmaceut. Sci. 96, 1468–1479.
Kim, D.K., Kang, D.H., 2018. UVC LED irradiation effectively inactivates aerosolized viruses, bacteria, and fungi in a chamber-type air disinfection system. Appl. Environ. Micorobiol. 84.
Kitagawa, H., Nomura, T., Naum, T., Omori, K., Shigemoto, N., Sakaguchi, T., Ohge, H., 2020. Effectiveness of 222 nm-ultraviolet light on disinf ecting SARS-CoV-2 surface contamination. Am. J. Infect. Control 49, 299–301.
Liu, Q., Lim, J.Y.C., Xue, K., Yew, P.Y.M., Owst, C., Chee, P.L., Loh, X.J., 2020. Sanitizing agents for virus inactivation and disinfection. View, 1.
Merriam, V., Gordon, M.P., 1967. Pyrimidine dimer formation in ultraviolet irradiated TMV-RNS. Photochem. Photobiol. 6, 309–319.
Nguyen, T.M.H., Suwan, P., Kootnatep, T., Beck, S.E., 2019. Application of a novel, continuous-feeding ultraviolet light-emitting diode (UV-LED) system to disinfect domestic wastewater for discharge or agricultural reuse. Water Res. 153, 53–62.
Nunoya, S.S., Zhang, H., Lai, A.C.K., 2020. Comparison of disinfection performance of UVC LED and conventional upper-room UVGI systems. Indoor Air 30, 180–191.
Raeiszadeh, M., Adeli, B., 2020. A critical review on ultraviolet disinfection systems for virus inactivation and disinfection. View, 1.
Rheinboldt, M.D., Anderle, H., Peter Matthiessen, H., Barnard, I.R.M., Eadie, E., Wood, K., 2020. Further evidence that far-UVC for disinfection is unlikely to cause erythema or pre-mutagenic DNA lesions in skin. Photodermatol. Photoimmunol. Photomed. 36, 476–477.
Song, K., Mohseni, M., Taghpiour, F., 2016. Application of ultraviolet light-emitting diodes (UV-LEDs) for water disinfection: a review. Water Res. 94, 341–349.
Storm, N., McKay, L.G.A., Downs, S.N., Johnson, R.I., Birru, D., de Samber, M., Willaert, W., Cennini, G., Griffiths, A., 2020. Rapid and complete inactivation of SARS-CoV-2 by ultraviolet-C disinfection. Sci. Rep. 10, 1–5.
Vijgen, L., Kayeants, E., Moes, E., Mes, P., Duxon, G., Van Ranst, M., 2005. Development of one-step, real-time, quantitative reverse transcriptase PCR assays for absolute quantitation of human coronaviruses OC43 and 229E. J. Clin. Microbiol. 43, 11727–11734.
Welch, D., Buonanno, M., Grilj, V., Shuryak, I., Crickmore, C., Bigelow, A.W., Randers-Pehrson, G., Brenner, D.J., 2020a. Far-UVC light: a new tool to control the spread of airborne-mediated microbial diseases. Sci. Rep. 8, 2752.
Welch, D., Spotnitz, H.M., Brenner, D.J., Randers-Pehrson, G., Buonanno, M., Shuryak, I., 2018b. Far-UVC light applications: sterilization of MRSA on a surface and...
inactivation of aerosolized influenza virus. In: Dai, T. (Ed.), Light-Based Diagnosis and Treatment of Infectious Diseases. SPIE, p. 60.
Werbin, H., Hidalgo-Salvatierra, O., Sear, J., McLaren, A.D., 1966. Comparative photoreactivation of ultraviolet light-inactivated tobacco mosaic virus ribonucleic acid on Chenopodium, pinto bean, and tobacco plants. Virology 28, 202–207.
Woo, P.C.Y., Huang, Y., Lau, S.K.P., Yuen, K.Y., 2010. Coronavirus genomics and bioinformatics analysis. Viruses 2, 1804–1820.
Wu, L.Z., Sheng, Y.B., Xie, J. Bin, Wang, W., 2008. Photoexcitation of tryptophan groups induced reduction of disulfide bonds in hen egg white lysozyme. J. Mol. Struct. 882, 101–106.