

Suppression of Airborne Viral Epidemic Spread by UVC light barriers

Giovanni Agati^a, Franco Fusi^b, Giacomo Insero^b, Barbara Patrizi^c, Angela Pirri^a, Mauro Pistello^d, Simona Pollini^{e, f}, Ilaria Baccani^{e, f}, Sara Cuffari^{e, f}, Paola Quaranta^d, Giovanni Romano^b, Francesca Rossi^{*a}, Giovanni Scirè^a, Guido Toci^c, Matteo Vannini^c

^a Istituto di Fisica Applicata, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, Sesto Fiorentino, Firenze, Italy 50019; ^b Università degli Studi di Firenze, Dip. Scienze

Biomediche Sperimentali e Cliniche "Mario Serio", Viale Morgagni 50, Firenze, Italy, 50134;

^c Istituto Nazionale di Ottica, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, Sesto Fiorentino, Firenze, Italy 50019; ^d Università degli Studi di Pisa, Dipartimento di

Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Via Savi 10, Pisa, Italy 56126; ^e Università degli Studi di Firenze, Dip. Medicina Sperimentale e Clinica, Largo

Brambilla 3, Firenze, Italy, 50134; ^f Azienda Ospedaliero-Universitaria Careggi, Largo Brambilla 3, Firenze, Italy, 50134;

ABSTRACT

The use of light for sterilization is very well known in the scientific literature. However, the recent pandemic outbreak and the antimicrobial resistance question drew attention to this topic: to design new light sources for preventing viral epidemic spread is of utmost importance, as an alternative use of chemicals and drugs. Here we present the preliminary ex vivo studies aiming at verifying the potential of new UVC light sources as barriers to the spread of airborne viruses and bacteria. The emitted light is at very short wavelengths (around 220 nm): optical penetration in biological media is limited to a few micrometers, thus preventing the possible damages to the skin and the cornea; the absorption of RNA/DNA shows a minimum at 230 nm, increasing at shorter wavelengths. In this study we optimized a UVC commercial excimer lamp to design a light barrier. The sterilization efficacy has been tested in vitro in cultured *Staphylococcus aureus*, *Pseudomonas aeruginosa* and in *Sars-Cov-2*. The results point out a strong antimicrobial effect (>99.9% bacteria reduced) at $\square 15$ mJ/cm² (corresponding to 1 minute treatment time @0.25 mW/cm²). The designed prototype can thus be proposed as a light barrier for preventing contamination, reducing the risks for human beings.

Keywords: light barriers, UVC, antimicrobial effects, light sterilization

1. INTRODUCTION

The use of light for sterilization is very well known in the scientific literature and in the common popular use [1]. However, the recent pandemic outbreak and, before this, the antimicrobial resistance problem, drew attention to this topic: the possibility to use new light sources to support prevention of viral epidemic spread is of utmost importance nowadays, as an alternative use of chemicals and drugs [2].

The use of UVC light has been proposed in recent years for the environmental sterilization, but its application has been limited because of possible irreversible damage to human beings accidentally exposed to these wavelengths [1],[3]-[8].

Recently, commercial lamps emitting at UVC wavelengths shorter than 254 nm were proposed in the market. It has been observed that the optical penetration depth in biological media is very short (a few micrometers) at

wavelengths shorter than 230 nm, thus preventing the possible damages to the skin and the cornea. At the same time the absorption of RNA/DNA shows a minimum at 230 nm, but it increases at shorter wavelengths. This spectral range has thus been investigated in view of a possible application for environmental sterilization.

*f.rossi@ifac.cnr.it; phone 0039 055 5225337; bpnlab.ifac.cnr.it/

The virucidal effects of light in the wavelength range around 220 nm has been demonstrated *in vitro* in recent years [10]- [13]. In this work we present the design of a prototype used to sterilize the airborne virus and bacteria, spread in small droplets (diameter <40um) in air. Its antimicrobial effects in selected bacteria and viruses are demonstrated in *in vitro* experiments.

2. MATERIAL AND METHODS

2.1 Excimer lamp

Excimer lamps used in the experiments were produced by Ushio (model Care 222). The emitting elements are small quartz tube filled with the KrCl mixture and excited by a capacitive discharge. The lamp emits prevalently at 222 nm, also due to a short pass filter set on the output window, that cuts off the emission at wavelength > 240 nm. An image of the lamp is shown in the Figure 1. The UV intensity emitted by the lamp is about 4.5 mW/cm² as measured just in contact with the output window. The measurement was carried out with a UV extended photodiode power meter Thorlabs model S120VC. The output of the lamp was found very stable (variation <3%) even after some tens of hours of operation.

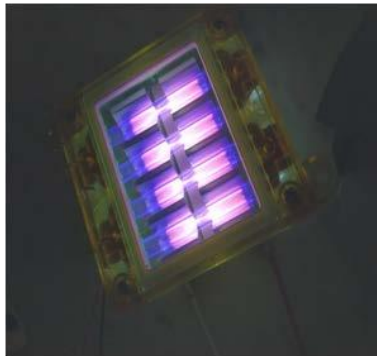


Figure 1. Image of the Ushio 222 lamp. The output window has a size of 44 x 59 mm.

2.2 Testing antiviral activity

All tests were performed using African green monkey kidney cells (Vero-E6) and titrated amounts of the SARS-CoV-2/ Human/ITA/PAVIA10734/2020 of Wuhan lineage (full sequence available on GISAID, accession no. EPI_ISL_568579, and GeneBank, accession no. M527178.1). Viral titer was calculated from the cytopathic effect (CPE) induced through limited dilution of the viral stock and using the Spearman-Kärber method. Titer of viral stocks produced were expressed by Median Tissue Culture Infectious Dose tissue/ml (TCID₅₀/ml). Tests were carried out using 10⁸ TCID₅₀/ml. To assess the antiviral activity of the lamp, virus suspended in PBS1x was plated in 12 well plate, 250µl for each well; samples were irradiated at 222 nm respectively for 2, 5 and 10 min. At the end of irradiation the viral supernatant were recovered and titred by using the Spearman-Kärber method. Not irradiated virus was used as negative control.

2.3 Testing antibacterial activity

Four strains (2 *S. aureus* and 2 *P. aeruginosa* strains) were selected as representatives of clinically relevant bacterial pathogens; wild-type reference strains and strains of clinical origin harbouring relevant antibiotic resistance mechanisms (e.g., carbapenem and oxazolidinones resistance enzymes) were included (Table 1). Strains (inoculum size ca. 1x10⁸ - 5x10⁸ CFU/ml) were irradiated using the excimer lamp at 222 nm at a fluence of 7.6, 15.2 and 30.4 mJ/cm². Viable cell counts were used to estimate photokilling activity.

3. RESULTS

The preliminary designed prototype shows a strong antiviral effect at 7.5 mJ/cm² (Figure 2).

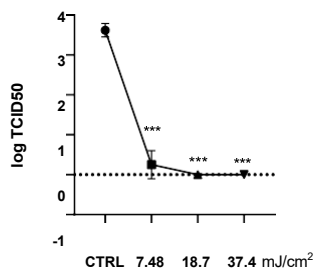


Figure 2. Antiviral effect of the lamp. The log of viral titer was plotted versus the energy density (i.e. 2, 5 and 10 minutes treatment time). Already after 2 minutes of irradiation, a reduction of the viral titer of about 4 logarithms compared to the negative control, is pointed out. Data are statistically significant (***pvalue =0.0001).

The preliminary designed prototype shows a strong antibacterial effect (more than 99.9% bacteria reduction, corresponding to a 3-log decrease in viable cells counts) at around 15 mJ/cm² dose. Photokilling activity was comparable in both the tested bacterial species, including antibiotic resistance strains of clinical origin (Figure 3).

Table 1. Features of the bacterial strains selected for photokilling activity testing.

Isolate	Species	Resistance phenotype ^a	Resistance genes ^b
ATCC 29213	<i>Staphylococcus aureus</i>	Wild-type reference strain	
4250	<i>Staphylococcus aureus</i>	LIN ^R	<i>cfr</i> , <i>poxtA</i>
ATCC 27853	<i>Pseudomonas aeruginosa</i>	Wild-type reference strain	
14-048	<i>Pseudomonas aeruginosa</i>	CARBA ^R	<i>bla</i> _{VIM-2}

^a CARBA^R: resistance to carbapenems; LIN^R: resistance to oxazolidinones.

^b When available, resistance genes variants were reported. *cfr*, *poxtA*: oxazolidinone resistance genes, *bla*_{VIM-2}: carbapenem resistance genes.

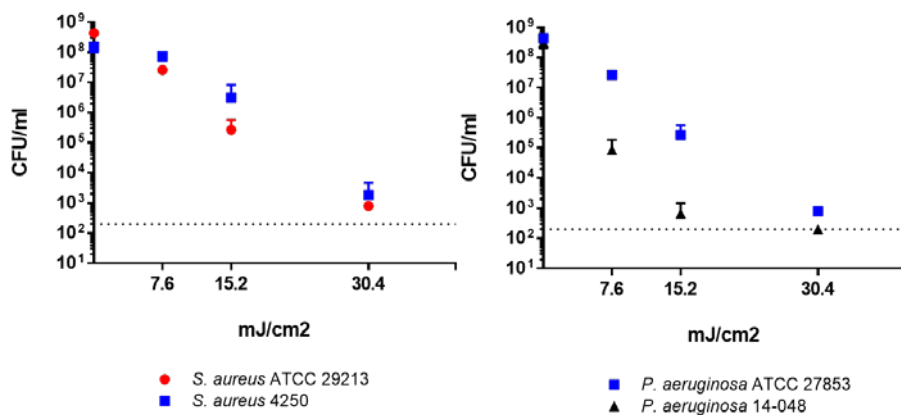


Figure 3. Photokilling of bacterial strains at different UV light fluences.

4. CONCLUSIONS

The preliminary ex vivo studies point out a high antimicrobial efficacy of commercial UVC excimer lamps. They were used to design a prototype that can be used as a light barrier to prevent the spread of airborne viruses and bacteria. The emitted light is at very short wavelengths (around 220 nm): its optical penetration in biological media is very short (a few micrometers), thus preventing the possible damages to the skin and the cornea; the absorption of RNA/DNA shows a minimum at 230 nm, thus increasing at shorter wavelengths. The device can thus be used in the future as a tool for decontamination of environments even in presence of human beings.

5. ACKNOWLEDGMENT

The research activity reported in this manuscript has been performed in the framework of the regional project "Suppression of Airborne Viral Epidemic Spread by Ultraviolet light barriers (SAVES-US)". This research project is funded by Tuscany Region.

REFERENCES

- [1] Thresh, J. C., Beale, J. F., "The practical sterilisation of potable waters by means of the ultraviolet rays of light.," *The Lancet* 176(4556), 1849-1851 (1910).
- [2] Fusi, F., Romano, G., "Shedding light on the restart," *Phys Med.* 77, 18-20 (2020).
- [3] Harm, W., "Biological Determination of the Germicidal Activity of Sunlight," *Radiation Research* 40(1), 63–69 (1969).
- [4] Reed, N. G., "The History of Ultraviolet Germicidal Irradiation for Air Disinfection," *Public Health Reports* 125(1), 15–27 (2010).
- [5] Tseng, C. C., Li, C. S., "Inactivation of Viruses on Surfaces by Ultraviolet Germicidal Irradiation," *Journal of Occupational and Environmental Hygiene* 4(6): 400–405 (2007).
- [6] Sliney, David. 2013. "Balancing the Risk of Eye Irritation from UV-C with Infection from Bioaerosols." *Photochemistry and Photobiology* 89(4): 770–76. <http://doi.wiley.com/10.1111/php.12093> (July 2, 2020).
- [7] Sterenborg, H. J. C. M., S. C. J. Putte, and J. C. Leun. 1988. "The Dose-Response Relationship of Tumorigenesis by Ultraviolet Radiation of 254 nm." *Photochemistry and Photobiology* 47(2): 245–53. <http://doi.wiley.com/10.1111/j.1751-1097.1988.tb02722.x> (July 2, 2020).
- [8] Balasubramanian, D. 2000. "Ultraviolet Radiation and Cataract." *Journal of Ocular Pharmacology and Therapeutics* 16(3): 285–97. <https://www.liebertpub.com/doi/abs/10.1089/jop.2000.16.285> (June 21, 2020).
- [9] Beck, S. E., Rodriguez, R. A., Linden, K. G., Hargy, T. M., Larason, T. C., and Wright, H. B. (2014). Wavelength dependent UV inactivation and DNA damage of adenovirus as measured by cell culture infectivity and long range quantitative PCR. *Environ. Sci. Technol.* 48, 591–598. doi:10.1021/es403850b.
- [10] Buonanno, M., Welch, D., Shuryak, I., and Brenner, D. J. (2020). Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses. *Sci. Rep.* 10, 1–8. doi:10.1038/s41598-020-67211-2.
- [11] Kitagawa, H., Nomura, T., Nazmul, T., Kawano, R., Omori, K., Shigemoto, N., et al. (2021a). Effect of intermittent irradiation and fluence-response of 222 nm ultraviolet light on SARS-CoV-2 contamination. *Photodiagnosis Photodyn. Ther.* 33, 1–4. doi:10.1016/j.pdpdt.2021.102184.
- [12] Kitagawa, H., Nomura, T., Nazmul, T., Omori, K., Shigemoto, N., Sakaguchi, T., et al. (2021b). Effectiveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination. *Am. J. Infect. Control* 49, 299–301. doi:10.1016/j.ajic.2020.08.022.

[13] Ohashi, H. et al 2020 "State-of-the-art Technology: Inactivation of Pathogens Using a 222-nm Ultraviolet Light Source with an Optical Filter" *Journal of Science and Technology in Lighting* 44, 9-11