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Proceeding Paper

Photoinactivation of *Staphylococcus carnosus* on Surfaces by Irradiation with Blue and Violet Light †

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Abstract: To control the spread of bacteria and viruses in medical environments and everyday life on surfaces, suitable disinfection methods are required. Visible radiation in the violet or blue spectral range is known to exhibit an antimicrobial impact on microorganisms. However, so far most published studies were performed in liquids. In contrast, the sensitivity of microorganisms to visible radiation on surfaces were only investigated in a few studies. In order to transfer possible conclusions from irradiation in media to irradiation on surfaces and to apply visible light as a possible valid alternative for common disinfection methods, the log reduction doses for surfaces and liquids were compared in this study. The non-pathogenic *Staphylococcus carnosus* was selected as surrogate for the ESKAPE pathogen *Staphylococcus aureus*, as the experiments were performed in a S1 laboratory. The irradiations were performed with wavelengths of 403 nm (violet) and 453 nm (blue). The observed log reduction doses in liquids (literature with same strain and setup) and surfaces (this investigation) were 101.8 J/cm² and 14.0 J/cm² at 403 nm and 374.3 J/cm² and 112.8 J/cm² at 453 nm, respectively. The results suggest that the photosensitivity of *S. carnosus* on surfaces is much higher than in liquid with a ratio of 7.3 (violet) and 3.3 (blue). On the one hand, this demonstrates that irradiation on surfaces is more efficient than in liquids, especially in the violet spectral range. On the other hand, depending on the strength of the irradiation source, disinfection with visible irradiation is a useful alternative to conventional disinfection methods.

Keywords: visible irradiation; blue light; violet light; surface; bacteria; *Staphylococcus carnosus*; inactivation; disinfection

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1. Introduction

Multidrug-resistant pathogens (MDR pathogens) are emerging with increasing frequency in recent years. This is caused by the numerous and prophylactic administration of antibiotics. MDR pathogens are nonresponsive or only mildly responsive to antibiotics, thus posing a major therapeutic and hygienic problem [1]. One of the most famous MDR pathogens is methicillin-resistant *Staphylococcus aureus* (MRSA). The proportion of MRSA of all identified strains of *S. aureus* on clinical material increased from 1.1% in the 1990s to 20.3% in 2007 [2].

Alcohol-based disinfectants are applied in many areas to prevent the spread of multi-resistant pathogens such as MRSA, but also of non-multi-resistant pathogens. This reduces bacteria and viruses [3], but not all surfaces are suitable for cleaning with disinfectants. As an alternative, disinfection with radiation can be an option that offers several advantages over liquid disinfectants (e.g., less staff and cleaning costs or cleaning of surfaces where liquid cleaning agents can't be applied).

Disinfection with visible light (380–780 nm) is generally harmless to humans compared to disinfection with UV radiation and is therefore suitable for irradiation of premises frequented by people. The inactivation mechanism is based on endogenous photosensitizers such as flavins and porphyrins, which are excited by light absorption. As a result, reactive oxygen species (ROS), such as singlet oxygen or oxygen peroxides, are produced in an oxygen-containing environment. These highly reactive molecules damage internal cell structures, like cell membranes or cellular lipids and proteins, by oxidative processes and thus inactivate the cell [4–6].

For irradiation, wavelengths of 405 nm (violet) and 450 nm (blue) are selected, as these are most effective in the visible spectral range [7]. The absorption spectra of some photosensitizers match well with the spectra of blue and violet LED emissions e.g., with peaks at 407 nm (protoporphyrin IX) and 446 nm (riboflavin) [8].

The non-pathogenic relative *Staphylococcus carnosus* is chosen as test organism for this investigations because it exhibits a visible light photosensitivity similar to that of *S. aureus* [9] and it can be used without infection risk. This and the fact that Staphylococci can be found on almost any surface [10] are reason that *S. carnosus* is selected for this investigation. To draw a comparison and answer the question, as to which the sensitivity of bacteria increases when irradiation is applied to surfaces, existing data of irradiations in liquids from the literature (published by our group) with the exact same *S. carnosus* strain and the same culturing procedure are employed [11].

2. Material and Methods

2.1. Irradiation Setup

For irradiation with violet light, LEDs of LED-Engin, Inc. (California, Silicon Valley, USA) of the type LZ1-10UB00-00U7 with a peak emission wavelength at 403 nm were applied. To achieve an irradiance of 5.5 mW/cm², the current was set to 1 A. The LED GD CSSRM2.14 of OSRAM Opto Semiconductors Inc. (Munich, Germany) with a peak wavelength at 443 nm was taken for blue irradiation. The current was set around 120 mA to achieve an irradiation of also 5.5 mW/cm².

The irradiation setup includes a glass plate with dimensions 380 mm × 325 mm × 50 mm, an LED array (three rows: 3, 1, 3), a hollow pyramid covered with a high-reflective inner surface to achieve a homogeneous distribution of radiation [7], a fan to cool the glass plate from below, and a fan to cool the LEDs (4). The glass plate with the bacteria was positioned by an elevation of PMMA 6.5 cm above a black background, which prevents light reflections, so that air circulation keeps the plate cooled during the experiments (Figure 1).

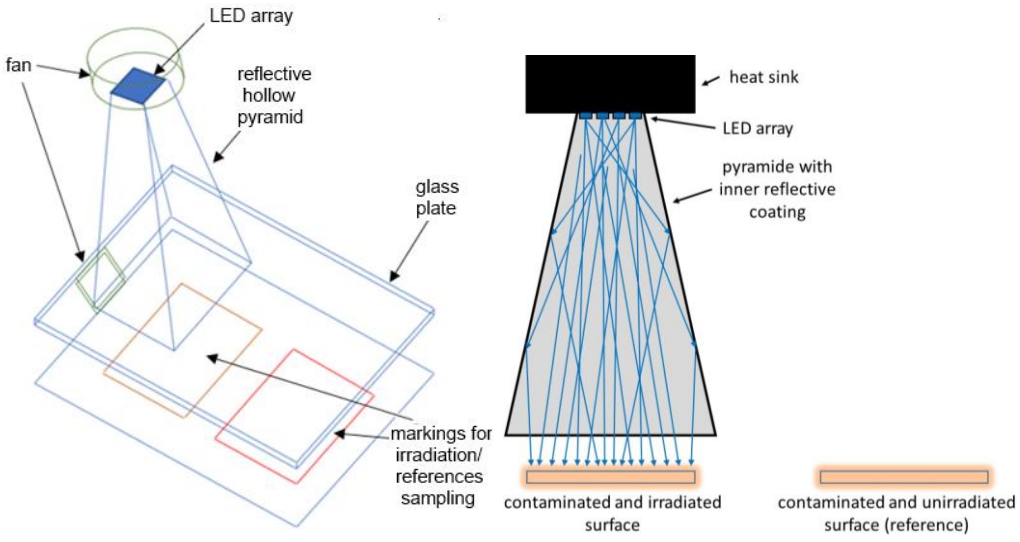

Figure 1. Schematic surface irradiation setup (**left**: 3D schema, **right**: cross section).

2.2. Microbiological Procedure

A colony of *S. carnosus* DSM 20501 (DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) was cultivated in M92 medium [12] at 37 °C until an OD of 0.33 was reached, which was equivalent to 1–5 ×10^8 colony forming units (CFU)/mL. Afterwards, 300 µL of the culture was diluted with 11.7 mL of phosphate-buffered saline (PBS) to a final concentration of approx. 10^5 CFU/mL.

A glass plate (380 mm × 325 mm × 50 mm) was employed as test surfaces. Prior to contamination they were sprayed with 70% ethanol and then irradiated with UVC (254 nm) for a few seconds. In order to distribute the bacteria quite homogeneously, an industrial paper towel (Glaeser, Ulm, Germany) was placed on the glass plate and 12 mL of the bacterial solution were evenly dispersed with a Pasteur pipette. After 2 h, the paper towel was dry, detached from the plate, and was removed.

For sampling, TSA contact plates (VWR-Chemicals, Leuven, Belgium) were used and the glass plate was divided in an irradiated area and a non-irradiated area. Due to the intended irradiances, a time of 1.5 h for 403 nm and 10 h for 453 nm were applied for the experiments. In order to reduce potential temperature effects during the experiments, the temperature of the irradiated glass plate was kept between 20 °C and 25 °C, controlled with an infrared thermometer Raynger MX4 (Raytek®, Berlin, Germany). At each sampling interval (every 15 min @ 403 nm and every 60 min from 2–4 h and a sample at 10 h @ 453 nm), a sample was taken at the irradiated area and a reference sample on the non-irradiated area. For 403 nm six runs were performed and nine for 453 nm. After sampling, the bacteria on the contact plates were cultivated at 37 °C in an incubator for at least 24 h bevor counting the colonies.

3. Results

An exponential reduction of *S.carnosus* was observed with both wavelengths, though they differed significantly. Due to the defined irradiation times, the irradiation doses up to 29.7 J/cm² were applied @ 403 nm and up to 198 J/cm² @ 453 nm. The average irradiation doses for the reduction of one log level were 14.0 J/cm² @ 403 nm and 112.8 J/cm² @ 453 nm (Figures 2 and 3).

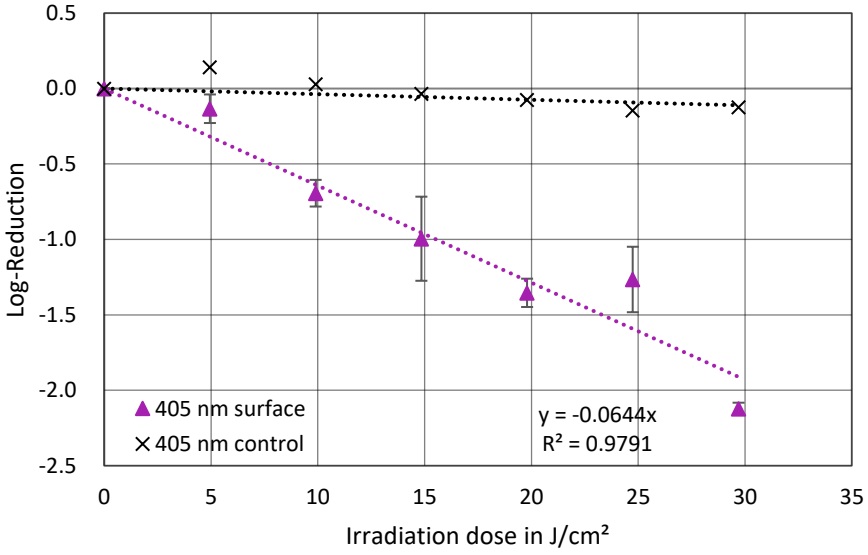

Figure 2. Irradiation of *S. carnosus* on surfaces with 403 nm (triangle) and control (cross). Each data point is the average of at least six independent runs, and for each run, two technical replicates were analysed. The error bars indicate the standard deviation of the single results.

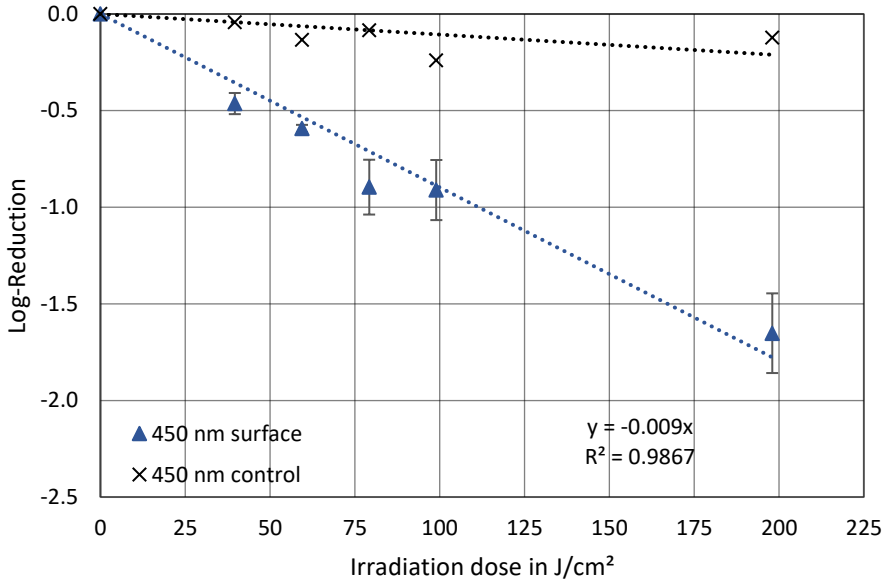

Figure 3. Irradiation of *S. carnosus* on surfaces with 453 nm (triangle) and control (cross). Each data point is the average of at least six independent runs, and for each run, two technical replicates were analysed. The error bars indicate the standard deviation of the single results.

4. Discussion

The surface results reveal an exponential reduction of *S. carnosus* with both wavelengths (violet and blue). Compared to the log reduction doses of the previously mentioned work with *S. carnosus* in (liquid) PBS [11], a factor of 7.3 (101.8 J/cm²/14.0 J/cm²) for violet and a factor of 3.3 (374.3 J/cm²/112.8 J/cm²) for blue were obtained, which indicates that *S. carnosus* are much more sensitive to visible irradiation on dry surfaces than in liquids.

The reason for this increased sensitivity might be the fact, that during the irradiation on surfaces, the bacteria were exposed to forced drought. Dehydration leads to conformational changes in proteins that can be detected by Fourier transform infrared spectroscopy [13]. In addition, it changes the nature of DNA, partially transforming it from B-DNA to A-DNA, which is more susceptible to blue light than B-DNA [14,15]. Dehydration also leads to oxidative stress [16] that produces partially reduced or activated forms of oxygen, i.e., ROS [17,18], resulting in dysfunction of organisms and damage to cell membranes. Since no reduction is detectable on the non-irradiated samples, the effect of the dryness is not sufficient to inactivate the bacteria. If violet or blue radiation is added, the effect of this could be synergistically enhanced by the dryness, which would explain the difference between dry surface and suspension. Nevertheless, this possible synergy seems to differ between the wavelengths.

5. Conclusions

Based on the results violet light seems to be useful for practical applications, such as disinfection lightning in hospitals or general workplaces. However, violet light alone cannot replace a standard light source the fact that it does not achieve required color temperature, color rendering index and illuminance [19,20].

Therefore, several possibilities would be conceivable for a realistic application of violet irradiation that also has a sufficient reduction:

- Violet LEDs are applied together with white LEDs to achieve a sufficient color rendering index and the required illumination, which has already been attempted in other investigations [21]. This would ensure a continuous irradiation and a sufficient reduction of pathogens.

- Violet LEDs are installed in room lighting, but are switched on only temporarily, when neither working persons, or in the case of hospitals also patients, are present in the affected area. This means that the irradiance can be adjusted as required within the limits of the technical possibilities.

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