INTRODUCTION

The disinfection properties of short-wave-length visible light were discovered more than 100 years [1, 2]. They have mostly been ignored because, compared to disinfection with ultraviolet (UV) radiation as emitted by mercury vapor lamps, high doses are required that have long been difficult to achieve in technically feasible times. Unfortunately, UV radiation also affects human cells and might lead to skin cancer, photokeratitis and cataractogenes, so another kind of antimicrobial radiation, which is less harmful to humans, would be desirable. The development of powerful violet and blue light emitting diodes (LEDs) in recent years has restarted research into the antimicrobial properties of short-wavelength visible light. By now, it has been demonstrated that all investigated bacteria, fungi and probably even viruses can be inactivated if the irradiation dose is sufficiently [3÷8].

The basics of the mechanism of action are understood meanwhile and schematically illustrated in Figure 1. Naturally occurring photosensitizers (PS) such as porphyrins and flavins absorb violet or blue light and generate so-called reactive oxygen species (ROS) in the presence of oxygen. These are radicals that attack virtually all relevant structures in the cell and can thus lead to its death [3÷5, 9÷12].

Maclean and coworkers successfully investigated the antimicrobial effect of violet light in hospital environments in several studies [13÷15]. Violet light with a wavelength of about 405 nm has a significantly stronger antimicrobial effect compared to blue light [6], and is at the same time better tolerated by human cells than ultraviolet radiation [16]. However, violet light generates effects similar to black light on fluorescent materials and can therefore be perceived as irritating and interferes with the viewer’s color perception. Therefore, Gillespie et al. have proposed a white LED illumination
composed of pulsed violet, green, yellow, and red LEDs [17]. This white illumination demonstrated improved color rendering and a simultaneous disinfecting effect on the clinical relevant pathogens Staphylococcus aureus and Pseudomonas aeruginosa.

Rohan et al. and Rutala et al. performed similar successful studies in hospital environments with 405 nm LEDs [18, 19]. Rutala et al. [19] investigated the antimicrobial effect of white light with a noticeable staphylococci reduction even for low irradiation doses. These results fit to an own study, in which we investigated the disinfecting properties of a touch screen, and observed that a highly luminous blue screen was capable of pronounced bacterial reduction, while a white luminous screen, for which the light was a combination of red, green, and blue emissions, was even slightly more effective [20].

The study presented here aims to:
1. Investigate the disinfecting properties of commercial warm-white and cool-white LEDs, which both consist of blue LEDs and phosphorescent layers, in comparison to the antimicrobial properties of pure blue LEDs and whether green, yellow or red light portions of white LEDs deliver a significant contribution to the disinfection performance.
2. Furthermore, the question is addressed whether it is possible to combine a conventional white LED illumination with violet LEDs to increase the antimicrobial effect without changing the perceived color impression particularly. This would pose a significant technical improvement to the approach of Gillespie et al. [17]. The approach seems to be realistic, since the human eye sensitivity at 405 nm, a typical wavelength of violet LEDs, is about a factor of 1 000 lower than in the spectral range 520 - 590 nm, in which conventional white LEDs emit to a large extent.

MATERIALS AND METHODS

Irradiation Setup

The following high-power LEDs were employed for the irradiation experiments: a warm-white LED type CXB1512-0000-000F0HN430G of Cree (Durham, North Carolina (USA)), a cool-white LED type Cree CXB1310-0000-000F0HN265E, a blue LED type Cree XPEBRY-L1-0000-00Q01, and a violet LED type LZ1-00UB00 of LEDengin (San Jose, California (USA)).

The white and blue LEDs were selected to have similar peak wavelengths in the blue spectral region around 450 nm. For the comparison of the antimicrobial impact of the warm-white, cool-white, and blue LED, the irradiance was adjusted using a DT-Blue 475 nm short-pass filter from Qioptiq (Goettingen, Germany) so that the blue component of the irradiance was 6.3 mW/cm² for all white and blue LEDs. In this way, it could be investigated whether only the blue emissions are responsible for the potential antimicrobial effect of white LEDs.

For the experiments on the additional effect of violet LEDs, the irradiances of the
warm-white LED and the violet LED were adjusted so that the irradiance in the violet-blue spectral region (below 480 nm) was 5 mW/cm². The spectra of the applied LEDs and the potentially involved bacterial photosensitizers can be found in Figure 2. The homogeneous irradiation of the bacterial samples was achieved with a pyramid-shaped mirror arrangement as described previously [7, 21]. Because of the potential influence of the violet LED on human color perception color coordinates, correlated color temperature and color rendering indices (CRI) of the warm-white LED, the violet LED and the combination of both were calculated.

Microbiological Experiments

Since it is restricted to work with pathogens in the available laboratory, *Staphylococcus carnosus* (DSM 20501) was selected as a non-pathogenic bacterium that is related to the methicillin-resistant *Staphylococcus aureus* (MRSA), which is notorious in hospitals but slightly less sensitive to visible light [7]. Concentrations in the range of $10^6$ bacteria per ml were prepared in phosphate-buffered saline solution (PBS) and 3 ml were irradiated in glass beakers at a temperature of approximately 25 °C. At the beginning and during the course of irradiation, 100 μl samples were taken and plated out on agar plates at different dilution levels. Surviving bacteria showed up after 24 h at 37 °C in the incubator as visible and countable colonies, from which the bacterial concentration at the time of sampling could be calculated. As a control, non-irradiated bacterial samples were also always examined in parallel to the irradiation experiment. Due to the known high variability of such microbiological experiments, each individual sample was plated out at least three times at each dilution level and each series of experiments was performed three times separately.

RESULTS

The results of the photoinactivation of *S. carnosus* by warm-white, cold-white and blue LED emission are presented in Figure 3 as a function of the blue irradiation dose. At first glance, warm-white seems to have a slightly stronger antimicrobial impact than the blue and cold-white illumination, but the error bars reveal a high variation in the underlying results of the single experiments and one should keep in mind, that the given irradiance is just the blue part of the LED emission. The results of both white LEDs are close to the results of the blue LED. This indicates that the disinfecting effect of the white LEDs is predominantly caused by their blue emission components.

The blue irradiation dose necessary for 1 and 3 log-reductions (90% and 99.9% reduction) of *S. carnosus* is about 130 J/cm² and 210 J/cm², respectively. The contribution of the blue part of the cool-white LED emission is approximately 24% of its total irradiance. For the warm-white
this blue fraction is three times lower with a percentage of about 8%. Therefore, a cool-white LED should have an approximately three times higher disinfecting impact at the same total irradiance than a warm-white one.

The antimicrobial effect of the warm-white and the violet LED, and the combination of both is given in Figure 4. A 1 log-reduction for staphylococci is reached after about 8 h, 4.3 h and 2.9 h for the warm-white LED, the violet LED and the LED combination. Concerning the violet irradiation doses the 1 and 3 log-reductions required approximately 77 and 162 J/cm².

Table 1 and Figure 5 illustrate the human color impression of the warm-white and the violet LED, and the combination of both. There is almost no difference in the color coordinates, correlated temperature and color rendering between the white LED and the combination with the violet LED. Even a hypothetic tenfold increase in the violet irradiance would only lead to moderate changes in the total color perception.
DISCUSSION

The results reveal that warm-white and cool-white LEDs exhibit antimicrobial properties, which are predominantly caused by their blue components and cool-white LEDs are supposed to be more efficient for the same total irradiance.

Operating theatres in hospitals represent an obvious field of application. Pathogen reduction is of great importance there and, at the same time, high illuminance levels of 10 000 - 100 000 lumens/m² are required [23], which would be a maximum of up to 10 lm/cm². If this illuminance is generated just with the warm-white LED, this corresponds to a blue irradiance of approx. 2.3 mW/cm². A 90% staphylococci reduction thus requires about 15.7 h, which seems to be of limited use in this form even for an automatic overnight disinfection. In combination with the violet LED (additional 2.3 mW/cm² of 405 nm), however, this time would already decrease to about 8.4 h. The simulated combination of a warm-white LED (2.3 mW/cm² of 450 nm) and a 10x stronger violet LED 23 mW/cm² of 405 nm), a further reduction in the range of about 1 h is feasible and this does not have to be the limit, if the violet irradiation is further increased.

In these considerations, 1 log-reduction data of *S. carnosus* in liquids were presupposed: 77 J/cm² for violet light and 130 J/cm² for blue light. These assumptions are rather pessimistic. In our own previous investigations, the medically more significant pathogen *S. aureus* turned out to be more sensitive to light for both spectral ranges [7]. Applying 405 nm in a hospital setting, Rutala et al. observed a 90% staphylococcus reduction on surfaces for a dose as little as 14.5 J/cm² [19], and Gillespie et al. reported that less than 4 J/cm² were needed for a log-reduction in their *S. aureus* experiments [17].

In addition to hospital and healthcare settings, applications of white-violet combinations in domestic environments such as bathrooms or kitchens are also conceivable. For example, campylobacter, food pathogens that frequently cause intestinal infections, are very sensitive to violet light and require only a dose of 3.5 J/cm² of 405 nm irradiation for a 90% reduction [24]. Technically, a kitchen work area could be equipped with an appropriate antimicrobial lighting. Just 10 W of violet illumination could generate an irradiance of about 4 mW/cm² on an area of 60 x 40 cm², and thus reduces the previously mentioned campylobacters by 90% within 15 min. Applications in which white and violet do not necessarily radiate simultaneously all the time are also conceivable. For example, it would be imaginable to have a white-violet shower illumination that only shines white during the shower process and then automatically switches to violet-white or violet for a selected duration, afterwards.

**Table 1.** CIE (Commission Internationale de l’Éclairage) xy color coordinates, correlated color temperatures (CCT) and color rendering indices (CRI) calculated for spectra of the warm-white, the violet, the combined warm-white and violet LED and an additional hypothetic combination of the warm-white LED with a ten times stronger violet one

| LED                | CIE x   | CIE y   | CCT [K] | CRI |
|--------------------|---------|---------|---------|-----|
| Violet             | 0.172   | 0.028   | –       | –   |
| Warm-white         | 0.451   | 0.415   | 2880    | 98  |
| Warm-white & violet| 0.445   | 0.406   | 2901    | 98  |
| Warm-white & 10x violet| 0.397 | 0.341   | 3256    | 92  |

**Fig. 5.** Representation of the different tested LED illuminations in a CIE color diagram. Included is a hypothetic additional combination of the warm-white LED and a 10x stronger violet LED (Modified according to [22])
CONCLUSION

White LEDs and the combination of white and violet LEDs do not replace UV irradiation in its antimicrobial effect and short application duration, but allow many possible approaches for pathogen reduction and infection prevention in medical and domestic environments in the immediate vicinity of humans.

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