



Proceeding Paper

Assessment of Visible Violet Light for Hand Disinfection †

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Abstract: (1) Background: Hand hygiene with chemical disinfectants is an important measure to reduce the spread of infections, but frequent use can cause skin irritation. In recent years, it has become widely accepted that visible light can also have an antimicrobial effect, and visible light has even been applied to the disinfection of wounds. The present study aims to evaluate whether hand disinfection with visible light is a realistic alternative to chemical disinfectants. (2) Methods: Human hands were irradiated with a dose of 10 or 33 J/cm² of visible violet light (405 nm) for 3 or 10 min, respectively. The reducing effect of the visible violet light was determined by comparing the contact agar plate results of irradiated and non-irradiated hands. Comparative experiments with a conventional hand disinfecting gel were also performed. Applicable standards were consulted to evaluate skin exposure to the irradiation. (3) Results: Irradiation of the hands with 10 and 33 J/cm² resulted in an average reduction of microorganisms on the skin of 0.43 and 0.76 log-levels, respectively. These disinfection results with visible violet light are far behind that of the disinfectant gel, which achieved a reduction of 2.17 log-levels. Additionally, due to legal limits, a 3-min irradiation can only be performed five times per day and a 10-min procedure even only once. (4) Conclusion: Since the irradiation doses applied up to now have not provided a high antimicrobial effect and an increase of the dose in a short time is not arbitrarily possible without heating the hand unpleasantly, visible light of 405 nm seems rather unsuited for repeated hand disinfection.

Keywords: hand disinfection; photoinactivation; endogenous photosensitizer; blue light; violet light

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1. Introduction

Hand hygiene is one of the most important measures in hospitals to prevent nosocomial infections [1–3]. Doctors and nursing staff have to disinfect their hands up to 100 times a day for this purpose [4]. This is usually accomplished with alcohol-based liquid disinfectants, which, however, can lead to unpleasant skin irritations that reduce user compliance [1–3,5,6].

In a previous study, it was investigated whether Far-UVC irradiation (200–230 nm) could be an alternative to conventional hand disinfection [7], since Far-UVC has a strong antimicrobial effect, and due to favorable spectral properties is already absorbed in the outermost skin layer and does not harm vital human cells. In principle, the approach works, especially when gloves are worn, but on the one hand the multiple daily application in Europe is limited by applicable standards and on the other hand no studies exist for the effects of a longer lasting frequent Far-UVC irradiation of the skin.

In contrast to UV radiation, visible light is considered to be much less harmful to humans. Nevertheless, visible blue and violet light in particular also exhibit antimicrobial properties and are capable of inactivating bacteria and fungi, among others [8–13]. This effect is based on the fact that microorganisms contain endogenous photosensitizers such as flavins and porphyrins, which absorb blue and violet light and subsequently generate

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so-called reactive oxygen species. These attack membranes, proteins and DNA in the cell, and if the resulting damage is great enough, the microorganism dies [14–17]. These properties of visible light have already been investigated for potential therapeutical applications like wound disinfection [18–20].

In the context of the present study, it should therefore be investigated whether hand disinfection is also possible with visible violet light of wavelength 405 nm. At this wavelength, strong LEDs exist and the most important photosensitizers (porphyrins) feature strong absorptions, so that this wavelength seems to be the most suitable for the potential application of hand disinfection.

For the irradiation of skin with visible light with wavelengths above 400 nm, there are in principle no legal irradiation limits for daily use. However, 405 nm LEDs are not monochromatic and also exhibit weak emissions in the UV range (below 400 nm) for which irradiation limits exist [21]. The extent to which these limits restrict the application of violet light for skin disinfection is also being determined.

2. Materials and Methods

2.1. Hand Disinfection with 405 nm Irradiation

The applied, home-built light source was already described in detail in [22]. An area of about 9.5×9.5 cm² was irradiated from below and above by 128 LEDs type NVSU119CT of Nichia (Tokushima, Japan) on printed circuit boards as illustrated in Figure 1.

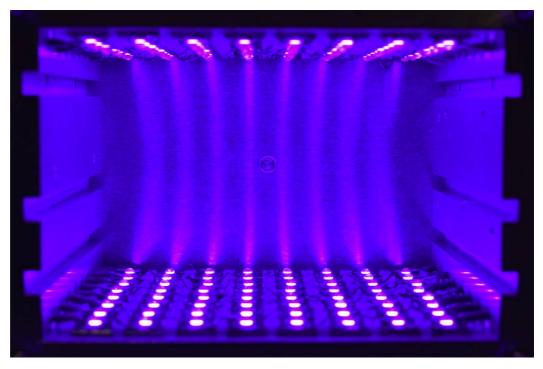


Figure 1. Photograph of the 405 nm illumination device with 64 LEDs at the top and 64 LEDs at the bottom.

Table 405. nm. At a total LED current of 2 A each LED board generated an irradiance of 55 mW/cm² with the spectral distribution given in Figure 2. This was not the maximum possible current or the highest possible irradiance, but one that could be well sustained during the irradiation tests without the skin becoming too hot. Irradiation durations of 3 and 10 min were applied, corresponding to irradiation doses of 10 and 33 J/cm². In laboratory tests on *Staphylococcus aureus* and *Staphylococcus epidermis*, these violet light doses were sufficient for bacterial reduction on the order of 1–2 log-levels [8].

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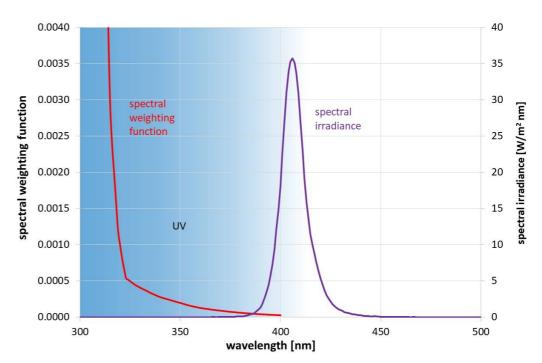


Figure 2. Applied spectral irradiance $E_{\lambda}(\lambda)$ on the skin and spectral weighting function $S(\lambda)$ according to DIN EN 1500 [23] for calculating the effective irradiance H_{eff}.

Since the 405 nm device and the 55 mm agar plates employed are not large enough to irradiate and sample the whole hand, only the microbial contamination of the 3 middle fingers was examined. For this purpose, three fingers—each of a non-disinfected and a disinfected hand—were pressed onto a Caso contact agar plate of VWR/Avantor (Darmstadt, Germany) and rolled slightly forward. Afterwards both plates were incubated for about 24 h at 37 °C and the number of visible colonies were counted and compared to each other. Both samples were always from the same person and prior to the disinfection procedure and sampling, both hands were rubbed against each other to reach more or less the same contamination on both hands.

As mentioned above there are limits for the daily skin exposure by UV radiation [21]. The effective irradiance H_{eff} defined as

$$H_{eff} = \int_{0.180 \, nm}^{t} S(\lambda) E_{\lambda}(\lambda, t) \, d\lambda \, dt$$

with the spectral irradiance $E_{\lambda}(\lambda,t)$ and the spectral weighting function $S(\lambda)$ [21], both illustrated in Figure 2, is limited to 30 J/m² per day. This allowed to calculate the possible number of disinfection procedures per day. A 1 min exposure based on the data in Figure 2 leads to an effective irradiance of 1.74 J/m².

2.2. Hand Disinfection with Commercial Disinfection Gel

For comparison, the conventional hand disinfection with a commercial disinfection gel was also investigated. Both hands were rubbed against each other to even the microbial concentrations on both hands. Then the three middle fingers of one hand were pressed on the Caso contact agar plates. Afterwards, 3 mL of the disinfection gel OSA VITA HANDHYGIENE GEL of OSA Brands (Schorndorf, Germany), approved according to the standard DIN EN 1500, were applied as described in DIN EN 1500 [23]. Subsequently, the three middle fingers of the other hand were pressed on an agar plate and after 24 h at 37 °C the colony number were evaluated.

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3. Results

For each disinfection approach (10 J/cm 2 @405 nm-33 J/cm 2 @405 nm-disinfection gel) at least 8 runs were performed and analyzed. Figure 3 gives an example for contact agar plates for a hand that was irradiated for 10 min with 405 nm and an unirradiated hand.

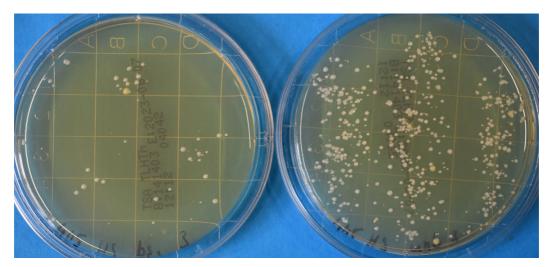


Figure 3. Photograph of contact agar plates for a hand that was irradiated for 10 min with 405 nm (**left**) and an unirradiated hand (**right**).

However, the 405 nm irradiation results were not always thus obvious but exhibited large variations. The mean log-reduction after 3 min of 405 nm irradiation was 0.429, which is a reduction of about 63%. A 10-min irradiation led to an average log-reduction of 0.757 or a drop of 82.5% in the detected number of microorganisms. In comparison, the hand disinfection gel inactivated 99.3% of the microorganisms that corresponds to a reduction of 2.168 log-steps. The results of the different approaches with their average and median reductions and the scattering of the results are illustrated in the box plot in Figure 4.

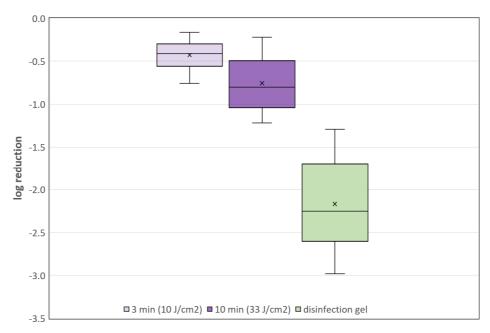


Figure 4. Box-Plots with average and median log-reduction and scattering of the single results for all three disinfection approaches.

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Concerning the daily irradiation limit according to the directive 2006/25/EC [21], this irradiation intensity may be applied for 1033 s or 17.2 min. Accordingly, only five 3 min or one 10 min disinfection procedures would be allowed per day.

4. Discussion

Hand disinfection with the commercial gel provides good reduction results with more than 2 log-levels. In contrast, the antimicrobial effect of the visible violet light is rather sobering. The reduction effect is clearly visible, but at least with the two 405 nm irradiation doses applied, the effect of the commercial gel cannot be achieved.

Also, this dose cannot realistically be increased arbitrarily. On the one hand, the duration of the application speaks against it. Already now, the shorter of the two irradiation times was 3 min. On the other hand, if for example the irradiance is doubled to 110 mW/cm², in order to halve the application time, a significant warming of the skin is to be expected, since 110 mW/cm² corresponds to the maximum irradiation at midday in summer. There is also a legal limit to the skin irradiation intensity to prevent skin burns by infrared and visible light. Slightly simplified, this limit is not exceeded, as long as the irradiation intensity stays below 355 mW/cm², which is about 6.5 times above the current intensity.

The dose cannot be increased arbitrarily either by elongation of the irradiation, as this is also limited by the European directive due to the UV components of the LED emission. A maximum daily irradiation of 57 J/cm² would be possible for these LEDs. It has not been experimentally verified here, but it can be assumed that even this dose would not have achieved the reduction effect of the disinfection gel, since it does not even correspond to a doubling of the examined maximum dose of 33 J/cm².

It would still be conceivable to apply 450 nm LEDs, which do not exhibit UV emissions. Based on known log-reduction doses for 405 and 450 nm [8,9] it is to be expected that even higher 450 nm irradiation doses and thus longer irradiation durations would be necessary. However, application of very high doses of 450 nm or an even higher wavelength may be complicated by another effect. Human cells also contain endogenous photosensitizers and it is therefore not surprising that high irradiation doses of visible light can also have a skin damaging effect [24,25].

Author Contributions:.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Technische Hochschule Ulm (No. 2023-01 of 4th of July 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data is available upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Händehygiene in Einrichtungen des Gesundheitswesens: Empfehlung der Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) beim Robert Koch-Institut (RKI). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz **2016**, 59, 1189–1220. https://doi.org/10.1007/s00103-016-2416-6.
- 2. Stadler, R.N.; Tschudin-Sutter, S. What is new with hand hygiene? *Curr. Opin. Infect. Dis.* **2020**, *33*, 327–332. https://doi.org/10.1097/QCO.000000000000054.
- 3. Lotfinejad, N.; Peters, A.; Tartari, E.; Fankhauser-Rodriguez, C.; Pires, D.; Pittet, D. Hand hygiene in health care: 20 years of ongoing advances and perspectives. *Lancet Infect. Dis.* **2021**, 21, e209–e221. https://doi.org/10.1016/S1473-3099(21)00383-2.
- 4. Centers for Disease Control and Prevention. Clean Hands Count for Safe Healthcare. Available online: https://www.cdc.gov/patientsafety/features/clean-hands-count.html (accessed on August 2023).

Biol. Life Sci. Forum **2023**, 31, x

5. Kramer, A.; Hübner, N.-O.; Assadian, O. Anforderungen an die chirurgische Handedesinfektion und veraendertes Prozedere: Requirements on surgical hand disinfection and modified procedures. *GMS Krankenhaushyg Interdiszip* **2007**, *2*, Doc55.

- 6. Labadie, J.C.; Kampf, G.; Lejeune, B.; Exner, M.; Cottron, O.; Girard, R.; Orlick, M.; Goetz, M.L.; Darbord, J.-C.; Kramer, A. Recommendations for surgical hand disinfection -- requirements, implementation and need for research. A proposal by representatives of the SFHH, DGHM and DGKH for a European discussion. *J. Hosp. Infect.* **2002**, *51*, 312–315. https://doi.org/10.1053/jhin.2002.1243.
- 7. Hessling, M.; Sicks, B.; Lau, B. Far-UVC Radiation for Disinfecting Hands or Gloves? *Pathogens* **2023**, 12, 213. https://doi.org/10.3390/pathogens12020213.
- 8. Hessling, M.; Spellerberg, B.; Hoenes, K. Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths—A review on existing data. *FEMS Microbiol. Lett.* **2016**, 364, fnw270. https://doi.org/10.1093/femsle/fnw270.
- 9. Tomb, R.M.; White, T.A.; Coia, J.E.; Anderson, J.G.; MacGregor, S.J.; Maclean, M. Review of the Comparative Susceptibility of Microbial Species to Photoinactivation Using 380-480 nm Violet-Blue Light. *Photochem. Photobiol.* **2018**, *94*, 445–458. https://doi.org/10.1111/php.12883.
- Ashkenazi, H.; Malik, Z.; Harth, Y.; Nitzan, Y. Eradication of Propionibacterium acnes by its endogenic porphyrins after illumination with high intensity blue light. FEMS Immunol. Med. Microbiol. 2003, 35, 17–24. https://doi.org/10.1111/j.1574-695X 2003 tb00644 x
- 11. Guffey, J.S.; Wilborn, J. In vitro bactericidal effects of 405-nm and 470-nm blue light. *Photomed. Laser Surg.* **2006**, 24, 684–688. https://doi.org/10.1089/pho.2006.24.684.
- Maclean, M.; MacGregor, S.J.; Anderson, J.G.; Woolsey, G. High-intensity narrow-spectrum light inactivation and wavelength sensitivity of Staphylococcus aureus. FEMS Microbiol. Lett. 2008, 285, 227–232. https://doi.org/10.1111/j.1574-6968.2008.01233.x.
- 13. Dai, T.; Hamblin, M.R. Visible Blue Light is Capable of Inactivating Candida albicans and Other Fungal Species. *Photomed. Laser Surg.* **2017**, *35*, 345–346. https://doi.org/10.1089/pho.2017.4318.
- Feuerstein, O.; Ginsburg, I.; Dayan, E.; Veler, D.; Weiss, E.I. Mechanism of visible light phototoxicity on Porphyromonas gingivalis and Fusobacterium nucleatum. *Photochem. Photobiol.* 2005, 81, 1186–1189. https://doi.org/10.1562/2005-04-06-RA-477.
- Amin, R.M.; Bhayana, B.; Hamblin, M.R.; Dai, T. Antimicrobial blue light inactivation of Pseudomonas aeruginosa by photo-excitation of endogenous porphyrins: In vitro and in vivo studies. *Lasers Surg. Med.* 2016, 48, 562–568. https://doi.org/10.1002/lsm.22474.
- 16. Plavskii, V.Y.; Mikulich, A.V.; Tretyakova, A.I.; Leusenka, I.A.; Plavskaya, L.G.; Kazyuchits, O.A.; Dobysh, I.I.; Krasnenkova, T.P. Porphyrins and flavins as endogenous acceptors of optical radiation of blue spectral region determining photoinactivation of microbial cells. *J. Photochem. Photobiol. B* **2018**, *183*, 172–183. https://doi.org/10.1016/j.jphotobiol.2018.04.021.
- 17. Cieplik, F.; Spath, A.; Leibl, C.; Gollmer, A.; Regensburger, J.; Tabenski, L.; Hiller, K.-A.; Maisch, T.; Schmalz, G. Blue light kills Aggregatibacter actinomycetemcomitans due to its endogenous photosensitizers. *Clin. Oral Investig.* **2014**, *18*, 1763–1769. https://doi.org/10.1007/s00784-013-1151-8.
- 18. Zhang, Y.; Zhu, Y.; Gupta, A.; Huang, Y.; Murray, C.K.; Vrahas, M.S.; Sherwood, M.E.; Baer, D.G.; Hamblin, M.R.; Dai, T. Antimicrobial blue light therapy for multidrug-resistant Acinetobacter baumannii infection in a mouse burn model: Implications for prophylaxis and treatment of combat-related wound infections. *J. Infect. Dis.* **2014**, 209, 1963–1971. https://doi.org/10.1093/infdis/jit842.
- 19. Dai, T.; Gupta, A.; Huang, Y.-Y.; Yin, R.; Murray, C.K.; Vrahas, M.S.; Sherwood, M.E.; Tegos, G.P.; Hamblin, M.R. Blue light rescues mice from potentially fatal Pseudomonas aeruginosa burn infection: Efficacy, safety, and mechanism of action. *Antimicrob. Agents Chemother.* **2013**, *57*, 1238–1245. https://doi.org/10.1128/AAC.01652-12.
- Plattfaut, I.; Demir, E.; Fuchs, P.C.; Schiefer, J.L.; Stürmer, E.K.; Brüning, A.K.E.; Opländer, C. Characterization of Blue Light Treatment for Infected Wounds: Antibacterial Efficacy of 420, 455, and 480 nm Light-Emitting Diode Arrays Against Common Skin Pathogens Versus Blue Light-Induced Skin Cell Toxicity. *Photobiomodul. Photomed. Laser Surg.* 2021, 39, 339–348. https://doi.org/10.1089/photob.2020.4932.
- Richtlinie 2006/25/EG über Mindestvorschriften zum Schutz von Sicherheit und Gesundheit der Arbeitnehmer vor der Gefährdung durch physikalische Einwirkungen (künstliche optische Strahlung). Amtsblatt der Europäischen Union, 2006; pp. 38-59.
- 22. Lau, B.; Becher, D.; Hessling, M. High Intensity Violet Light (405 nm) Inactivates Coronaviruses in Phosphate Buffered Saline (PBS) and on Surfaces. *Photonics* **2021**, *8*, 414. https://doi.org/10.3390/photonics8100414.
- DIN EN 1500; Chemische Desinfektionsmittel und Antiseptika_- Hygienische H\u00e4ndedesinfektion_- Pr\u00fcryfverfahren und Anforderungen (Phase 2/Stufe_2); Deutsche Fassung EN_1500:2013. Beuth Verlag GmbH: Berlin, Germany, 2017.
- Pourang, A.; Tisack, A.; Ezekwe, N.; Torres, A.E.; Kohli, I.; Hamzavi, I.H.; Lim, H.W. Effects of visible light on mechanisms of skin photoaging. *Photodermatol. Photoimmunol. Photomed.* 2022, 38, 191–196. https://doi.org/10.1111/phpp.12736.
- 25. Cole, C.; Forbes, P.D. Examining the role of visible light in photocarcinogenesis—Lessons from the past. *J. Photochem. Photobiol.* **2023**, *17*, 100201. https://doi.org/10.1016/j.jpap.2023.100201.

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