Effectiveness of the blue led in the photoinactivation of *Staphylococcus aureus* and *Staphylococcus epidermidis* in vitro

Eficácia do led azul na fotoativação de *Staphylococcus aureus* e *Staphylococcus epidermidis* in vitro

Eficacia del led azul en la fotoactivación de *Staphylococcus aureus* y *Staphylococcus epidermidis* in vitro

Received: 01/11/2022 | Reviewed: 01/20/2022 | Accept: 01/27/2022 | Published: 01/28/2022

Juliana Teixeira Pedroso
ORCID: https://orcid.org/0000-0002-2521-1352
Universidade do Vale do Paraíba, Brazil
E-mail: juliana.teixeira.pedroso@hotmail.com

Edna Ponce
ORCID: https://orcid.org/0000-0002-8658-4363
Universidade do Vale do Paraíba, Brazil
E-mail: poncé0702@gmail.com

Isabelle de Paula Ribeiro
ORCID: https://orcid.org/0000-0002-4278-9594
Universidade do Vale do Paraíba, Brazil
E-mail: isabelle.isa27@gmail.com

Juliana Guerra Pinto
ORCID: https://orcid.org/0000-0002-7356-1576
Universidade do Vale do Paraíba, Brazil
E-mail: juguerra@univap.br

Alejandro Guillermo Miñán
ORCID: https://orcid.org/0000-0001-8864-8415
Facultad de Ciencias Exactas, Argentina
E-mail: agminan@gmail.com

Juliana Ferreira-Strixino
ORCID: https://orcid.org/0000-0001-7128-6817
Universidade do Vale do Paraíba, Brazil
E-mail: juferreira@univap.br

Abstract

One possibility of treatment in Aesthetics for folliculitis is a blue LED, as it acts on microbial control. Studies describe that the blue LED, with 405-470nm wavelength, has a bactericidal effect when irradiated in certain bacteria such as *Staphylococcus aureus*. This study aimed to evaluate the 450 nm blue LED’s efficacy as a modality in aesthetic treatments in the photoinactivation of the planktonic strains of *S. aureus* and *S. epidermidis* with power densities of 97, 110, 156, and 200 mW/cm² in different energy densities. Bacterial suspensions of *S. aureus* (ATCC 25923) and *S. epidermidis* (ATCC 12228) were plated in 24-well plates and irradiated with other energy and power densities. After irradiation, each bacterial suspension was diluted in a phosphate buffer solution in a 96-well plate. Aliquots of 10 µL were collected from this dilution and streaked, in triplicate, in Brain Heart Infusion agar plates and incubated for 24h/37 °C. CFU counts were expressed in log10/mL and submitted to ANOVA and Tukey statistical tests. The energy and power densities used were insufficient to cause an antimicrobial effect on *S. aureus* or *S. epidermidis* planktonic cultures with a single light application.

Keywords: Phototherapy; Blue led; *Staphylococcus aureus*; *Staphylococcus epidermidis*; Antimicrobial; Photoinactivation.

Resumo

Uma possibilidade de tratamento em Estética para foliculite é o uso do LED azul, pois atua no controle microbiano. Estudos descrevem que o LED azul, com comprimentos de onda de 405-470nm, tem efeito bactericida quando irradiado em determinadas bactérias como *Staphylococcus aureus*. Este estudo teve como objetivo avaliar a eficácia dos LED’s azuis de 450 nm como modalidade em tratamentos estéticos na fotoinativação das cepas planctônicas de *S. aureus* e *S. epidermidis* com densidades de potência de 97, 110, 156 e 200 mW/cm² em diferentes densidades de energia. Suspensões bacterianas de *S. aureus* (ATCC 25923) e *S. epidermidis* (ATCC 12228) foram semeadas em placas de 24 poços e irradiadas com diferentes densidades de energia e potência. Após a irradição, cada suspensão bacteriana foi diluída em uma solução tampão de fosfato em uma placa de 96 poços. Aliquotas de 10 µL foram coletadas desta diluição.
e semeadas, em triplicata, em placas de ágar Brain Heart Infusion e incubadas por 24h/37°C. As contagens de UFC foram expressas em log10/mL e submetidas aos testes estatísticos ANOVA e Tukey. As densidades de energia e potência utilizadas foram insuficientes para causar efeito antimicrobiano em culturas planctônicas de S. aureus ou S. epidermidis com uma única aplicação de luz.

Palavras-chave: Fototerapia; Led azul; Staphylococcus aureus; Staphylococcus epidermidis; Antimicrobiano; Fotoinativação.

Resumen
Un posible tratamiento para la foliculitis, empleado en estética, es el uso de radiación LED azul ya que limita el crecimiento microbiano. Diversos estudios han reportado que la radiación LED azul, con longitudes de onda en el rango de 405-470nm, tiene un efecto bactericida sobre ciertos patógenos bacterianos como Staphylococcus aureus. El presente estudio tiene por objeto evaluar la eficacia de la radiación LED azul a 450 nm (usada habitualmente en tratamientos estéticos) en la fotoinactivación de cultivos planctónicos de S. aureus y S. epidermidis empleando diferentes densidades de potencia (97, 110, 156 y 200 mW/cm2) y densidades de energía. Las suspensiones bacterianas de S. aureus (ATCC 25923) y S. epidermidis (ATCC 12228) se sembraron en placas de 24 pocillos y se irradiaron con diferentes densidades de energía y potencia. Finalizada la irradiación, se llevó a cabo la enumeración de cada suspensión bacteriana por dilución seriada en solución buffer fosfato. Luego se tomaron alícuotas de 10 µL de cada dilución para sembrarlas en placas de agar infusión Cerebro-Corazón y se incubaron durante 24 h a 37°C. Los recuentos de UFC se expresaron en escala logarítmica (log10/mL) y se realizó el análisis estadístico de ANOVA y test de Tukey. Los resultados indicaron que las densidades de energía y potencia utilizadas fueron insuficientes para causar un efecto antimicrobiano en cultivos planctónicos de S. aureus o S. epidermidis con una sola aplicación de radiación azul.

Palabras clave: Fototerapia; Led azul; Staphylococcus aureus; Staphylococcus epidermidis; Agente antimicrobiano; Fotoinactivación.

1. Introduction

Bacterial infections have been a constant concern in public health. As bacteria develop antibiotic resistance, it significantly increases hospitals' morbidity and mortality, leading to alternative treatments. Skin infections are very prevalent in clinical practice and have an effective presentation, etiology, and severity variability. Most cases of bacterial skin infection are caused by Gram-positive bacteria such as Staphylococcus aureus (Lister & Horswill, 2014). Folliculitis is a skin condition that affects the hair follicle, usually caused by S. aureus bacteria generating acne-like lesions, manifested by pus, follicular hyperemia, and may or may not present pain (Albuquerque et al., 2019). S. aureus lives commensally on the skin, nodules, and human mucosa as an opportunistic pathogen that can infect, invade, persist and replicate in many humans. It is a Gram-positive, aerobic, spherically shaped bacteria called coconuts, with approximately 0.5 to 1.5 µm in diameter, non-flagellate and organized in clusters, known to be very virulent and has a potent capacity to develop resistance to antibiotics (Bumah, Masson-Meyers, Cashin, & Enwemeka, 2015; Rupel et al., 2019). This important infectious agent uses open wounds as an entry site for infections and can generate more severe invasive conditions (Monaco, Araujo, Cruciani, Coccia, & Pantosti, 2017).

Staphylococcus epidermidis is a species best known as a symbiotic colonizer of the human skin microbiota, helping to protect this environment against pathogens and maintain balance with other diners. However, exogenous or endogenous factors, such as injury, stress, pollution, hormonal and pH changes, can affect skin homeostasis, favoring the development of inflammatory skin diseases such as acne, atopic dermatitis, rosacea, and psoriasis (Brown & Horswill, 2020; Claudel et al., 2019).

Recent research seems to confirm the beneficial role of S. epidermidis in the pathophysiology of acne by limiting skin colonization by Cutibacterium acne and inflammation. However, the imbalance in favor of the S. epidermidis microorganism can also result in other health consequences, such as nosocomial infections at a rate almost as high as that of S. aureus (Claudel et al., 2019).

Blue LED therapy (Light Emitting Diode) has been gaining more and more space because of its microbial reduction capacity, with a non-pharmacological approach and without the involvement of exogenous photosensitizers (Dai et al., 2013, 2012). LED has proved to be an alternative to laser and has obtained approval for its use by the Food and Drug Administration (FDA) in the United States of America for not offering human risks. LED is safe, non-toxic, non-invasive, and there are no
reports of side effects in the literature. Biological effects depend on parameters such as wavelength, energy density, power density, irradiation time, continuous or pulsed wave mode, and pulse patterns (Barolet, 2008).

One possibility of treatment in Aesthetics for folliculitis is a blue LED, as it acts on microbial control. It was described in previous studies that blue light (visible), with wavelengths of 405-470 nm, when irradiated in certain bacteria such as S. aureus, leads to a photosensitization of endogenous intracellular porphyrins that stimulate the production of reactive oxygen species, predominantly singlet oxygen (1O2), leading the cell to death. (Ashkenazi, Malik, Harth, & Nitzan, 2003; Hamblin & Hasan, 2004; Lipovsky, Nitzan, Gedanken, & Lubart, 2010; Papageorgiou, Katsambas, & Chu, 2000).

The objective of this study was to evaluate the efficacy of blue LED, as a modality of application in aesthetic treatments for photoinactivation of S. aureus and S. epidermidis strains, in vitro, with the wavelength of 450±10 nm, at different energy densities.

2. Methodology
2.1 Bacterial cultures and photo-inactivation treatments

This study selected S. aureus and S. epidermidis as representative opportunistic pathogens responsible for skin infections. The strains of S. aureus (ATCC 25923) and S. epidermidis (ATCC 12228) were kept in a freezer at -20 ºC in Brain Heart Infusion (BHI) broth suspension containing 5% glycerol. Each bacterial solution was first prepared by reactivating the strains later added to the BHI broth in conical Falcon-type tubes, remaining incubated for 24 hours in an incubator at 37 ºC. The tubes were centrifuged at 3500 rpm for 15 minutes, and the supernatant was discarded. Next, bacterial pellets of S. aureus and S. epidermidis were resuspended in sterile phosphate buffer solution (PBS) to obtain a bacterial density comparable to the 0.5 tubes on the MacFarland scale (1.5 x 10^8 CFU/mL). In the case of S. epidermidis, the bacterial suspension was adjusted at 1.5 x 10^8 CFU/mL by adding PBS. Both bacterial solutions were centrifuged at 3500 rpm for 15 minutes, and the supernatant was discarded.

The contents of the tubes were resuspended with 300 µL sterile PBS for each group to be irradiated. Thus, 300 µL of bacterial suspension of each group were separated into three wells (100 µL per well) of a 24-well plate, which was irradiated with blue light, and then 100 µL for each well were diluted in 900 µL of sterile PBS.

The plates were irradiated using the equipment and parameters described in Table 1. The control groups were processed similarly to the irradiated groups in all stages.

<table>
<thead>
<tr>
<th>Device</th>
<th>Nº LEDs</th>
<th>λ (nm)</th>
<th>Power density (mW/cm²)</th>
<th>Energy density (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biolable (Biolpixel50)</td>
<td>48</td>
<td>450</td>
<td>97 ± 110</td>
<td>5,10,15 ± 25</td>
</tr>
<tr>
<td>Biolable (Homonado)</td>
<td>54</td>
<td>455</td>
<td>156</td>
<td>5,10,15 ± 25</td>
</tr>
<tr>
<td>Elite Olympus (DMC)</td>
<td>4</td>
<td>450</td>
<td>200</td>
<td>6,12,18 ± 24</td>
</tr>
</tbody>
</table>

Source: Authors.
Finally, the viable bacteria in the irradiated groups and controls were enumerated in BHI agar plates by serial dilution and plate counting method. The CFU counts were expressed in (log_{10}/ml).

The temperatures for each power density and energy density were measured with a digital thermometer during irradiations. All tests were performed in triplicate to ensure data accuracy.

2.2 Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and Tukey statistical test (Bioestat 5.0 software) to evaluate differences between groups. A p-value of <0.05 was considered statistically significant.

3. Results and Discussion

As the objective of this study was to verify the effect of the antimicrobial action of blue light, the temperatures for each power density and energy density were measured, monitoring the thermal change during irradiations (figure 3). The temperatures were measured during irradiation. It can be observed that in Biotable homemade, 156 mW/cm², there was practically no temperature change about the increase in energy density. On the other hand, an increase in temperature was observed in the Elite (DMC) and Biopdi450 devices as the energy density increased. The highest temperature registered was 51.7°C in 200 mW/cm² at 24 J/cm².

![Figure 1: Variation of temperature in different energy and power densities after exposure to blue light LED.](Source: Authors.)

In this study, two opportunistic pathogens of the genus Staphylococcus (S. aureus and S. epidermidis) responsible for bacterial skin infections were selected as model microorganisms. In addition, the photoinactivation assays were performed, taking into account the maximum number of bacteria isolated in patients that suffered atopic dermatitis (AD) (Brown, 2020).

Thus the initial inoculums for S. aureus and S. epidermidis were ~ 1.5 x 10⁸ bacteria/mL. Next, the planktonic bacteria were exposed to blue light doses between 5 and 25 J/cm² using three light source devices (Table 1).

The results of bacterial growth after applying blue LED (Figure 2) indicate a reduction in the number of viable cells, this factor being dependent on the fluency and power density applied. For the S. aureus strain (Figure 2A), a more significant reduction was observed in the group treated with 5 J/cm² and 200 mW/cm², of approximately 2 log, while the treatments with 10, 15, and 25 J/cm² and 200 mW/cm², showed a slight reduction. The second most effective treatment was 5 J/cm² and 156.3 mW/cm², reducing over one log. Although a statistically significant reduction was observed in the other treatments, it cannot be considered a bactericidal effect. For this strain of S. aureus, an effect on bacterial viability dependent on the potency density
used was demonstrated since, at the fluence of 5 J/cm² at the potency densities of 97 and 110 mW/cm², no reduction was observed in the same proportion of those treated with 156.3 and 200 mW/cm².

The observed behavior was not similar for the *S. epidermidis* strain (Figure 2B). The best result was achieved with 25 J/cm² and 97 mW/cm² with a reduction of approximately 1 log, while all other treatments showed a similar reduction pattern of less than one log. Therefore, for *S. epidermidis*, no more significant effect was demonstrated for higher power densities in any of the fluences tested.

These results demonstrate that the temperature variations observed in the study do not imply a change in the viability of both strains. The observed effects are only a result of the interaction with the LED at 450 nm.

**Figure 2.** Viability evaluation of *S. aureus* (A) and *S. epidermidis* (B) in CFU/ml after applying different fluences and power densities. Symbols represent a significant difference p<0.01 between treatments and their respective controls.

Source: Authors.
Phototherapy involves the application of visible light using irradiation parameters considered therapeutic. These parameters involve the power density measured in W/cm², or mW/cm² is the light output power per unit area, it can also be called intensity or fluence rate; energy density measured in J/cm² is the amount of energy per unit area transferred to matter, also known as fluence or light dose. In addition, these parameters modulate the tissue response, either minimizing the thermal effect, stimulating or inhibiting the tissue response (Ribeiro, 2004).

In the present study, the blue LED with a wavelength of 450±10 nm was used to inactivate strains of *S. aureus* in vitro because it was demonstrated in previous studies that the values between 453 and 480 nm are less cytotoxic for human cells (Opländer et al., 2011; Rupel et al., 2019) and also because they are used in phototherapy equipment in the area of Aesthetics.

*S. aureus* is the most common pathogen in skin infections (Oyama et al., 2020; Santajit & Indrawattana, 2016; Tong, Davis, Eichenberger, Holland, & Fowler, 2015). At the same time, *S. epidermidis* is the most abundant bacterial colonizer of healthy human skin, which is attributed to an essential role in preventing skin colonization of bacterial pathogens. However, current evidence suggests that colonization by specific strains of *S. epidermidis* may either help or damage the skin barrier (Brown & Horswill, 2020). Patients with AD are often highly colonized with *S. aureus* at lesional sites (7 x 10⁶ bacteria/cm²). However, some studies reported that these patients could be highly colonized by *S. epidermidis* rather than *S. aureus* (Byrd et al., 2017; Hon, Tsang, Pong, Leung, & Ip, 2016). Moreover, it has been reported that the skin inflammation severity tends to correlate with the number of bacteria in the site of infection (Travers et al., 2012). In this sense, the effectiveness of the blue light treatment was evaluated, taking into account the bacterial density present in skin infections caused by opportunistic pathogens such as *S. aureus* and *S. epidermidis*. Therefore to mimic the number of bacteria present at the lesional site during the bacterial infection, the initial inoculums of *S. aureus* and *S. epidermidis* were 1 x 10⁷ and 10⁶ bacteria/cm², respectively.

The results of our work indicated that no microbial reduction above 2 log was observed in the irradiated groups with power densities of 97.110, 156, and 200 mW/cm² at different energy densities (5, 10, 15, and 25 J/cm²) when compared to the control groups. There were no statistical differences with the energy density of 25 J/cm² between the power densities used, inferring that in this energy density, the power density, after a certain point, is not relevant for the antimicrobial effect. Other studies used parameters similar to this study (405 nm, 100 mW/cm², and power densities between 1 and 60 J/cm²) in strains of methicillin-resistant *S. aureus*. In this sense, Enwemeka et al. reduced 50% (2-fold reduction) the initial inoculum (5x10⁶ CFU/mL) at 12 J/cm², which is in agreement with our results (Enwemeka, Williams, Hollosi, Yens, & Enwemeka, 2008). Moreover, they observed that the microbial reduction does not double or triple at densities of 24 J/cm² and 36 J/cm², and the fluence-dependent effect is non-linear. Bumah achieved 100% reduction with an exposure of 3x10⁶ CFU/mL, although, with denser bacterial solutions, 5x10⁶ CFU/mL, two exposures at 50 J/cm² were necessary to obtain the same microbial reduction.

There was no significant microbial reduction at 450 nm, at 97 mW/cm², and 10-15 J/cm² with a single light application in this study. Comparing the results obtained by Enwemeka et al., it is believed that shorter wavelengths in the range of visible blue light are an essential parameter for the antimicrobial effect. In addition, Bumah achieved a reduction of colonies in higher energy densities, in which it is believed that there is a more preponderant thermotoxic action than the phototoxic action.

Maclean et al. achieved a 5 log reduction with the parameters of 405 nm, 10 mW/cm², and 36 J/cm², but the exposure time, 60 to 90 minutes, was too long to be applied to people. (Maclean, MacGregor, Anderson, & Woolsey, 2009).

Lipovsky et al. and Maclean et al. also achieved significant microbial reduction: 415 nm, 100 mW/cm², 120 J/cm² (90% reduction) and 400 nm, 300 mW/cm², and 450 J/cm² (3 log reduction), respectively. As the energy density used by both is relatively high, it is believed that the high thermal factor must have been an adjunct to the inhibition of bacterial growth (Lipovsky et al., 2010; Maclean, MacGregor, Anderson, & Woolsey, 2008).

Froes et al. achieved a satisfactory microbial reduction in the treatment of folliculitis. However, the result was not only due to the application of blue light. The protocol consisted of 4 sessions, with the application of blue LED (4 Joules/40s) and
infrared (32s), using antifoliculitis cream with ammonium lactate and essential oil of tea tree (Froes-Meyer et al., 2018).

It is important to note that all irradiated groups received a single exposure to blue light in the present study. Therefore, the temperature for each group was checked to ensure that the thermal effect was not an adjuvant factor in the inactivation of the bacteria. The temperature in the Biotable homemade remained in the range of 20-25 °C, and the highest temperature reached during the exposures was 51.7°C with the Elite Olympus (DMC) device.

It was observed that the results in the three devices used, Biopdi450, biotable manufactured by the laboratory and Elite Olympus (DMC) - the latter used in the area of Aesthetics - were similar, suggesting that there was standard behavior of response to irradiation in the face of increased irradiation parameters, i.e., a nonsignificant reduction.

It is necessary to analyze how many irradiations are necessary to define the parameters of emission of blue light that inhibit S. aureus without causing harmful effects to human cells. In power and energy densities, the thermal effect is accentuated, and it is not desired in applications in patients in the Aesthetic area. The choice of wavelength seems relevant to avoid damage to human cells, as there are studies that report that wavelengths between 400-420 nm can induce different degrees of intracellular oxidative stress17. Therefore, it is recommended to use the range from 450 to 470 nm, which is safer. However, the choice of energy and power density parameters still causes controversy among the scientific community.

4. Conclusion

This study demonstrated that the energy and power densities used were insufficient to cause a bactericidal effect of S. aureus planktonic cultures in a single application. Studies describe the efficacy of blue light as a promising tool to inhibit the growth of S. aureus in vitro. However, further studies are needed to establish protocols, parameters, and adjustments for the best application.

Acknowledgments

The authors would like to thank the Research and Development Institute of the University of Vale do Paraíba (IP&D - UNIVAP) and Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001.

References


