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ORIGINAL ARTICLE

The effect of combined curcumin-mediated photodynamic therapy and artificial skin on *Staphylococcus aureus*–infected wounds in rats

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Abstract

Healing wounds represent a major public health problem, mainly when it is infected. Besides that, the antibiotics misuse and overuse favor the development of bacterial resistance. This study evaluated the effects of antimicrobial photodynamic therapy (aPDT) combined with artificial skin on disinfection of infected skin wound in rats. Twenty-four Wistar rats were randomly distributed into 4 groups (n = 6): (i) control—untreated; (ii) aPDT—treated with curcumin-mediated aPDT (blue light); (iii) artificial skin—treated with artificial skin alcohol-based; and (iv) aPDT plus artificial skin—treated with aPDT associated with artificial skin alcohol-based. For the in vivo model, a full-thickness biopsy with 0.80 cm was performed in order to inoculate the microorganism *Staphylococcus aureus* (ATCC 25923). The aPDT was performed with a curcumin gel and a blue LED light (450 nm, 80 mW/cm^2) at the dose of 60 J/cm^2 and the treatment with alcohol-based artificial skin was done with the topical application of 250 μL. Additional animals were submitted to aPDT combined with the artificial skin. After treatments, the number of colony-forming units (CFU) and the damage area were determined. Data were analyzed by two-way repeated measures ANOVA and Tukey tests. The highest reduction of the bacterial viability was observed in the PDT plus artificial skin group (4.14 log10), followed by artificial skin (2.38 log10) and PDT (2.22 log10) groups. In addition, all treated groups showed higher relative area of wound contraction (36.21% for the PDT, 38.41% for artificial skin, and 35.02% for PDT plus artificial) in comparison with the control group. These findings provide evidence for the positive benefits of aPDT with blue light and curcumin associated with artificial skin to decontaminate and accelerate the wound contraction.

Keywords Curcumin · Blue light · Photochemotherapy · Artificial skin · Wound healing

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Introduction

Skin and soft tissue infections (SSTIs) are among the most common infections and causes of emergency care. According to the Infectious Disease Society of America (IDSA), between 2005 and 2010, more than 3 million patients have received medical care in the emergency department for SSTI per year. It represents a 3-fold increase compared to the preceding 15 years—leading to a higher morbidity and healthcare costs. The majority of SSTI are caused by bacteria and, among them, *Staphylococcus aureus* is one of the most frequent pathogen isolated from contaminated wounds [1, 2].

The goal of the antimicrobial therapies is to eradicate the causative organisms, avoiding complications and preventing recurrences. The conventional treatment is based on an incision and drainage as well as antibiotic therapy [2]. However, the antibiotics misuse and overuse during infection treatments,

the lack of new antibiotics, and the poor infection prevention are accelerating the development of new resistant bacteria [3]. Moreover, multiple antibiotic-resistant strains are becoming more frequently isolated and the available drugs are not effective to inactivate them [4]. The development of approaches that can selectively inactivate bacteria limiting the spread of resistance should be investigated. In this context, antimicrobial photodynamic therapy (aPDT) and artificial skins are promising alternative therapeutic approaches.

aPDT is a technique based on the photoactivation of a photosensitizer (PS) in the presence of oxygen, to induce cell death. There are two different mechanisms described in the literature responsible for the microorganism inactivation by aPDT. These mechanisms lead to deoxyribonucleic acid (DNA) damage and damage to the cytoplasmic membrane, which allows leakage of cellular contents or inactivation of membrane transport system and enzymes [5].

Several in vivo studies have demonstrated the potential of aPDT to treat infected wound using a range of PSs and light sources, such as red wavelength (laser or non-coherent light) with toluidine-O blue [6], methylene blue [7], highly pure chlorin e6 [8], polycationic PS conjugate between poly-L-lysine, and chlorin e6 [9], among others [10, 11]. The use of natural compounds to mediate aPDT has also been employed. Curcumin is a natural yellow-orange dye obtained from the root of *Curcuma longa* L., an old Indian spice that exhibits antibacterial, antiviral, and antifungal effects [12]. Curcumin associated with light may be able to increase some of these properties. In vitro [13] and clinical trial [14] studies have shown that the combination of curcumin and blue LED light was able to significantly reduce the total number of colony-forming units (CFU) of oral microorganisms. However, to the best of our knowledge, the effects of blue light and curcumin for infected wound healing have not been investigated.

Artificial skins, also named skin substitutes or wound dressing, can be used as an adjuvant treatment to favor the skin healing process. It refers to a biological or synthetic scaffold engineered to enhance wound healing or even to provide permanent skin replacements. It is known for treating massive burns and deep skin wounds, by limiting infection and fluid loss as well as reducing inflammatory responses and scarring [15–17]. Various materials have been employed for skin substitute fabrication, such as elastin [18], collagen [19], chitosan [20], antibiotic-loaded collagen-hyaluronic acid matrix [21], polyvinyl alcohol [22], and platelet-rich plasma [23].

Considering the potential of curcumin-mediated aPDT to inactivate microorganisms and the positive use of artificial skin to hold wound contraction, the aim of this study was to evaluate the effects of aPDT with blue light and curcumin associated with artificial skin alcohol-based in rat preclinical model to disinfect and contract wounds.

Materials and methods

Animals

This study was approved by the Ethics and Research Committee of the Federal University of São Carlos (N. 053/2012). All animal procedures were performed in accordance with the principles in the Guide for the Care and Use of Laboratory Animals. Twenty-four 3-month-old male Wistar (albino) rats weighing between 350 and 450 g were used. Food (Nuvilab CR1, Nuvital Nutrientes S/A, Brazil) and water were available ad libitum. The cages were maintained in controlled temperature (22 ± 2 °C) and humidity (70%) with a 12/12-h light-dark photoperiod. Animals were group-housed and randomly distributed into four groups (n = 6 per group): (i) control group, (ii) PDT group, (iii) artificial skin group, and (iv) PDT plus artificial skin group. The study timeline is shown in Fig. 1.

Microorganism, animal model

The reference strain of *Staphylococcus aureus* ATCC 25923 (Rockville, MD) was used to induce the wound infection. The bacteria were frozen at − 20 °C in a tube containing brain heart infusion (BHI) broth and were reactivated in Mannitol agar (Difco Laboratories, Detroit, Mich., EUA) at 37 °C for 24 h. The cells were cultured in BHI broth (Laboratório Difco, Detroit, Mich., EUA) at 37 °C for 18 h. Then, cells were concentrated by centrifugation (3000 rpm) during 5 min and the supernatant was discarded. The cells were resuspended in distilled water (5 mL) and the number of viable cells in suspension was determined using a Neubauer chamber and trypan blue under an optical microscope (Carl Zeiss, Jena, Germany). The *Staphylococcus aureus* inocula was prepared in mid-log phase = 25×10^7 cells, as previously described [24].

For the wound induction and infection, the rats were anesthetized with an intramuscular injection (90 mg/kg of ketamine (Agener União, Brasília, DF, Brazil) plus 10 mg/kg of xylazine (Bayer, São Paulo, SP, Brazil)) and a 0.80-cm diameter punch was used to remove a circular full-thickness skin on the dorsal surface. Immediately after the skin removal, each wound was inoculated with 100-μL suspension of *Staphylococcus aureus* into the wound bed.

Treatments

Four days after the wound induction and infection, animals received the proposed treatments. For the PDT treatment, 0.06 mL of 1.5% curcumin gel (PDT Pharma, Cravinhos, SP, Brazil) was topically applied on the wound and incubated for 20 min, covered with plastic wrap and aluminum foil (Fig. 2). After the incubation, the wound was illuminated with

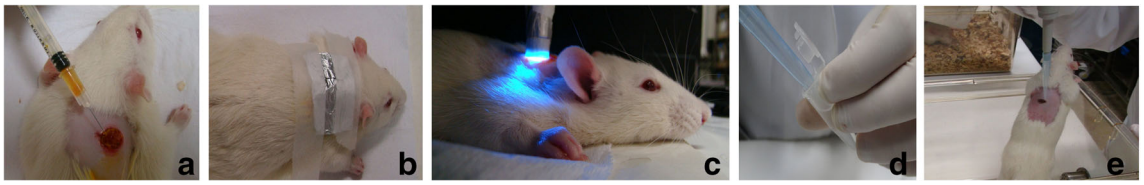

Fig. 1 aPDT procedure and artificial skin treatment. The curcumin gel was topically applied on the wound (**a**). It was incubated and covered with plastic wrap and aluminum foil (**b**). After the incubation, the wound was illuminated with a blue LED (**c**). After aPDT, the artificial skin (**d**) was applied with a pipette onto the wound site (**e**)

a LED-based device at 450 ± 30 nm, 80 mW/cm^2 developed by the Optics Group of the São Carlos Institute of Physics (IFSC), University of São Paulo (USP). The wound was irradiated (Fig. 2) for 12.5 min. and the dose delivered was ~ 60 J/cm^2. A single session of the PDT was performed on the fourth day of the study.

After aPDT, an aliquot of 250-μL artificial skin was applied with a pipette onto the wound site (Fig. 2) (PDT plus artificial skin group). The artificial skin was applied for 4 consecutive days. The artificial skin used in this study is a non-commercial, natural, biocompatible, and biodegradable product [25]. It is a dense and transparent liquid composed of water (33%), polyvinyl alcohol (16%), and hydrated alcohol (51%). This artificial skin was obtained by increasing the temperature from 20° to 75 °C, when a final dense liquid is obtained.

Additional animals received only one session of aPDT (PDT group) or only artificial skin therapy during 4 consecutive days (artificial skin group). The control group consisted of animals with no treatment.

Microbiological analysis

To recover the microorganisms, the wounds were swabbed twice for 20 s in each period of evaluation (pre-contamination—day 1; pre-treatment—day 3; and post-treatment—day 8). Each collection was made in duplicate. The sterile cotton swabs (Absorve, Cotia, SP, Brazil) were transferred to tubes containing 1 mL of sterile saline. The samples underwent serial dilutions and 25-μl aliquots of each dilution were plated on brain heart infusion agar (BHIA; Difco Laboratories, Detroit, Mich.) in quadruplicate and incubated under microaerophilic conditions for 24 h at 37 °C. After incubation, the total number of colony-forming units per milliliters (CFU/mL) was determined.

Wound measurements

The animals were anesthetized (90 mg/kg of ketamine (Agener União, Brasília, Brazil) plus 10 mg/kg of xylazine (Bayer, São Paulo, Brazil)) and standardized photographs (same resolution and focal distance of 20 cm (Nikon SLR camera D40 X, 10.2 megapixel, Tokyo, Japan)) were used to measure the wound area at each timepoint (pre-contamination, pre-treatment, and post-treatment) in all groups. An adhesive tape of 1 cm^2 was positioned on a flat surface next to the wound as a reference. Afterwards, the wound area was determined using the ImageJ (Rasband, WS, ImageJ, National Institutes of Health, Bethesda, MD; http://imagej.nih.gov/ij/). Wound areas were calculated in absolute and relative terms. The relative area of wound contraction was determined using the following formula: relative area of wound contraction (%) = [initial damage area (cm^2) − contracted area (cm^2) / initial damage area (cm^2) × 100].

After completion of the protocol, on day 8, the animals were sacrificed with intracardiac injection of 1 ml of potassium chloride (3 M KCL), under general anesthesia (90 mg/kg of ketamine (Agener União, Brasília, DF, Brazil) plus 10 mg/kg of xylazine (Bayer, São Paulo, SP, Brazil)).

Statistical analysis

The log10 (CFU/mL) values were used in the statistical analysis. The descriptive analysis of the data was performed and, then, Shapiro-Wilk and Levene's tests were used to analyze the normality and homogeneity of variance. Two-way repeated measures ANOVA with post hoc Tukey tests were used to compare the log10 values and the damaged area at each timepoint. The independent factors were group (with four levels: control, PDT, skin artificial, and PDT plus artificial skin groups) and time ((i) with two levels for log10 (CFU/

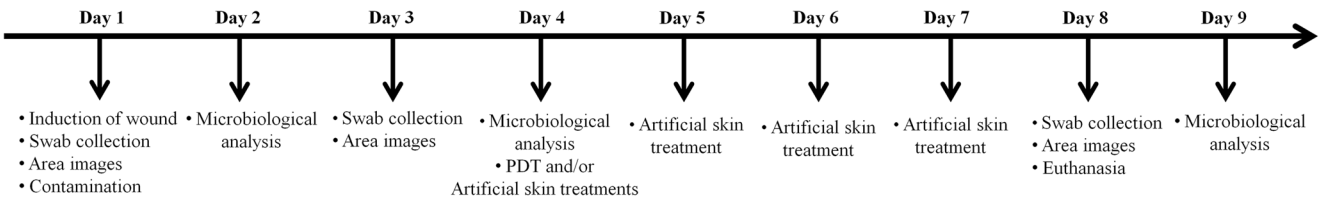

Fig. 2 The study timeline

ml) and relative area of wound contraction: pre-treatment and post-treatment; and (ii) with three levels for damaged area: pre-contamination, pre-treatment, and post-treatment, which were also considered a repeated measurement (intragroup differences)). The delta of CFU or relative area of wound contraction between the situations before and after the treatments (Δ = post-treatment – pre-treatment) was performed for intergroup comparisons using a one-way ANOVA with post hoc Tukey tests. The Statistic for Windows Release 7 software (Statsoft Inc., Tulsa, OK) was used for the statistical analysis and the significance level was set at 5% ($p < 0.05$).

Results

CFU counting

At pre-contamination period, it was observed that all wounds were pathogen free. The swabs collected during the pre-contamination period were performed to identify any possible contamination during the surgical procedure to induce the wound. Three days after the wound infection (pre-treatment), all groups showed bacterial growth (approximately 4–6 log10 CFU/mL), confirming the success establishment of the infection (Table 1). The PDT and the artificial skin groups showed a similar log reduction (approximately 2 log reduction); no significant difference was found between the groups ($p > 0.05$). Moreover, there was a higher reduction of the viability (approximately 4 log) when both techniques were combined (Fig. 3), showing significant difference compared with other groups ($p < 0.05$). The microbial reduction (log10 CFU/mL) showed intragroup (Table 1, $p < 0.001$) and intergroup differences (Fig. 3a, $p < 0.05$) for all treated groups.

Wound areas

The relative areas of wound contraction for all groups were maintained at around 35–45% before treatment; however, following the appropriate treatments, the values of areas contracted around 80% compared to the pre-treatment area for all groups (PDT, skin, PDT+skin) while the control group remained almost unchanged (Table 1). However, no significant differences between aPDT, artificial skin, or both techniques combined were found related to wound shrinkage when comparing the difference between the relative area of wound contraction post-treatment and the relative area of wound contraction pre-treatment (Fig. 3b). The damage area (absolute values) showed intragroup differences (Table 1, $p < 0.05$) for all the groups; however, the relative area of wound contraction (relative data) showed intragroup (Table 1, $p < 0.001$) and intergroup (Fig. 3b, $p < 0.001$) differences for all the treated groups unless for the control group.

Discussion

The emergence of resistant bacteria to the available antibiotics has led to the failure of the wound infections' treatment. The

Table 1 Effects of PDT and/or artificial skin on the Log 10 CFU/ml and the wound area

	Pre-contamination	Pre-treatment	Post-treatment
Log 10 CFU/ml			
Control group	–	5.23 ± 1.17	5.27 ± 0.37
PDT group	–	5.94 ± 0.61	3.71 ± 0.40[a]
Artificial skin group	–	4.96 ± 0.28	2.58 ± 0.69[a]
PDT + artificial skin group	–	5.75 ± 0.53	1.56 ± 0.78[a]
Wound area (cm^2)			
Control group	0.85 ± 0.32	0.54 ± 0.29	0.49 ± 0.22[b]
PDT group	0.77 ± 0.40	0.41 ± 0.18[a]	0.15 ± 0.08[bc]
Artificial skin group	0.55 ± 0.12	0.33 ± 0.04[a]	0.13 ± 0.03[bc]
PDT + artificial skin group	0.80 ± 0.38	0.33 ± 0.14 [a]	0.13 ± 0.03[bc]
Relative area of wound contraction (%)			
Control group	–	36 ± 20	42 ± 13
PDT group	–	44 ± 19	79 ± 4[a]
Artificial skin group	–	36 ± 17	75 ± 8[a]
PDT + artificial skin group	–	45 ± 24	80 ± 9[a]

[a] Significant difference compared with pre-treatment (repeated measures ANOVA with post hoc Tukey, $p < 0.001$)

[b] Significant difference compared with pre-treatment (repeated measures ANOVA with post hoc Tukey, $p < 0.05$)

[c] Significant difference compared with pre-contamination (repeated measures ANOVA with post hoc Tukey, $p < 0.05$)

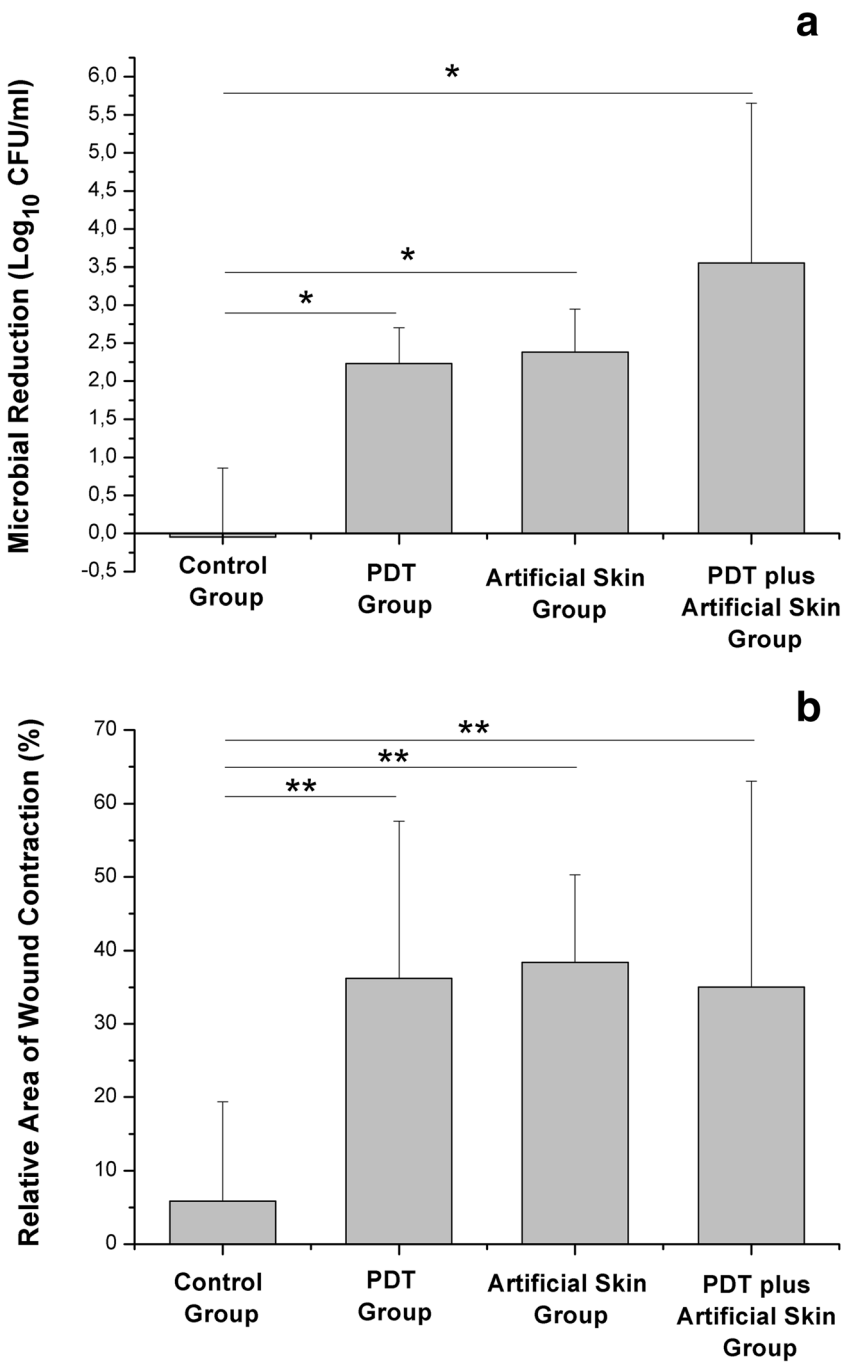

Fig. 3 The delta (post–pre = Δ) between key variable before and after treatment. Microbial reduction (**a**) and contracted wound (**b**). Significant intergroup difference (one-way ANOVA with post hoc Tukey, $*p < 0.05$ and $**p < 0.001$)

efforts for the development of alternative therapies have been turned an urgent need. The use of aPDT for this purpose has been intensively investigated evaluating a range of PSs, light sources, and in combination with others strategies. However, to the author's knowledge, this was the first study showing the potential of single aPDT session combined with an artificial skin as an alternative treatment for *Staphylococcus aureus* skin infection. Here, the curcumin-mediated aPDT was performed at low concentration of PS and low light dose and it was observed that aPDT or artificial skin was able to reduce approximately 2 log10 of CFU. When both treatments were combined, approximately 4 log10 of reduction was achieved, showing clinical significance. Previous studies of Lanzafame et al. [26] showed the potential of blue light combined with collagen-embedded flavins on methicillin-resistant *Staphylococcus aureus* in mice, inactivating 2–3 log10 of CFU. Zolfaghari et al. [7] also showed the antimicrobial potential of methylene blue with red laser on methicillin-resistant *Staphylococcus aureus* in mouse. Topaloglu et al. [27] observed around 90% of bacterial reduction on abrasion wound after aPDT using 808-nm laser and indocyanine green. All these results corroborate the findings in the current study,

showing aPDT as a promising therapy for bacterial infection, and more interestingly, the use of artificial skin to potentiate the effectiveness and healing.

The presence of high concentrations of *Staphylococcus aureus* in acute and chronic wounds promotes changes in the gene expression of the immune response [28] and develops virulence factors, specifically proteases, to overcome immune defenses and destroy the host's connective tissue, weakening the wound contraction response [29]. In the current study, the treated animals with aPDT and/or artificial skin showed reduction of CFU and higher wound contraction, suggesting that a bacterial control contributed to a tissue repair [30, 31].

The aPDT protocol developed in this study indicates optimal curcumin concentration and blue light intensity, promoting bacterial reduction and enabling wound contraction with single application. Curcumin (negative potential), when photoactivated, promotes electrostatic interaction with the cell wall of *Staphylococcus aureus* (gram-positive) and provides increased porosity and better permeation of curcumin in the cytoplasmic membrane [32]. aPDT parameters in wound healing of animals varied in the PS incubation time from 15 to 90 min, with an intensity of 84 to 300 mW/cm^2 and fluency from 6 to 450 J/cm^2 [33]. The 15-min time taken by DAI et al. [34] was similar to the present study (12.5 min); however, the amount of energy to sensitize chlorine (c6) was 240 J/cm^2 (100 mW/cm^2), differing from the current study that used 60 J/cm^2 (80 mW/cm^2). Okada et al. [35] reported only a slight inflammation on the supraspinous layer of mice's oral mucous membranes when curcumin at 1 M in glycerin was applied in combination with 400 mW/cm^2 for 5 min. In the present study, there was only one application of aPDT, demonstrating excellent antibiotic response and stimulating the wound to contract after 4 days of therapeutic intervention. Likewise, CHEN et al. [31], when applying aPDT with short application time and fluency of 15 J/cm^2, promoted bactericidal action with ZnPc-(Lys)5 and optimized the contraction of the wounds, but with 12 daily applications. In this sense, the photoactivation of curcumin demonstrated the need for less irradiation time, fluence, and intensity, when comparing with the physical parameters of the other aPDT [33].

According to Zolfaghari et al. [7], the in vivo bacterial killing by aPDT can be affected by some factors: (1) binding of PS to host material, resulting generation of singlet oxygen where there is no bacteria; (2) absorption of the light by the host tissue, hindering bacterial inactivation; and (3) quenching of singlet oxygen by the host molecules, protecting the bacteria. Additionally, according to Dai et al. [11], it is possible the in vivo formation of a complex biofilm on the surface of the host and this microbiological community is another factor that limits PS access and, as a consequence, it decreases the PS concentration in deep sites of tissues. Therefore, the in vivo evaluation of a treatment is an important step prior to clinical recommendation, since animal models mimic human infections and are a valuable tool to establish effective parameters of new antimicrobial therapies. For this reason, the positive results observed in the current study hold great potential for clinical application in the future.

The mechanism involved in the bacterial photokilling mediated by curcumin has been described and is related to the formation of reactive oxygen species (ROS) such as singlet oxygen [36], hydroxyl radical [37], or hydrogen peroxide [38]. Besides the antimicrobial effect, aPDT may promote cell death caused by the impairment of microcirculation and inflammatory responses [10]. The efficiency of aPDT reduces the healing time of wounds [11]; on the other hand, aPDT can have deleterious effects on connective tissue and increase the healing time of wounds [10]. In addition, aPDT did not provoke any healing inhibition and harmful effect. Dovigo et al. [39] concluded that curcumin-mediated aPDT was effective for in vivo inactivation of *Candida albicans* without harming the host tissue of mice. For this reason, curcumin-mediated aPDT can be safely used for wound care management, including leg ulcers, diabetic foot ulcers, and pressure sores. The benefit of choosing aPDT with curcumin provides low cost of PS, local bactericidal effect, and absence of systemic complications when compared to antibiotics. Moreover, Curcumin as PS can be easily incorporated into creams or solutions that make its topical application very convenient. Its quantum efficiency is high, meaning it has a great capacity to convert the energy of photons into free radicals. It is also of great compatibility with human tissues and has good penetrability, allowing decontamination even for an internal portion of the contaminated layers of the wounds. There are several studies that indicate curcumin as an excellent option for use as a microbicide in PDT [40, 41].

Regarding the artificial skin, in the study of Li, Wang, and Wu [21], a polyvinyl alcohol (PVA)–based artificial skin was used. PVA is one of the most frequently and the oldest synthetic polymer employed as wound dressings, wound management, drug delivery systems, artificial organs, and contact lenses. In the current study, when the artificial skin was applied on the wound, it solidified and mimicked the mobility of the skin as an elastomer. Besides that, this artificial skin exhibits important properties that accelerate the healing, such as the presence of microporous that do not block oxygen delivery to tissue and as an alcohol; it is antiseptic, avoiding further bacterial contamination. According to Korsmeyer and Peppas [42], another advantage of this artificial skin is non-toxicity, non-carcinogenicity, and good biocompatibility. Kang YO et al. [30] observed accelerated healing using chitosan-coated polyvinyl alcohol compound for wound dressing, and the authors attributed this positive result to the greater surface area and microporous structure, promoting cell attachment and proliferation in the tissue. For this reason, PVA-based artificial skin is a valid option to be employed in the wound healing process and, moreover, to be associated with antimicrobial treatments to enhance the effectiveness against microorganisms.

Although there was microbial reduction in the current study, aPDT plus artificial skin did not have additional effects on wound contraction, showing a similar result to isolated therapies. In this context, the photobiomodulation [43] with red and/or infrared radiation could be used to improve the protocol established here and will be investigated in the future.

Lack of groups (curcumin alone and blue light alone) and absence of histology and physiology assessments (e.g., cytokines, growth factors, and antioxidants activities) are considered limitations in the current study. Additional groups and several wound healing measurements should be considered in future studies to determine optimal approaches to wound management.

Therefore, recent technological advances may promote the treatment and closure rapid of wounds as well as a functional and aesthetically satisfactory scar. In this context, aPDT and artificial skins are an alternative therapeutic approach.

Conclusion

In conclusion, these findings provide evidence for the positive benefits of aPDT mediated by curcumin and blue light in association with artificial skin, where bacterial inactivation and accelerated healing of the wounds were observed in Wistar rats, without causing any side effect to the tissue. Future clinical studies should be carried out for effectiveness of the treatment protocol.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Ethics and Research Committee of the Federal University of São Carlos (N. 053/2012). All animal procedures were performed in accordance with the principles in the Guide for the Care and Use of Laboratory Animals.

References

1. Pallin DJ, Egan DJ, Pelletier AJ, Espinola JA, Hooper DC, Camargo CA Jr (2008) Increased US emergency department visits for skin and soft tissue infections, and changes in antibiotic choices, during the emergence of community-associated methicillin-resistant Staphylococcus aureus. Ann Emerg Med 51(3):291–298
2. Chahine EB, Sucher AJ (2015) Skin and soft tissue infections. In: Murphy JE, Lee MW (eds) PSAP Infectious Diseases. American College of Clinical Pharmacy, pp 5–26
3. Dai T, Gupta A, Huang YY, Sherwood ME, Murray CK, Vrahas MS, Kielian T, Hamblin MR (2013) Blue light eliminates community-acquired methicillin-resistant Staphylococcus aureus in infected mouse skin abrasions. Photomed Laser Surg 31(11): 531–538
4. Gould IM, Bal AM (2013) New antibiotic agents in the pipeline and how they can overcome microbial resistance. Virulence 4(2):185–191
5. Hamblin MR, Hasan T (2004) Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci 3(5):436–450
6. Garcia VG, de Lima MA, Okamoto T, Milanezi LA, Júnior EC, Fernandes LA, de Almeida JM, Theodoro LH (2010) Effect of photodynamic therapy on the healing of cutaneous third-degree-burn: histological study in rats. Lasers Med Sci 25(2):221–228
7. Zolfaghari PS, Packer S, Singer M, Nair SP, Bennett J, Street C, Wilson M (2009) In vivo killing of Staphylococcus aureus using a light-activated antimicrobial agent. BMC Microbiol 9:27
8. Park JH, Ahn MY, Kim YC, Kim SA, Moon YH, Ahn SG, Yoon JH (2012) In vitro and in vivo antimicrobial effect of photodynamic therapy using a highly pure chlorin e6 against Staphylococcus aureus Xen29. Biol Pharm Bull 35(4):509–514
9. Hamblin MR, O'Donnell DA, Murthy N, Contag CH, Hasan T (2002) Rapid control of wound infections by targeted photodynamic therapy monitored by in vivo bioluminescence imaging. Photochem Photobiol 75(1):51–57
10. Lambrechts SA, Demidova TN, Aalders MC, Hasan T, Hamblin MR (2005) Photodynamic therapy for Staphylococcus aureus infected burn wounds in mice. Photochem Photobiol Sci 4(7):503–509
11. Dai T, Tegos GP, Zhiyentayev T, Mylonakis E, Hamblin MR (2010) Photodynamic therapy for methicillin-resistant Staphylococcus aureus infection in a mouse skin abrasion model. Lasers Surg Med 42(1):38–44
12. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K (2014) A review on antibacterial, antiviral, and antifungal activity of curcumin. Biomed Res Int 186864. https://doi.org/10.1155/2014/186864
13. Dovigo LN, Pavarina AC, Ribeiro AP, Brunetti IL, Costa CA, Jacomassi DP, Bagnato VS, Kurachi C (2011) Investigation of the photodynamic effects of curcumin against Candida albicans. Photochem Photobiol 87(4):895–903
14. Leite DP, Paolillo FR, Parmesano TN, Fontana CR, Bagnato VS (2014) Effects of photodynamic therapy with blue light and curcumin as mouth rinse for oral disinfection: a randomized controlled trial. Photomed Laser Surg 32(11):627–632
15. Halim AS, Khoo TL, Mohd. Yussof SJ (2010) Biologic and synthetic skin substitutes: an overview. Indian J Plastic Surg 43(Suppl): S23–S28
16. Nathoo R, Howe N, Cohen G (2014) Skin substitutes: an overview of the key players in wound management. J Clin Aesthet Dermatol 7(10):44–48
17. Varkey M, Ding J, Tredget EE (2015) Advances in skin substitutes-potential of tissue engineered skin for facilitating anti-fibrotic healing. J Funct Biomater 6(3):547–563
18. Daamen WF, Veerkamp JH, van Hest JCM, Van Kuppevelt TH (2007) Elastin as a biomaterial for tissue engineering. Biomaterials 28:4378–4398
19. Miyata T, Taira T, Noishiki Y (1992) Collagen engineering for biomaterial use. Clin Mater 9:139–148
20. Dai NT, Williamson MR, Khammo N, Adams EF, Coombes AG (2004) Composite cell support membranes based on collagen and

polycaprolactone for tissue engineering of skin. Biomaterials 25(18):4263–4271
21. Park SN, Kim JK, Suh H (2004) Evaluation of antibiotic-loaded collagen-hyaluronic acid matrix as a skin substitute. Biomaterials 25(17):3689–3698
22. Li JK, Wang N, Wu XS (1998) Poly(vinyl alcohol) nanoparticles prepared by freezing-thawing process for protein/peptide drug delivery. J Control Release 56(1-3):117–126
23. Rozman P, Bolta Z (2007) Use of platelet growth factors in treating wounds and soft-tissue injuries. Acta Dermatovenerol Alp Pannonica Adriat 16(4):156–165
24. Burkatovskaya M, Castano AP, Demidova-Rice TN, Tegos GP, Hamblin MR (2008) Effect of chitosan acetate bandage on wound healing in infected and noninfected wounds in mice. Wound Repair Regen 16(3):425–431
25. Janota R (2015) Película protetora epidérmica corpórea antibacteriana, processo de obtenção de película protetora epidérmica corpórea antibacteriana e modo de aplicação. BR patent no. BR 10 2012 023591 9 A2. BR: INPI
26. Lanzafame RJ, Stadler I, Cunningham R, Muhlbauer A, Griggs J, Soltz R, Soltz BA (2013) Preliminary assessment of photoactivated antimicrobial collagen on bioburden in a murine pressure ulcer model. Photomed Laser Surg 31(11):539–546
27. Topaloglu N, Güney M, Yuksel S, Gülsoy M (2015) Antibacterial photodynamic therapy with 808-nm laser and indocyanine green on abrasion wound models. J Biomed Opt 20(2):28003
28. Xu Z, Hsia HC (2018) The impact of microbial communities on wound healing: a review. Ann Plast Surg 81(1):113–123
29. Lindsay S, Oates A, Bourdillon K (2017) The detrimental impact of extracellular bacterial proteases on wound healing. Int Wound J 14(6):1237–1247
30. Kang YO, Yoon IS, Lee SY, Kim DD, Lee SJ, Park WH, Hudson SM (2010) Chitosan-coated poly(vinyl alcohol) nanofibers for wound dressings. J Biomed Mater Res B Appl Biomater 92(2):568–576
31. Chen Z, Zhang Y, Wang D, Li L, Zhou S, Huang JH, Chen J, Hu P, Huang M (2016) Photodynamic antimicrobial chemotherapy using zinc phthalocyanine derivatives in treatment of bacterial skin infection. J Biomed Opt 21(1):18001
32. Agel MR, Baghdan E, Pinnapireddy SR, Lehmann J, Schäfer J, Bakowsky U (2019) Curcumin loaded nanoparticles as efficient photoactive formulations against gram-positive and gram-negative bacteria. Colloids Surf B Biointerfaces 1(178):460–468
33. Sun Y, Ogawa R, Xiao BH, Feng YX, Wu Y, Chen LH, Gao XH, Chen HD (2019) Antimicrobial photodynamic therapy in skin wound healing: a systematic review of animal studies. Int Wound J 17(2):285–299
34. Dai T, Tegos GP, Lu Z, Huang L, Zhiyentayev T, Franklin MJ, Baer DG, Hamblin MR (2009) Photodynamic therapy for Acinetobacter baumannii burn infections in mice. Antimicrob Agents Chemother 53(9):3929–3934
35. Okada N, Muraoka E, Fujisawa S, Machino M (2012) Effects of curcumin and capsaicin irradiated with visible light on murine oral mucosa. In Vivo 26(5):759–764
36. Ribeiro AP, Pavarina AC, Dovigo LN, Brunetti IL, Bagnato VS, Vergani CE, Costa CA (2013) Phototoxic effect of curcumin on methicillin-resistant Staphylococcus aureus and L929 fibroblasts. Lasers Med Sci 28(2):391–398
37. Kunchandy E, Rao MNA (1990) Oxygen radical scavenging activity of curcumin. Int J Pharm 58:237–240
38. Dahl TA, Mcgowan WM, Shand MA, Srinivasan VS (1989) Photokilling of bacteria by the natural dye curcumin. Arch Microbiol 151:183–185
39. Dovigo LN, Carmello JC, de Souza Costa CA, Vergani CE, Brunetti IL, Bagnato VS, Pavarina AC (2013) Curcumin-mediated photodynamic inactivation of Candida albicans in a murine model of oral candidiasis. Med Mycol 51(3):243–251
40. da Silva AP, Carbinato FM, Bagnato VS, Inada NM (2015) A promising strategy for the treatment of onychomycosis with curcumin and photodynamic therapy. J Pharm Pharmacol 3:434–437
41. Soares JM, Silva KOO, Inada NM, Bagnato VS, Blanco KC (2020) Optimization for microbial incorporation and efficiency of photodynamic therapy using variation on curcumin formulation. Photodiagn Photodyn Ther 29:101652
42. Korsmeyer RW, Peppas NA (1981) Effect of the morphology of hydrophilic polymeric matrices on the diffusion and release of water soluble drugs. J Membr Sci 9(3):211–227
43. Corazza AV, Jorge J, Kurachi C, Bagnato VS (2007) Photobiomodulation on the angiogenesis of skin wounds in rats using different light sources. Photomed Laser Surg 25(2):102–106