Received: 18 September 2019 | Revised: 24 October 2019 | Accepted: 28 October 2019

DOI: 10.1111/iwj.13269

ORIGINAL ARTICLE

IWJ WILEY

Antimicrobial photodynamic therapy in skin wound healing: A systematic review of animal studies

Yan Sun[1] | Rei Ogawa[2] | Bi-Huan Xiao[1] | Yu-Xin Feng[1] | Yan Wu[1] | Liang-Hong Chen[3] | Xing-Hua Gao[1] | Hong-Duo Chen[1]

[1]Department of Dermatology, The First Hospital of China Medical University, Shenyang, China

[2]Department of Plastic, Reconstructive and Aesthetic Surgery, Nippon Medical School, Tokyo, Japan

[3]Department of Emergency Medicine, Shengjing Hospital of China Medical University, Shenyang, China

Correspondence
Liang-Hong Chen, Address: Department of Emergency Medicine, Shengjing Hospital of China Medical University, No.36 Sanhao Street, 110011, Shenyang, China.
Email: skill002@163.com,

Yan Wu, Department of Dermatology, The First Hospital of China Medical University, 155 North Nanjing Street, Shenyang, 110001, China.
Email: jlwuyan@126.com

Funding information
111 Project, Grant/Award Number: D18011; National Key Research and Development Program of China, Grant/Award Number: 2016YFC0901504

Abstract

Bacterial infection is a common wound complication that can significantly delay healing. Classical local therapies for infected wounds are expensive and are frequently ineffective. One alternative therapy is photodynamic therapy (PDT). We conducted a systematic review to clarify whether PDT is useful for bacteria-infected wounds in animal models. PubMed and Medline were searched for articles on PDT in infected skin wounds in animals. The language was limited to English. Nineteen articles met the inclusion criteria. The overall study methodological quality was moderate, with a low-moderate risk of bias. The animal models were mice and rats. The wounds were excisional, burn, and abrasion wounds. Wound size ranged from 6 mm in diameter to 1.5×1.5 cm^2. Most studies inoculated the wounds with *Pseudomonas aeruginosa* or methicillin-resistant *Staphylococcus aureus*. Eleven and 17 studies showed that the PDT of infected wounds significantly decreased wound size and bacterial counts, respectively. Six, four, and two studies examined the effect of PDT on infected wound-cytokine levels, wound-healing time, and body weight, respectively. Most indicated that PDT had beneficial effects on these variables. PDT accelerated bacteria-infected wound healing in animals by promoting wound closure and killing bacteria.

1 | INTRODUCTION

Wounds are mainly caused by trauma, surgery, and diseases, including diabetes mellitus and vascular diseases. They can have a profound deleterious effect on patient quality of life and are common. For example, it is estimated that, at any given time point, 1.5 to 2 million people in Europe have acute or chronic wounds that require medical care.[1] Moreover, several studies have shown that patients with wounds occupy 27% to 50% of acute hospital beds in Europe on any given day[2] and cost the United Kingdom approximately £5 billion annually in 2012/2013: the latter accounted for 3% of all British health care expenditure in 2012/2013.[3]

Infection is one of the most common complications of wounds that could delay wound healing. Bacterial infections are particularly frequent. The most common culprit is *Staphylococcus aureus*. The most common therapy for infected wounds is antibiotics. However, much to the alarm of health care workers all over the world,

© 2019 Medicalhelplines.com Inc and John Wiley & Sons Ltd

antibiotics have become increasingly less effective for bacterial wound infections because of the abuse of antibiotics and the subsequent emergence of increasing numbers of multi-resistant bacterial species such as *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus*.[4] Other classical local therapies for infected wounds include dressings, topical products, and—more recently—negative pressure therapy. However, all of these classical therapies, including antibiotics, are expensive and not consistently effective.[5] Thus, there is a great need for alternative therapies for infected wounds.

One possibility is photodynamic therapy (PDT).[6] This is not a new treatment as it has been used for about 100 years to topically treat various skin lesions, starting with superficial non-melanoma skin cancers.[7,8] PDT involves three key ingredients, namely, a photosensitiser,[9] light, and oxygen. The process starts with the topical application of the photosensitiser onto the target lesion. The application is followed by an incubation period that allows the abnormally proliferating cells in the lesion to take up the photosensitiser and convert it to a component in the heme biosynthetic pathway, called protoporphyrin-IX. When the lesion is illuminated with light of the appropriate wavelength, photoporphyrin-IX becomes activated and converts molecular oxygen into reactive oxygen species. This in turn causes the cells to die via necrosis or apoptosis. The preferential uptake of the photosensitiser by proliferating cells means that this therapy does not affect the surrounding normal tissue.[10]

Recently, several researchers showed that PDT improves wound healing in mice and humans,[11,12] has antimicrobial effects on bacteria isolated from infected human burn wounds,[13] and improves the healing of leg ulcers.[14] Human studies also showed that PDT is well tolerated by the patients.[12,14] Thus, PDT may be a suitable alternative to standard therapies for infected wounds.

In 2018, Nesi-Reis et al conducted a systematic review on the ability of PDT to improve (non-infected) chronic ulcers and superficial non-melanoma skin cancers in humans.[15] However, a systematic review on the antimicrobial effects of PDT and its ability to promote the healing of infected wounds has not been reported. Here, we conducted a systematic review to clarify whether PDT is useful for bacteria-infected wounds in animal models. We expect that this review will promote further research and ultimately aid the clinical treatment of wounds.

2 | MATERIALS AND METHODS

This study followed the guidelines for systematic reviews of animal studies that were suggested by Vries et al.[16]

Key Messages

- a systematic review is conducted to clarify whether photodynamic therapy (PDT) is useful for bacteria-infected wounds in animal models
- PubMed and Medline were searched, and the language was limited to English
- nineteen articles met the inclusion criteria PDT accelerated bacteria-infected wound healing in animals by promoting wound closure and killing bacteria

2.1 | Data sources and searches

The PubMed and Medline databases (from inception to May 2019) were searched for all PDT-related articles. The language was limited to English. The Medical Subject Headings (MeSH) terms were as follows: ("Wound Healing" OR "Re-Epithelialization" OR "Regeneration") AND ("Photochemotherapy" OR "Photochemistry" OR "Photosensitizing Agents"). The bibliographic references of the articles that were captured by this electronic database search were also searched manually to identify additional potential studies.

2.2 | Study selection

All articles on animals that reported the antimicrobial effects of PDT in skin wound healing and that had a PDT-untreated control group were eligible for inclusion. The titles and abstracts of all captured articles were screened for possible inclusion. Reviews, duplicates, human studies, studies on wounds that did not involve the skin, and studies irrelevant to our topic were excluded. After reading the full text of the remaining articles, all studies that compared animals treated with PDT alone to untreated control animals were included in the systematic review. Articles in which the PDT was only delivered in combination with another treatment were excluded. Articles that involved wounds that had not been inoculated with bacteria were also excluded. Two independent reviewers (Yan Sun and Bi-Huan Xiao) conducted this article selection procedure. Disagreements were resolved by discussions between these reviewers or by asking a third reviewer (Liang-Hong Chen) to help achieve consensus.

2.3 | Data extraction and quality assessment

Two reviewers (Yan Sun and Bi-Huan Xiao) independently extracted the following data from the included articles: first author, year of publication, country of research, animal species, type of wound, bacterial species, the photosensitiser and light parameters used for PDT, the primary and secondary study outcome measures, and the main results.

Two reviewers (Yan Sun and Bi-Huan Xiao) also independently assessed the methodological quality of the included studies. Disagreements about quality were resolved by discussions or by asking for help from a third reviewer (Liang-Hong Chen). Methodological quality was assessed by using the risk-of-bias tool of the Systematic Review Center for Laboratory animal Experimentation (SYRCLE), which involves 10 items: (a) animal allocation sequence generation, (b) comparison of baseline characteristics of the comparator animal groups, (c) allocation concealment, (d) random housing of the animals in the animal room, (e) investigator blinding, (f) random outcome assessment, (g) assessor blinding, (h) incomplete outcome data addressed, (i) free of selective outcome reporting, and (j) free of other sources of bias.[17] For each item, "yes" indicated a "low risk of bias," while "no" represented a "high risk of bias." If the information provided was not sufficient, the item was judged as "unclear," which was considered an "unclear risk of bias."

2.4 | Data synthesis

A meta-analysis was performed by using Review Manager 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). To determine the homogeneity of the outcome (body weight) of the included studies, χ^2 test was performed, and the I^2 statistic were calculated. The study outcomes were considered to be heterogenous if the χ^2 test P value was <.1 and the I^2 statistic was >50%. If heterogeneity was detected, the random-effects model was used. If homogeneity was observed, the fixed-effects model was used. The difference between the PDT-treated and untreated groups in terms of outcome was expressed relative to the outcome variability in the study by calculating the standard mean difference with 95% confidence intervals. P values of .05 were considered to indicate statistically significant differences between PDT-treated and untreated groups. To ensure the reliability and accuracy of the results, two independent reviewers (Yan Sun and Bi-Huan Xiao) synthesised the data separately.

3 | RESULTS

3.1 | Identification of eligible studies

A total of 779 articles were identified by the preliminary bibliographic search. After screening the title and abstract of each article, 738 were excluded because they were reviews, duplicates, human studies, involved non-skin wounds, or were irrelevant to our topic. The full text of the remaining 41 articles underwent detailed evaluation. Twenty-two articles with combined interventions or wounds not inoculated with bacteria were excluded. Finally, 19 articles that related to antimicrobial PDT in skin wound healing in animal models were included. The selection process is illustrated in Figure 1.

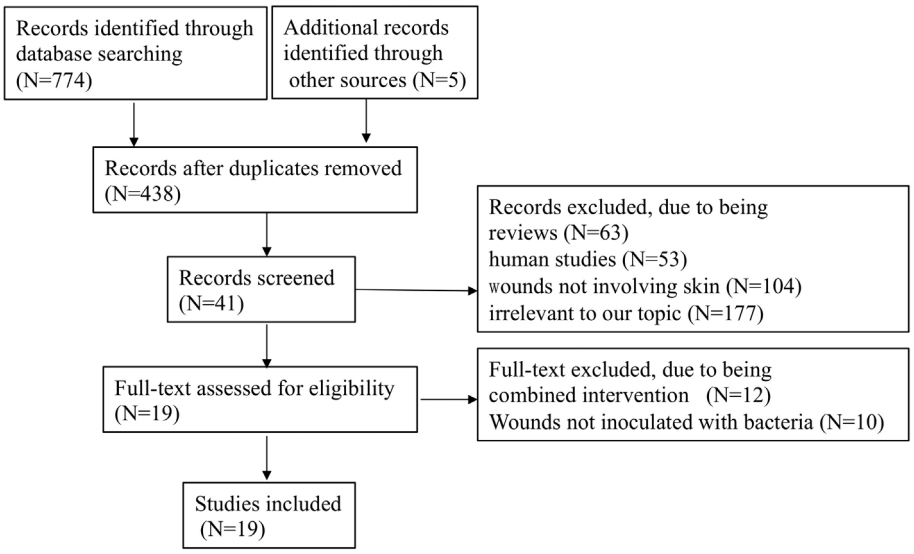

FIGURE 1 Flow diagram

3.2 | Characteristics of the included studies

All articles were written in English and were published between 2002 and 2018. The country in which the study was performed was the United States,[18-24] India,[25-28] China,[29-31] Japan,[32,33] Turkey,[34] Egypt,[35] and Italy.[36] The baseline characteristics of the studies are listed in Table 1.

3.3 | Methodological characteristics of the included studies

The quality of each study was assessed by applying the risk-of-bias tool from SYRCLE. Of the 19 studies, 26.3% generated an adequate allocation sequence (item 1),[29,31,34-36] 89.5% reported the baseline characteristics of the PDT-treated and untreated groups (including gender, age, and weight of the subjects) (item 2),[18-21,23-29,31-36] 5.3% adequately concealed allocation (item 3),[35] 36.8% reported that each animal was housed in a cage of its own in the animal room (item 4),[19,20,27,32,33,35,36] 73.7% addressed incomplete outcome data (item 8),[18-20,25-35] and all but one study[23] (94.7%) were free of selective outcome reporting (item 9). None of the studies indicated whether the investigators were blinded (item 5), the outcome assessments were random (item 6), or the assessors were blinded (item 7). All of the studies were free of other sources of bias. Figure 2 presents a summary of the methodological quality of the 19 studies.

3.4 | Animal models

The animal species used in the 19 studies were mice (n = 16)[18-29,31-33,36] and rats (n = 3).[30,34,35] Of the 16 mouse studies, nine used BALB/c mice,[18-21,23,24,29,31,36] 4 utilised Swiss albino mice,[25-28] two used C57BL/ksj db/db mice,[32,33] and one used CD1 mice.[36] One study[22] did not report the mouse strain that was used. Notably, three of these studies used diabetic mice to produced chronic wound models, namely, the two studies using C57BL/ksj db/db mice[32,33] and one study using Streptozotocin-injected Swiss albino mice.[25] Two studies used immunosuppressed mice; the low peripheral blood neutrophils of these mice created an environment that was more vulnerable to infection.[18,19] Of the rat studies, two used Wistar rats[34,35] and one used Sprague-Dawley rats.[30]

3.5 | Wounds

All wounds were generated on the dorsum of the animals. They were excisional wounds,[22-28,30-33,35,36] burn wounds,[20,21,29] and abrasion wounds.[18,19,34] The wound sizes ranged from 6 mm in diameter[32] to 1.5 × 1.5 cm^2.[31]

3.6 | Bacteria

To investigate the antimicrobial effect of PDT, the wounds in all studies were inoculated with one or two species of bacteria. The majority were *P. aeruginosa*[22,23,26-28,32] and methicillin-resistant *S. aureus*.[18,19,25,27,29,31,33,34,36] Others were unresistant *S. aureus* strains,[21,29,30,35,36] *Escherichia coli*,[22,24] and *Acinetobacter baumannii*.[20]

3.7 | Photosensitizers

The photosensitisers that are used for PDT can be classified biochemically as first-, second-, and third-generation photosensitisers.[37] The photosensitisers that were used in the studies were from the second generation with the exception of hypericin-laden nanoparticles, which was used in the study by Nafee et al[35] and which belong to the third-generation photosensitiser class. The second-generation photosensitiser that is most commonly used in recent times is 5-aminolevulinic acid. It was used in two studies.[32,33] Other used photosensitisers were 5-phenyl-10; 15, 20-tris (N-methyl-4-pyridyl) porphyrin chloride (PTMPP)[21]; sinoporphyrin sodium (also known as DVDMS)[29]; indocyanine green (ICG)[34]; pentalysine β-carbonylphthalocyanine zinc (ZnPc-$[Lys]_5$)[30]; zinc phthalocyanine tetrasulfonate (ZnPc-S_4)[30]; poly-L-lysine conjugate of chlorine p6 (pl-cp6),[25-28] β-lactamase-activated EtNBS (LAEtNBS)[31]; RLP068/Cl,[18,36] toluidine blue O (TBO)[18]; polyethylenimine-chlorin(e6) (PEI-ce6)[19,20]; and poly-L-lysine-chlorin e6 (pL-ce6).[22-24]

The photosensitisers were administered topically in 13 studies,[18-20,22-28,31,32,36] by subcutaneous injection in one study,[29] and by intraperitoneal injection in another study.[33] In one study, burn wounds were simultaneously treated by both topical application and injection under the wound.[21] The application route was not described in three studies.[30,34,35] The incubation time ranged from 15 minutes[20] to 90 minutes.[31]

TABLE 1 The baseline characteristics of included studies

Author	Animal	Wound	Bacteria	Photosensitiser	Light
Katayama et al[32]	Male C57BL/ksj db/db mice	Excisional wounds	*PA*	0.1% or 0.5% ALA; topical	410 nm LED, 164.5 mW/cm^2, 6 or 9 J/cm^2
Mai et al[29]	Female BALB/c mice	Burn wounds	*S. aureus* or *MRSA*	10, 5, or 2 μM DVDMS, injected subcutaneously, 75 min	635 nm semiconductor laser, 50 J/cm^2, 300 mW/cm^2
Chen et al[30]	Sprague–Dawley rats	Excisional wounds	*S. aureus*	1 mM ZnPc-$(Lys)_5$ or ZnPc-S_4, 25 μL, 30 min	680 nm, 100 mW, 15 J/cm^2, 5 min
Sahu et al[25]	Female Swiss albino mice	Excisional wounds	*PA*	200 μM pl-cp6, topical, 30 min	(660 ± 25) nm red light, 100 mW/cm^2, 60 J/cm^2, 10 min
Sahu et al[26]	Male and female diabetic swiss albino mice	Excisional wounds	*MRSA*	200 μM pl-cp6, 20 μL, topical, 30 min	(660 ± 25) nm red light, 100 mW/cm^2, 60 or 120 J/cm^2, 10 or 20 min
Topaloglu et al[34]	Female Wistar albino rats	Abrasion wounds	*MRSA*	500, 1000, 2000 μg/mL ICG, 50 μL, 30 min	808 nm diode laser, 450 J/cm^2, 15 min
Morimoto et al[33]	Male C57BL/ksj db/db mice	Excisional wounds	*MRSA*	50 or 200 mg/kg 5-ALA, injected intraperitoneally	410 nm LED, 164.5 mW/cm^2, 10 or 50 J/cm^2
Fu et al[31]	Male BALB/c mice	Excisional wounds	*MRSA*	200 μM LAEtNBS or EtNBS-COOH, 50 μL, topical, 90 min	(640 ± 10) nm LED, 90 J/cm^2, 30 min
Sahu et al[24]	Female Swiss albino mice	Excisional wounds	*MRSA or PA*	100 or 200 μM pl-cp6, 20 μL, topical, 30 min	(660 ± 25) nm, ~100 mW/cm^2, 60 or 120 J/cm^2, 10 or 20 min
Nafee et al[35]	Female Wistar rat	Excisional wounds	*S. sureus*	0.124 μM HY-DMSO or HY-NPs, 50 μL	23.5 J/cm^2
Sahu et al[28]	Female Swiss albino mice	Excisional wounds	*PA*	200 μM pl-cp6, 25 μL, topical, 30 min	(660 ± 25) nm red light, ~100 mW/cm^2, 60 or 120 J/cm^2, 10 or 20 min
Vecchio et al[18]	Immunosuppressed female BALB/c mice	Abrasion wounds	*MRSA*	75 μM RLP068/Cl or TBO, 40 μL, topical, 30 min	(690 ± 15) nm or (630 ± 15) nm noncoherent light, 100 mW/cm^2, 84 J/cm^2, 14 min
Simonetti et al[36]	CD1 mice or BALB/c mice	Excisional wounds	*MRSA* or *S. aureus*	0.01%, 0.1%, 0.3%, or 0.5% RLP068/Cl, 25 μL, topical, 1 h	698 nm diode laser, 120 mW/cm^2,60 J/cm^2
Dai et al[19]	Immunosuppressed female BALB/c mice	Abrasion wounds	*MRSA*	400 μM PEI-c_{e6}, 50 μL, topical	(660 ± 15) nm non-coherent light, 100 mW/cm^2, 360 J/cm^2
Dai et al[20]	Female BALB/c mice	Burn	*A. baumannii*	PEI-c_{e6}, 50 μL, topical, 15 min	(660 ± 15) nm non-coherent light, 100 mW/cm^2, 240 J/cm^2
Demidova et al[22]	Mice	Excisional wounds	*E. coli* or *PA*	pl-ce6, 50 μL, topical, 30 min	660 nm diode laser, 100 mW/cm^2, 165 or 240 J/cm^2
Lambrechts et al[21]	Male BALB/c mice	Burn	*S. aureus*	500 μM PTMPP, 100 μL, topical and injected	(635 ± 15)nm, 84 mW/cm^2, 211 or 423 J/cm^2, 42 or 82 min

(Continues)

TABLE 1 (Continued)

Author	Animal	Wound	Bacteria	Photosensitiser	Light
Hamblin et al[23]	Male BALB/c	Excisional wounds	*PA*	200 μM pl–c_{e6}, 50 μL, topical, ≥30 min	665 nm diode laser, 100 mW/cm^2, 240 J/cm^2
Hamblin et al[24]	Male BALB/c	Excisional wounds	*E. coli*	100 μM pl–c_{e6}, 50 μL, topical, 30 min	665 nm diode laser, 100 mW/cm^2, 160 J/cm^2, 27 min

Abbreviations: *A. baumannii*, Acinetobacter baumannii; ALA, aminolevulinic acid; DVDMS, sinoporphyrin sodium; *E. coli*, *Escherichia coli*; EtNBS-COOH, 5-(30 -Carboxypropylamino)-9-diethylaminobenzogurepheno-thiazinium chloride; ICG, indocyanine green; LAEtNBS, b-lactamase Activated EtNBS; HY-DMSO, hypericin-dimethyl sulfoxide; HY-NPs, hypericin-laden nanoparticles; LED, light-emitting diode; MRSA, methicillin-resistant *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa*; PEI- c_{e6}, polyethylenimine-chlorin e6; pl-ce6, poly-L-lysine-chlorin(e6); pl-cp6, poly-L-lysine conjugate of chlorine p6; PTMPP, 5-Phenyl-10,15,20-tris(N-methyl-4-pyridyl)porphyrin chloride; *S. aureus*, *Staphylococcus aureus*; TBO, toluidine blue O; ZnPc-$(Lys)_5$, pentalysine β-carbonylphthalocyanine zinc; ZnPc-S_4, Zinc phthalocyanine tetrasulfonate.

3.8 | Light

The light wavelengths that were used in most studies belonged to the red light range. Two studies used 410-nm light-emitting diodes (LEDs),[32,33,38] one study used an 808-nm diode laser,[34] and one study did not mention the wavelength of the light source.[35] When visible light was used, the fluence ranged from 6 [32] to 450 J/cm^2,[34] and the energy density ranged from 84[21] to 300 mW/cm^2.[29] For the 808-nm diode laser, the fluence was 450 J/cm^2.[34]

3.9 | Treatment methods, outcome measures, and main results

The treatment methods, outcome measures, and the main results are listed in Table 2. The outcomes after PDT that were measured were wound size, bacterial numbers in the wound, wound cytokine levels, wound-healing time, and body weight.

3.10 | Wound size

Eleven studies examined the effect of PDT on infected wounds at various times after treatment.[18,21,23,24,28,30-35] As expected, several studies showed that bacterial infections delayed wound healing: as a result, the untreated infected wounds had significantly greater wound sizes at various assessment time points than the uninfected wounds.[28,33] With regard to the effect of PDT on wound size, 1 of the 11 studies showed that PDT did not differ from various controls in terms of reducing wound size.[24] However, the remaining 10 studies all found that the PDT-treated bacterially infected wounds were significantly smaller at various time points than the infected wounds that had not been treated[18,21,28,32,35] or had been treated with photosensitiser only[18,21,30,31,33] or light only.[18]

In one study, PDT was combined with topical 1% silver sulfadiazine cream and compared with the cream on its own: the combination treatment performed significantly worse in terms of reducing wound size than the cream.[21] In two other studies, topical 0.5% $AgNO_3$[23] or 2% mupirocin[34] on their own served as positive controls for PDT: in both cases, PDT reduced wound size better than these drugs.

Two studies showed that PDT reduced infected ulcer size so effectively that it was not only significantly smaller than the size of untreated bacteria-infected ulcers, but it was similar to the size of uninfected control wounds at most time points.[32,33] Both studies also showed that altering PDT parameters affected efficacy in

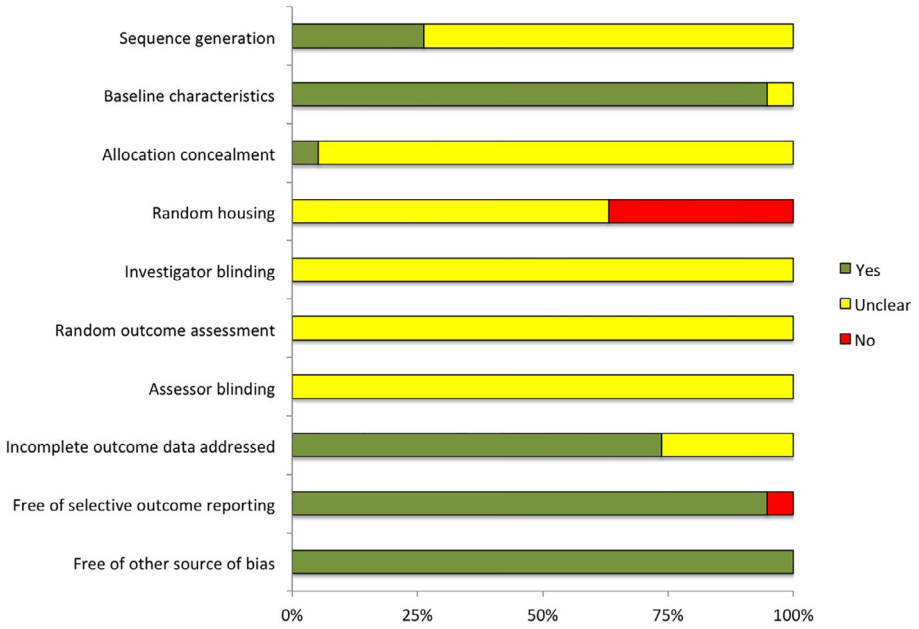

FIGURE 2 The methodological quality of included studies

terms of wound size: PDT with higher photosensitiser concentrations or light fluences reduced infected ulcer size better than when these parameters were set at lower levels.[32,33] Moreover, Nafee et al. showed that, when a second-generation photosensitiser (hypericin) was loaded onto nanoparticles (thus creating a third-generation photosensitiser preparation), it yielded smaller wound sizes than when the wound was treated with the same concentration of the photosensitiser diluted in dimethyl sulfoxide (DMSO).[35]

3.11 | Bacterial measurements

Seventeen studies measured the effect of PDT on wound bacteria counts or bioluminescence.[18-24,26,28-36] All showed that PDT reduced the infection. Specifically, the PDT-treated infected wounds had significantly ($P < .05$) lower bacterial counts at various time points than infected control wounds that had not been treated[19,20,22-24,26,28,29,35,36] or had been treated with photosensitiser only[18,22-24,28,30,34] or light only.[23,24,31,34]

In several studies, topical or injected antibacterial drugs served as positive controls for PDT: in all cases, PDT performed similarly or better in terms of reducing bacterial levels.[23,32,33,36] In one study, PDT + silver sulfadiazine cream was not as effective as the cream alone in terms of reducing the bacterial infection.[21]

Several studies examined the effect of altering PDT parameters on bacterial counts. One study showed that PDT with 0.01% of the photosensitiser RLP68/Cl did not reduce bacterial counts, but PDT with 0.1% or 0.3% RLP68/Cl reduced it by ≥83% ($P < .05$). PDT with 0.5% RLP68/Cl did not reduce the counts further.[36] However, another study showed that PDT with the photosensitiser ICG at concentrations of 500, 1000, and 2000 μg/mL reduced the bacterial viability equally well ($P > .05$).[34] Furthermore, Nafee et al. showed that hypericin-loaded nanoparticles (a third-generation photosynthesiser preparation) lowered the bacterial counts better ($P < .05$) than the same concentration of hypericin diluted in DMSO.[35]

3.12 | Cytokine levels

Six studies evaluated the effect of PDT on the levels of various cytokines in infected wounds.[25-29,35] They showed that, compared with no treatment, PDT significantly increased the wound levels of β-fibroblast growth factor,[26,29] transforming growth factor (TGF)-β1,[29] vascular endothelial growth factor (VEGF),[29,35] VEGF-A,[25] hydroxyproline,[27,29] alkaline phosphatase,[26] fibroblast growth factor-2,[26] nitric oxide,[25] platelet-derived growth factor,[35] and cyclooxygenase-2[35] while significantly ($P < .05$) decreasing the wound levels of interleukin (IL)-6,[28,29] IL-1α,[26] IL-1ß,[26] IL-2,[26] malondialdehyde,[29] toll-like receptor-4,[26] nuclear factor kappa B (NF-κB)-p65,[25] phosphorylated NF-κB-p65,[25] phosphorylated-IKB-α,[25] phosphorylated p38 mitogen-activated protein kinase,[25] metalloproteinase-8 and -9,[27] and tumour necrosis factor-α.[28,29] Nafee et al. showed that that, when the photosynthesiser hypericin was loaded onto nanoparticles, it reduced TNF-α expression more markedly ($P < .05$) than when the wound was treated with the same concentration of hypericin diluted in DMSO.[35]

TABLE 2 The treatment methods, outcome measures, and the main results of included studies

Author	Treatment methods			Outcome measures	Main results
	T	C			
		C_b	C_u		
Katayama et al[32]	T1:0.1%ALA + 0.005% EDTA-2Na + 9 J/cm^2 (N = 5); T2:0.5%ALA +0.005% EDTA-2Na + 9 J/cm^2 (N = 5); T3:0.5% ALA+0.001% EDTA-2Na + 9 J/cm^2 (N = 5); T4:0.5%ALA+0.005% EDTA-2Na + 6 J/cm^2 (N = 5)	C_b1:NT (N = 5); C_b2: piperacillin-tazobactam (N = 5)	C_u:NT (N = 5)	1, 2	1:T2 < T1 < C_b1, T2 < T4; T2 vs C_u, T3 vs C_b1 ($P > .05$); 2:T2 (2 log10 units) < C_b2 (7 log10 units), C_b1 vs C_b2 ($P > .05$)
Mai et al[29]	T1:10 μMDVDMS + 50 J/cm^2 (once per day, 2 days) (N = 8); T2:5 μMDVDMS + 50 J/cm^2 (once per day, 2 days) (N = 8); T3: 2 μM DVDMS+50 J/cm^2 (once per day, 2 days) (N = 8)	C_b: 0.1mlPBS (N = 8)		2, 3, 4(a), 5, 6, 7, 8, 9, 20	2:T < C_b; 3:T > C_b, highest in T1 on Day 7; 4(a): T < C_b, lowest in T1 on Day 3; 5: T < C_b, lowest in T1; 6: T > C_b, increased gradually; 7: T > C_b, highest in T1; 8: T > C on Day7, 14 and 21; 9: T < C on Day 7, 14, and 21; 20. T1 vs T2 vs T3 vs C_b ($P > .05$)
Chen et al[30]	T1:1μMZnPc-$(Lys)_5$ + 15 J/cm^2 ; T2: ZnPc-S_4 + 15 J/cm^2	C_b1:1μMZnPc- $(Lys)_5$; C_b2: ZnPc-S4		1, 2, 10	1: smallest in T1; 2: lowest in T1; 10: T1 (1.4-fold) < C_b1 (2.2-fold) < T2/C_b2 (2.8-fold)
Sahu et al[25]	T: 200 μM pl-cp6 + 60 J/cm^2	C_b: NT	C_u: NT	2, 3, 4(b), 4(c), 4(d), 11, 12(a), 12(b), 13	2: T < C_b; 3: T > C_b; 4(b), 4(c), 4(d): C_b > C_u, T < C_b on Day 5; 11: C_b > C_u, T < C_b on Day 2; T < C_b, C_b > C_u (2-fold) on Day 5; 12(a): T > C_b > C_u on Day 2; C_b > T, T vs C_u ($P > .05$) on Day 5; 12(b): C_b > C_u, 12(b) < 12 (a) on Day 2; 13: C_b < C_u (1.5-fold on Day 2 and 4-fold on Day 5), T > C_b
Sahu et al[26]	T: 20 μL 200 μM pl-cp6 + 60 J/cm^2 (once per day, 3 days)	C_b1: NT; C_b2: $AgNO_3$; C_b3: AG	C_u1: 20 μl 200 μM pl-cp6 + 60 J/cm^2 on Day 2; C_u2: 20 μl 200 μM pl-cp6 + 120 J/cm^2 on Day 2	6(b), 12(c), 12(d), 12(e), 14, 15	6(b): increased in C_u1, decreased in C_u2, increased in T by 1.75-fold, lower in C_b2 and C_b3 than T; 12(c), 12(d), 12(e), 15: T < C_b2 < C_b3, C_b2 > C_b1; 14: increased in C_u1 by 1.5-fold, decreased in C_u2 by 1.5-fold, increased in T, C_b1 vs C_b2 ($P > .05$)
Topaloglu et al[34]	T1-1: 500 μg/mL ICG + 450 J/cm^2 (N ≥ 8) T1-2: 500 μg/mL ICG + 450 J/cm2 (N = 5); T2: 1000 μg/mL ICG + 450 J/cm^2 (N ≥ 8); T3: 2000 μg/mL ICG + 450 J/cm^2 (N ≥ 8)	C_b1:NT (N ≥ 8); C_b2:450 J/cm^2 (N ≥ 8); C_b3: ICG (N ≥ 8); C_b4: mupirocin (N = 5)		1, 2,	1: T1-2 < C_b4; 2: reduced 90% in T1-1/T3/T4, T1-1 vs T2 vs T3, C_b2 vs C_b1 ($P > .05$)

(Continues)

TABLE 2 (Continued)

Author	Treatment methods			Outcome measures	Main results
	T	C			
		C_b	C_u		
Morimoto et al[33]	T1: 50 mg/kg 5-ALA+50 J/cm^2 (N = 5); T2-1: 200 mg/kg 5-ALA+50 J/cm^2 (N = 5); T2-2: 200 mg/kg 5-ALA+50 J/cm^2 (N = 3); T3: 200 mg/kg 5-ALA+10 J/cm^2 (N = 5)	C_b1-1:NT (N = 5); C_b1-2: NT (N = 3); C_b2: 50 J/cm^2 (N = 5); C_b3: 200 mg/kg 5-ALA (N = 5); C_b4-1: VCM (N = 5); C_b4-2: VCM (N = 3)	C_u: NT (N = 5)	1, 2	1: C_b1-1 > C_u, C_b2 > T1 > T2-1, T3 > T2-1, C_b4-1 > C_u; T2-1 vs C_u, C_b3 vs C_b1-1, C_b4-1 vs C_b1-1($P > .05$) 2: T2-2 (2 log10 units of reduction) < C_b1-2 on Day 7
Fu et al[34]	T1: 200 μM LAEtNBS+90 J/cm^2; T2: 200 μM EtBS-COOH+90 J/cm^2	C_b: PBS + 90 J/cm^2		1, 2, 16	1, 2: T1/T2 < C_b; T1 vs T2 ($P > .05$); 16: T1 (14.8 ± 1.5)/T2 (14.5 ± 1.7) < C_b (21.0 ± 2.3), T1 vs T2 ($P > .05$)
Sahu et al[24]	T1: pl-cp6 + 60 J/cm^2 (*MRSA*); T2: pl-cp6 + 120 J/cm^2 (*MRSA*); T3: pl-cp6 + 60 J/cm^2 (*PA*)	C_b1: NT(*MRSA*); C_b2: pl-cp6 (*MRSA*); C_b3: NT(*PA*)	C_u	8, 17(a), 17(b)	8:T1/T2 > C_b1/C_b2 (3-fold), C_b1/C_b2 < C_u on Day 18; 17(a), 17(b): C_b3 > C_u,C_b3 > T3
Nafee et al[35]	T1: HY-DMSO+23.5 J/cm^2 (once per day, 5 days) (N = 4); T2: HY-NPs + 23.5 J/cm^2 (once per day, 5 days) (N = 4)	C_b: NT (N = 4)		1, 2, 5, 6(a), 18, 19	1: T2 < T1 < C_b on Day 10; 2: T2 < T1 < C_b; 5: T2 < T1 6(a), 18, 19: T1/T2 > C_b
Sahu et al[28]	T1: 200 μM pl-cp6 + 60 J/cm^2 (N = 6); T2: 200 μM pl-cp6 + 120 J/cm^2 (N = 6)	C_b1: NT (N = 6); C_b2: 200 μM pl-cp6 (N = 6)	C_u (N = 6)	1, 2, 4(a), 5, 16	1: C_b1 > C_u on Day 2 and decreased gradually; T1/T2 reduced continuously, significant different till Day 10; 2: C_b1/C_b2 increased by 30% at 24 h and decreased by 80% at 72 h, T1/T2 reduced by 1.5/2.0 log at 24 h, T1/T2 < C_b1/C_b2 at 240 h; 4(a), 5: C_b1 > C_u (1.9 times and 3 times higher, separately), T1/T2 < C_b1 at 24 and 96 h (4–5 times lower); 16: T1/T2 < C_b1 (4–5 days lesser)
Vecchio et al[18]	T1:75 μM PLP068/Cl + 84 J/cm^2 (N = 6); T2:75 μM TBO + 84 J/cm^2 (N = 6)	C_b1 (N = 6); C_b2: 84 J/cm^2 (N = 6); C_b3: 75 μM		1, 2	1: T1 (22%) < T2 (76%) < C_b3 (78%) < C_b4 (89%) < C_b2 (98%) < C_b1 (100%) on Day 4; 2: T1 decreased 2.9 log unit, while C_b3

(Continues)

TABLE 2 (Continued)

Author	Treatment methods			Outcome measures	Main results
	T	C			
		C_b	C_u		
		PLP068/Cl (N = 6); C_b4: 75 μM TBO (N = 6)			decreased 0.85 log unit, T2 decreased 1.0 log unit, while C_b4 negligible reduced; T1 was the lowest
Simonetti et al[36]	T1: 0.01% PLP68/Cl + 60 J/cm^2 (N = 6); T2: 0.1% PLP68/Cl + 60 J/cm^2 (N = 6); T3-1: 0.3% PLP68/Cl + 60 J/cm^2 (N = 6); T3-2: 0.3% PLP68/Cl + 60 J/cm^2 (N = 12); T4: 0.5% PLP68/Cl + 60 J/cm^2 (N = 6)	C_b1: NT (N = 12); C_b2-1: placebo gel (N = 6); C_b2-2: placebo gel (N = 12); C_b3:	teicoplanin(N = 12)		2
2: Day 2: RLP068 / Cl gave a dose-related reduction, T1	(no change) > T2(83% reduction) > T3-1, T2 ($3.3 \times 10^6 \pm 4.0 \times 10^6$ CFU/mL) < C_b1 ($1.0 \times 10^9 \pm 9.6 \times 10^8$ CFU/mL), T3-1 vs T4, C_b1 vs C_b2-2 Day 9: C_b1 ($2.1 \times 10^9 \pm 2.5 \times 10^9$ CFU/mL)/C_b2-2 ($9.5 \times 10^8 \pm 8.0 \times 10^8$ CFU/mL) > C_b3 ($4.7 \times 10^7 \pm 3.9 \times 10^7$ CFU/mL) > T3-2 ($2.6 \times 10^7 \pm 2.0 \times 10^7$ CFU/mL), C_b1 vs C_b2-2($P > .05$)				
Dai et al[19]	T: 400μMPEI-c_{e6} + 360 J/cm^2 (N = 10)	C_b: NT (N = 12)		2, 16, 20	2: T reduced 2.7 log10 reduction, T1 < C_b(1.3 log10 lower); 16: T (5.6 ± 5.1) < C_b (14.2 ± 2.6); 20: T(6.1 ± 3.4%) < C_b (11.6 ± 4.0%) on Day 2
Dai et al[20]	T1: PEI-ce6 + 240 J/cm^2 1 day after infection; T2: PEI-ce6 + 240 J/cm^2 on Day 0 (N = 7); T3: PEI-ce6 + 240 J/cm^2 on Day 1 (N = 11); T4: PEI-ce6 + 240 J/cm^2 on Day 2 (N = 6); T5: PEI-ce6 + 240 J/cm^2 on Day 1 and Day 2(N = 9)	C_b1: NT; C_b2: PEI-ce6; C_b3: PBS + 240 J/cm^2		2	2: T1 reduced 1.8 log unit, C_b2 reduced less than 0.9 log unit, Cb1 (1.02×10^6RLU) > T1 (2.73×10^6RLU); T2 decreased 3.6 log units in a light exposure-dependent manner, T3/T4 decreased 1.7 long unit, T5 decreased 1.7/2.7 log unit on Day1/2
Demidova et al[22]	T: 200 μM pl-ce6 + 240 J/cm^2 (N = 10)	C_b1: NT (N = 10); C_b2: 200 μM pl-ce6 (N = 10) C_b3: 240 J/cm^2 (N = 10)		2	2: T < C_b2 < C_b1, C_b1 vs C_b3 ($P > .05$)
Lambrechts et al[21]	T1: 500 μM PTMPP+ PBS + 211 J/cm^2 (N = 3); T2-1: 500 μM PTMPP+25% DMSO/PBS + 211 J/cm^2 (N = 5); T2-2: 500 μM PTMPP +25% DMSO/PBS + 211 J/cm^2 (N = 3); T3:	C_b1: NT (N = 3); C_b2: 500 μM PTMPP (N = 3);	C_u (N = 3)	1, 2, 16, 21	1: T5 < C_b2 on Day 18, T5 < C_b1 on Day 12, 16, and 18, T5 < C_b4 on Day 12 and 18; C_b2 < C_b1 on Day 6 and 14; C_b2 < C_b4 on Day 6, C_b2 < T4 from Day 8 to 16; C_b1 > C_b3

(Continues)

TABLE 2 (Continued)

Author	Treatment methods			Outcome measures	Main results
	T	C			
		C_b	C_u		
	500 μM PTMPP+25% DMSO/PBS + 423 J/cm^2 (N = 4); T4: 500 μM PTMPP+25% DMSO/PBS + 211 J/cm^2 + AgSD (N = 3); T5: 500 μM PTMPP+25% DMSO/PBS +211/423 J/cm^2) (N = 5)	C_b3: light (N = 3); C_b4: AgSD (N = 3)			on Day 12, 14 and 16; $C_b3 < C_b4$ on Day 6, $C_b3 <$ T4 on Day 12, 16 and 18; $C_b2 <$ T4 on Day 12 2: T1 (70% reduction) > T2 (2 $\log_{10}$ unit reduction); $C_b1 < C_b3$ on Day 12, $C_b1 > C_b2$ on Day 2, T3 < C_b4/T4 on Day 3; 16: C_b4 (19 ± 4.6) < C_b3 (27.7 ± 2.9), $C_b4 <$ T5 (28.8 ± 3.6), C_b1 (22.7 ± 1.2) < T5, C_b2 (22.7 ± 2.3) < T5, T4 (26.7 ± 2.3); 21: T2-2 vs T3, C_b4 (13.0 ± 5.57 days) vs T2-1/T2-2 (18.5 ± 5.65) vs T4 (21.3 ± 4.16) ($P > .05$)
Hamblin et al[23]	T1: 200 μM pl-c_{e6} + 240 J/cm^2 (N = 10); T2: 200 μM pl-c_{e6} + 240 J/cm^2 (half *PA*) (N = 6)	C_b1: NT (N = 10); C_b2: 240 J/cm^2 (N = 10); C_b3: 200 μM pl-c_{e6} (N = 10); C_b4: $AgNO_3$ (N = 10); C_b5: $AgNO_3$ (half *PA*) (N = 6)	C_u1: NT (N = 6); C_u2: 200 μM pl-c_{e6} + 240 J/cm^2 (N = 6); C_u3: $AgNO_3$(N = 6)	1, 2	1: T1 < C_b4, T2 < C_b5; C_u3 vs C_u2, C_u3 vs C_u1 ($P > .05$) 2: T1 produced a fluence-dependent loss of luminescence, T1 < $C_b3 < C_b1/C_b2$, T1 < C_b4; C_b1 vs C_b2 ($P > .05$)
Hamblin et al[24]	T: 100 μM pl-c_{e6} + 160 J/cm^2 (N = 6)	C1-1: NT (N = 6); C1-2: 100 μM pl–c_{e6} (N = 6); C1-3: 160 J/cm^2 (N = 6)		1, 2	1: T was the smallest ($P > .05$); 2: T was the lowest and showed a semilogarithmic light dose-dependent reduction

Abbreviations: AG, aminoguanidine; $AgNO_3$, silver nitrate; AgSD, silver sulfadiazine ; C, control group; C_b, bacteria-infected group; C_u, un-infected group; NT, no treatment; T, treatment group; VCM,vancomycin; 1, wound size; 2, bacteria measurement; 3, β-fibroblast growth factor (FGF); 4(a), Interleukin (IL)-6; 4(b),IL-1α; 4(c), IL-1β; 4(d), IL-2; 5, tumour necrosis factor (TNF)-α; 6(a), vascular endothelial growth factor (VEGF); 6(b), VEGF-A; 7, transforming growth factor (TGF)-β1; 8, hydroxyproline (Hyp); 9, malondialdehyde (MDA); 10, blood flow; 11, toll-like receptor (TLR-4); 12(a), nuclear factor kappa B (NF-κB)-p50; 12(b), NF-κB-p105; 12(c), phospho-IKB-α, 12(d), NF-κB p65; 12(e), phospho-NF-κB p-65; 13, alkaline phosphatase (ALP); 14, nitric oxide (NO); 15, phospho-p38 MAPK; 16, wound-healing time; 17(a), metalloproteinase (MMP)-8; 17(b), MMP-9; 18, platelet-derived growth factor (PDGF); 19, cyclooxygenase (COX)-2; 20, body weight; 21. infection time.

3.13 | Wound-healing time

Four studies compared PDT-treated and untreated infected wounds in terms of wound-healing time.[19,21,28,31] Three showed that PDT significantly accelerated wound healing.[19,28,31] In contrast, Lambrechts et al. showed that PDT increased the wound-healing time of infected wounds compared with no treatment and photosensitiser alone. Notably, light by itself tended to slow infected wound healing as well, albeit not as much as PDT. It was suggested that the slow infected wound-healing time of PDT reflected damage caused by the light treatment, which involved high radiation energy (84 mW/cm^2, 42/82 minutes).[21]

We were able to perform a meta-analysis of the studies by Lambrechts et al.[21] and Dai et al.[19] because the data were available. We had to use the random-effects model because the I^2 value was 88% and the χ^2 P value was .003. There was no significant difference between the two studies in terms of wound-healing time (SMD = −0.29 [95% CI −4.13, 3.54], P = .88).

3.14 | Body weight

Two studies examined the effect of PDT on the body weight of mice with infected wounds.[19,29] Dai et al. found that, over the first 4 days of infection, the untreated control mice exhibited a rapid loss of weight that troughed on day 2. The PDT-treated mice exhibited a similar pattern, but the weight loss was significantly less. For example, on day 2, the mean body weights of the PDT-treated and untreated mice were 11.6 ± 4.0% and 6.1 ± 3.4%, respectively (P = .0009).[19] In contrast, Mai et al. found that mice with untreated infected wounds exhibited progressive increases in body weight over the first 5 days. Moreover, the body weight of mice with PDT-treated infected wounds exhibited similar patterns, regardless of the concentration of the photosensitiser. This suggests that PDT does not have any observable side effects.[29]

4 | DISCUSSION

Physiological wound healing is a process that repairs damaged tissues. It involves three somewhat overlapping sequential phases, namely, the inflammatory, proliferative, and remodelling phases. Many cells, molecules, and biochemical events are involved in this process.[39,40] During the inflammatory phase, immune cells in the wound bed (eg, neutrophils and macrophages) release a variety of growth factors and chemokines to remove contaminating microbes.[41] During the proliferative phase, fibroblasts proliferate in the wound bed, and keratinocytes migrate inward from wound margins. Both cell types release a variety of cytokines that promote reepithelisation and angiogenesis. Finally, during the remodelling phase, collagen is deposited, and water is reabsorbed. This increases the strength of the scar and reduces its thickness.[42]

If any step of this physiological repair process is hampered, or aberrant activities occur, wound healing can be delayed. This can lead to the formation of chronic wounds.[9,43] Many factors contribute to delayed wound healing, including diabetes mellitus, vascular insufficiency, local pressure, protease deregulation, reduced growth factor activity, inflammation, and concurrent infection.[44] Infections with bacteria have a particularly deleterious effect on wound healing, especially when the wound already exhibits delayed wound healing.[45] The four most common bacteria in wounds are Enterobacteriaceae family members, *Enterococcus* species, *P. aeruginosa*, and *Staphylococcus* species.[46] Mixed infections with these bacteria are often observed. Bacterial infections slow down wound healing because they prolong the inflammatory phase[47] and produce virulence factors, such as enterotoxins, haemolysins, matrix metalloproteinases, and hyaluronidase, that overcome host defences, promote bacterial proliferation, and aggravate local tissue destruction.[48] The studies included in our systematic review used one or more of the bacterial species listed above to create wound infections. In all cases, PDT decreased the bacterial counts in the wound.[18-24,26,28-36] This effect was particularly notable when the photosensitiser was loaded onto nanoparticle carriers.[35] Thus, PDT has marked microbicidal effects on bacterial infections in wounds.

The photosensitiser is one of the three essential elements in PDT. These molecules are classified as first-, second-, and third-generation photosensitisers. The first-generation photosensitisers include hematoporphyrin derivatives and photofrin II and are somewhat effective in some tumours.[49] However, these photosensitisers have low cell selectivity and high cutaneous phototoxicity. These limitations led to the development of the second-generation photosensitisers, which have high photosensitivity, a narrow absorption spectrum, and good tissue selectivity. The structure of most second-generation photosensitisers are based on porphyrin and include benzoporphyrins, purpurins, phthalocyanines, chlorines, and protoporphyrin-IX. Recently, the third-generation photosensitisers were generated by gene engineering or nanotechnology to further improve PDT outcomes. All but one of the studies included in our systemic review used second-generation photosensitisers. All of these studies showed that PDT accelerated the closure and

healing of bacteria-infected wounds. The remaining study by Nafee et al. used a third-generation photosensitiser, namely, hypericin loaded onto nanoparticles. They showed that it killed wound bacteria and promoted the closure and healing of the infected wounds significantly better than the second-generation formulation of the photosensitiser (hypericin diluted in DMSO).[35]

After incubation, pro-drug photosensitisers are converted into protoporphyrin-IX. The absorption spectra of protoporphyrin-IX include a maximal peak at 410 nm (Soret band) and four smaller peaks (Q bands) between 500 and 630 nm.[50] Several kinds of lasers[51,52] and LEDs[12,53] have been used to excite the photosensitiser in PDT. Lasers can emit monochromatic light with high fluency and target lesions more precisely, thereby inducing little damage to adjacent tissues. LEDs are made of electronic components and provide a narrower spectrum of light irradiation. Compared with lasers, LEDs are smaller, cheaper, and easier to operate and have a larger irradiation field. All of the studies that were included in this systematic review used lasers or LEDs as the light source. The majority of the light wavelengths were in the range of the absorption peaks of protoporphyrin-IX.

Several studies have shown that PDT can change the immune status of the target tissue.[54,55] First, it has a significant impact on neutrophil activation: after PDT, neutrophils gather in the target lesions. This reaction is due in part to upregulated TNF-α expression after PDT.[56] PDT also induces the release by various cells of pro-inflammatory cytokines, which activate other cells of the innate immune system[57] and induce monocytes, macrophages, and mast cells to accumulate in the PDT-treated lesion. This in turn activates CD8+ T cells and eliminates damaged cells and tissues.[58,59] One of the most important cytokines in wound healing is TGF-β. It participates in the entire wound-healing process. Specifically, it promotes the epithelial-mesenchymal transition and induces keratinocytes to migrate from the wound margins towards the centre. It also stimulates the chemotaxis of fibroblasts in the wound bed, promotes their synthesis of collagen, and induces them to differentiate into myofibroblasts.[55,60] The studies included in this systematic review showed that PDT of infected wounds increased their β-fibroblast growth factor, TGF-β1, and VEGF levels while decreasing their IL-6, IL-1α, IL-1β, IL-2, malondialdehyde, toll-like receptor-4, NF-κB, and TNF-α. Thus, PDT effectively alters the inflammatory environment of infected wounds; these changes may directly or indirectly increase bacterial killing and promote wound closure.

Several human studies have shown that PDT is excellent at accelerating uninfected wound healing.[55,59] Our systematic review suggests that this effect may be due in part to the antimicrobial activity of PDT, which may prevent infections from taking hold and preventing wound closure. It also shows that PDT is effective in even heavily infected wounds. In addition, PDT is non-invasive and safe[61] because the photosensitiser is absorbed selectively by the proliferating target cells or tissues. Indeed, it has been shown that, after a photosensitiser is applied to an infected lesion, the resulting protoporphyrin-IX accumulates specifically in the bacteria; other parts of the lesion have low levels of this molecule.[33] The safety of PDT is also shown by the fact that PDT does not influence the body weight of animal models with infected wounds. Indeed, PDT exerted protective effects on the body weight of mice with *P. aureus*-infected wounds.[19,29]

5 | CONCLUSIONS

In conclusion, this systematic review demonstrated that PDT could accelerate the healing of bacteria-infected wounds in animals. However, there were some limitations in this study. First, the language was limited to English, and only two databases were included. This may have caused us to overlook other relevant publications. Second, some of the included studies were of low quality and/or did not describe the details of the treatment. Third, all included studies were in vivo experiments with mice and rats. Additional high-quality studies that examine the antimicrobial effect of PDT on skin wound healing in other species (including humans) are warranted.

ACKNOWLEDGEMENT

This work was supported by the National Key Research and Development Program of China (No.2016YFC0901504) and the 111 Project (D18011).

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

ORCID

Yan Sun https://orcid.org/0000-0003-1780-3363
Rei Ogawa https://orcid.org/0000-0003-3658-555X

REFERENCES

1. Lindholm C, Searle R. Wound management for the 21st century: combining effectiveness and efficiency. *Int Wound J*. 2016;13:5-15.
2. Posnett J, Gottrup F, Lundgren H, Saal G. The resource impact of wounds on health-care providers in Europe. *J Wound Care*. 2009;18(4):154-161.
3. Guest JF, Ayoub N, Mcilwraith T, et al. Health economic burden that wounds impose on the National Health Service in the UK. *BMJ Open*. 2015;5(12):e009283.

4. Kardas P, Devine S, Golembesky A, Roberts C. A systematic review and meta-analysis of misuse of antibiotic therapies in the community. *Int J Antimicrob ag*. 2005;26(2):106-113.
5. Duque APD, Pinto NDC, Mendes RD, et al. In vivo wound healing activity of gels containing *Cecropia pachystachya* leaves. *J Pharm Pharmacol*. 2016;68(1):128-138.
6. Pariser DM, Eichenfield LF, Bukhalo M, Waterman G, Jarratt M, Grp PS. Photodynamic therapy with methyl aminolaevulinate 80 mg g(−1) for severe facial acne vulgaris: a randomized vehicle-controlled study. *Brit J Dermatol.* 2016;174 (4):770-777.
7. Jerjes W, Hamdoon Z, Hopper C. Photodynamic therapy in the management of basal cell carcinoma: retrospective evaluation of outcome. *Photodiagnosis Photodyn Ther*. 2017;19:22-27.
8. Ding C, Ma YY, Wang P, Liu J. Multiple Bowen's diseases and basal cell carcinomas in a patient with acute promyelocytic leukemia treated with arsenic trioxide: a case report and effective treatment with photodynamic therapy. *Dermatol Ther*. 2018;31 (6):e12718.
9. Morton LM, Phillips TJ. Wound healing and treating wounds: differential diagnosis and evaluation of chronic wounds. *J Am Acad Dermatol*. 2016;74(4):589-605.
10. Ozog DM, Rkein AM, Fabi SG, et al. Photodynamic therapy: a clinical consensus guide. *Dermatol Surg*. 2016;42(7):804-827.
11. Garrier J, Bezdetnaya L, Barlier C, Grafe S, Guillemin F, D'Hallewin MA. Foslip (R)-based photodynamic therapy as a means to improve wound healing. *Photodiagn Photodyn*. 2011;8 (4):321-327.
12. Rosa LP, da Silva FC, Vieira RL, et al. Application of photodynamic therapy, laser therapy, and a cellulose membrane for calcaneal pressure ulcer treatment in a diabetic patient: a case report. *Photodiagnosis Photodyn Ther*. 2017;19:235-238.
13. Mahmoudi H, Pourhajibagher M, Alikhani MY, Bahador A. The effect of antimicrobial photodynamic therapy on the expression of biofilm associated genes in *Staphylococcus aureus* strains isolated from wound infections in burn patients. *Photodiagnosis Photodyn Ther*. 2019;25:406-413.
14. Mosti G, Picerni P, Licau M, Mattaliano V. Photodynamic therapy in infected venous and mixed leg ulcers: a pilot experience. *J Wound Care*. 2018;27(12):816-821.
15. Nesi-Reis V, Lera-Nonose DSSL, Oyama J, et al. Contribution of photodynamic therapy in wound healing: a systematic review. *Photodiagn Photodyn.* 2018;21:294-305.
16. de Rob BM, Vries CRH, Langendam MW, et al. A protocol format for the preparation, registration and publication ofsystematic reviews of animal intervention studies. *Evidence-Based Preclinical Med*. 2015;1(1):1-9.
17. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*. 2014;14:43.
18. Vecchio D, Dai TH, Huang LY, Fantetti L, Roncucci G, Hamblin MR. Antimicrobial photodynamic therapy with RLP068 kills methicillin-resistant Staphylococcus aureus and improves wound healing in a mouse model of infected skin abrasion. *J Biophotonics*. 2013;6(9):733-742.
19. Dai TH, Tegos GP, Zhiyentayev T, Mylonakis E, Hamblin MR. Photodynamic therapy for methicillin-resistant *Staphylococcus aureus* infection in a mouse skin abrasion model. *Laser Surg Med*. 2010;42(1):38-44.
20. Dai TH, Tegos GP, Lu ZS, et al. Photodynamic therapy for Acinetobacter baumannii burn infections in mice. *Antimicrob Agents Ch*. 2009;53(9):3929-3934.
21. Lambrechts SAG, Demidova TN, Aalders MCG, Hasan T, Hamblin MR. Photodynamic therapy for *Staphylococcus aureus* infected burn wounds in mice. *Photoch Photobio Sci*. 2005;4(7): 503-509.
22. Demidova TN, Gad F, Zahra T, Francis KP, Hamblin MR. Monitoring photodynamic therapy of localized infections by bioluminescence imaging of genetically engineered bacteria. *J Photoch Photobio B*. 2005;81(1):15-25.
23. Hamblin MR, Zahra T, Contag CH, McManus AT, Hasan T. Optical monitoring and treatment of potentially lethal wound infections in vivo. *J Infect Dis*. 2003;187(11):1717-1725.
24. Hamblin MR, O'Donnell DA, Murthy N, Contag CH, Hasan T. Rapid control of wound infections by targeted photodynamic therapy monitored by in vivo bioluminescence imaging. *Photochem Photobiol*. 2002;75(1):51-57.
25. Sahu K, Sharma M, Dube A, Gupta PK. Topical antimicrobial photodynamic therapy improves angiogenesis in wounds of diabetic mice. *Laser Med Sci*. 2015;30(7):1923-1929.
26. Sahu K, Sharma M, Gupta PK. Modulation of inflammatory response of wounds by antimicrobial photodynamic therapy. *Laser Ther*. 2015;24(3):201-208.
27. Sahu K, Sharma M, Sharma P, et al. Effect of poly-L-lysine-chlorin P6-mediated antimicrobial photodynamic treatment on collagen restoration in bacteria-infected wounds. *Photomed Laser Surg*. 2014;32(1):23-29.
28. Sahu K, Sharma M, Bansal H, Dube A, Gupta PK. Topical photodynamic treatment with poly-L-lysine-chlorin p6 conjugate improves wound healing by reducing hyperinflammatory response in *Pseudomonas aeruginosa*-infected wounds of mice. *Lasers Med Sci*. 2013;28(2):465-471.
29. Mai B, Gao Y, Li M, et al. Photodynamic antimicrobial chemotherapy for Staphylococcus aureus and multidrug-resistant bacterial burn infection in vitro and in vivo. *Int J Nanomedicine*. 2017;12:5915-5931.
30. Chen Z, Zhang YX, Wang D, et al. Photodynamic antimicrobial chemotherapy using zinc phthalocyanine derivatives in treatment of bacterial skin infection. *J Biomed Opt*. 2016;21(1): 18001.
31. Fu XJ, Zhu YQ, Peng YB, et al. Enzyme activated photodynamic therapy for methicillin-resistant *Staphylococcus aureus* infection both inv itro and in vivo. *J Photoch Photobio B.* 2014;136:72-80.
32. Katayama B, Ozawa T, Morimoto K, et al. Enhanced sterilization and healing of cutaneous pseudomonas infection using 5-aminolevulinic acid as a photosensitizer with 410-nm LED light. *J Dermatol Sci*. 2018;90(3):323-331.
33. Morimoto K, Ozawa T, Awazu K, et al. Photodynamic therapy using systemic administration of 5-Aminolevulinic acid and a 410-nm wavelength light-emitting diode for methicillin-resistant *Staphylococcus aureus*-infected ulcers in mice. *Plos One*. 2014;9(8):e105173.
34. Topaloglu N, Guney M, Yuksel S, Gulsoy M. Antibacterial photodynamic therapy with 808-nm laser and indocyanine green on abrasion wound models. *J Biomed Opt*. 2015;20(2): 28003.
35. Nafee N, Youssef A, El-Gowelli H, Asem H, Kandil S. Antibiotic-free nanotherapeutics: hypericin nanoparticles thereof for

improved in vitro and in vivo antimicrobial photodynamic therapy and wound healing. *Int J Pharm*. 2013;454(1):249-258.

36. Simonetti O, Cirioni O, Orlando F, et al. Effectiveness of antimicrobial photodynamic therapy with a single treatment of RLP068/cl in an experimental model of *Staphylococcus aureus* wound infection. *Brit J Dermatol*. 2011;164(5):987-995.
37. Kou J, Dou D, Yang L. Porphyrin photosensitizers in photodynamic therapy and its applications. *Oncotarget*. 2017;8(46): 81591-81603.
38. Alam M, Voravutinon N, Warycha M, et al. Comparative effectiveness of nonpurpuragenic 595-nm pulsed dye laser and microsecond 1064-nm neodymium:yttrium-aluminum-garnet laser for treatment of diffuse facial erythema: a double-blind randomized controlled trial. *J Am Acad Dermatol*. 2013;69(3): 438-443.
39. Kasuya A, Tokura Y. Attempts to accelerate wound healing. *J Dermatol Sci*. 2014;76(3):169-172.
40. Rahim K, Saleha S, Zhu X, Huo L, Basit A, Franco OL. Bacterial contribution in chronicity of wounds. *Microb Ecol*. 2017;73 (3):710-721.
41. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci Transl Med*. 2014;6(265):265sr6.
42. Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M. Biology and biomarkers for wound healing. *Plast Reconstr Surg*. 2016; 138(3 Suppl):18S-28S.
43. Lazarus GS, Cooper DM, Knighton DR, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol*. 1994;130(4):489-493.
44. Stojadinovic O, Pastar I, Nusbaum AG, Vukelic S, Krzyzanowska A, Tomic-Canic M. Deregulation of epidermal stem cell niche contributes to pathogenesis of nonhealing venous ulcers. *Wound Repair Regen*. 2014;22(2):220-227.
45. Gompelman M, van Asten SA, Peters EJ. Update on the role of infection and biofilms in wound healing: pathophysiology and treatment. *Plast Reconstr Surg*. 2016;138(3 Suppl):61S-70S.
46. Nolff MC, Reese S, Fehr M, Dening R, Meyer-Lindenberg A. Assessment of wound bio-burden and prevalence of multi-drug resistant bacteria during open wound management. *J Small Anim Pract*. 2016;57(5):255-259.
47. Wolcott RD, Rhoads DD, Dowd SE. Biofilms and chronic wound inflammation. *J Wound Care*. 2008;17(8):333-341.
48. Serra R, Grande R, Butrico L, et al. Chronic wound infections: the role of Pseudomonas aeruginosa and *Staphylococcus aureus*. *Expert Rev Anti Infect Ther*. 2015;13(5):605-613.
49. Gao H, Shi L, Yin H, et al. Evaluation of the effect of photodynamic therapy with hematoporphyrin monomethyl ether on VX2 tumors implanted in the rectal submucosa of rabbits. *J Photochem Photobiol B*. 2016;163:162-169.
50. Pottier RH, Chow YF, LaPlante JP, Truscott TG, Kennedy JC, Beiner LA. Non-invasive technique for obtaining fluorescence excitation and emission spectra in vivo. *Photochem Photobiol*. 1986;44(5):679-687.
51. Ricatto LG, Conrado LA, Turssi CP, Franca FM, Basting RT, Amaral FL. Comparative evaluation of photodynamic therapy using LASER or light emitting diode on cariogenic bacteria: an in vitro study. *Eur J Dent*. 2014;8(4):509-514.
52. Azizi A, Shohrati P, Goudarzi M, Lawaf S, Rahimi A. Comparison of the effect of photodynamic therapy with curcumin and methylene blue on *Streptococcus mutans* bacterial colonies. *Photodiagnosis Photodyn Ther*. 2019;27:203-209.
53. Sun Y, Chen L, Zhang Y, Gao X, Wu Y, Chen H. Topical photodynamic therapy with 5-aminolevulinic acid in Chinese patients with rosacea. *J Cosmet Laser Ther*. 2018;21(4):196-200.
54. Reginato E, Wolf P, Hamblin MR. Immune response after photodynamic therapy increases anti-cancer and anti-bacterial effects. *World J Immunol*. 2014;4(1):1-11.
55. Grandi V, Bacci S, Corsi A, et al. ALA-PDT exerts beneficial effects on chronic venous ulcers by inducing changes in inflammatory microenvironment, especially through increased TGF-beta release: a pilot clinical and translational study. *Photodiagnosis Photodyn Ther*. 2018;21:252-256.
56. Anzengruber F, Avci P, de Freitasa LF, Hamblin MR. T-cell mediated anti-tumor immunity after photodynamic therapy: why does it not always work and how can we improve it? *Photoch Photobio Sci*. 2015;14(8):1492-1509.
57. Gollnick SO, Evans SS, Baumann H, et al. Role of cytokines in photodynamic therapy-induced local and systemic inflammation. *Br J Cancer*. 2003;88(11):1772-1779.
58. Corsi A, Lecci PP, Bacci S, Cappugi P, Pimpinelli N. Early activation of fibroblasts during PDT treatment in leg ulcers. *G Ital Dermatol Venereol*. 2016;151(3):223-229.
59. Agostinis P, Berg K, Cengel KA, et al. Photodynamic therapy of cancer: an update. *CA Cancer J Clin*. 2011;61(4):250-281.
60. Weber CE, Li NY, Wai PY, Kuo PC. Epithelial-mesenchymal transition, TGF-beta, and osteopontin in wound healing and tissue remodeling after injury. *J Burn Care Res*. 2012;33(3):311-318.
61. Nestor MS, Berman B, Patel J, Lawson A. Safety and efficacy of Aminolevulinic acid 10% topical gel versus Aminolevulinic acid 20% topical solution followed by blue-light photodynamic therapy for the treatment of actinic keratosis on the face and scalp: a randomized. *Double-Blind Study J Clin Aesthet Dermatol*. 2019;12(3):32-38.

How to cite this article: Sun Y, Ogawa R, Xiao B-H, et al. Antimicrobial photodynamic therapy in skin wound healing: A systematic review of animal studies. *Int Wound J*. 2020;17: 285–299. https://doi.org/10.1111/iwj.13269