Lasers in Medical Science (2019) 34:779–791
https://doi.org/10.1007/s10103-018-2664-9

ORIGINAL ARTICLE

An improvement in acute wound healing in mice by the combined application of photobiomodulation and curcumin-loaded iron particles

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Received: 30 July 2018 / Accepted: 10 October 2018 / Published online: 5 November 2018

Abstract

Here, we examined the combined effect of pulse wave photobiomodulation (PBM) with curcumin-loaded superparamagnetic iron oxide (Fe_3O_4) nanoparticles (curcumin), in an experimental mouse model of acute skin wound. Thirty male adult mice were randomly allocated into 5 groups. Group 1 was served as the control group. Group 2 was a placebo and received distilled water, as a carrier of curcumin. Group 3 received laser (890 nm, 80 Hz, 0.2 J/cm^2). Group 4 received curcumin by taking four injections around the wound. Group 5 received laser + curcumin. One full-thickness excisional round wound was made on the back of all the mice. On days 0, 4, 7, and 14, bacterial flora, wound surface area, and tensile strength were examined and microbiological examinations were performed. In case of wound closure, the two-way ANOVA shows that wound surface area of entire groups decreased progressively. However, the decrease in laser + curcumin and laser groups, and especially data from laser + curcumin group were statistically more significant, in comparison with the other groups (F statistics = 2.28, sig = 0.019). In terms of microbiology, the two-way ANOVA showed that laser, and laser + curcumin groups have statistically a lower bacterial count than the curcumin, control, and carrier groups (F statistics = 35, sig = 0 = 000). Finally, the one-way ANOVA showed that laser + curcumin, curcumin, and curcumin significantly increased wound strength, compared to the control and carrier groups. Furthermore, laser + curcumin significantly increased wound strength, compared to the control, laser, and curcumin groups (LSD test, $p = 0.003$, $p = 0.002$, and $p = 0.005$, respectively). In conclusion, curcumin nanoparticles, pulse wave laser, and pulse wave laser + curcumin nanoparticles accelerate wound healing, through a significant increase in wound closure rate, as well as wound strength, and a significant decrease in *Staphylococcus aureus* counts. Furthermore, the statistical analysis of our data suggests that the combined treatment of pulse wave laser + curcumin nanoparticles enhances the wound closure rate, and wound strength, compared to the laser and curcumin nanoparticles alone.

Keywords Acute wound healing · Photobiomodulation · Low-level laser therapy · Wound closure · Microbial flora · Tensiometrical properties · Mouse

Introduction

Complex and chronic non-healing skin injuries have been always a financial concern for patients, insurance companies, and governments, imposing worth of billions dollars cost per year, in North America alone [1]. These are a challenge for physicians, and deplete considerable health care resources around the world [2, 3]. Skin injury repair is a composite process [4]. Scientists have studied comprehensively the mechanisms of skin repair, but further studies needed to elucidate the detailed mechanism [4]. Restoration of skin after an injury remains an extensive challenge, due to the complex construct of skin and the presence of many variant cell types [5].

In spite of new progresses in wound care products, such as wound dressings, ointments and solutions, adhesive tapes, bandages, medication, gauze and sponges, and wound cleansers, the old-style treatments based on natural derivative mixtures, such as herbal extracts, still are a

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fascinating substitute. These remedies propose novel options for the management of wounds [5]. The limitations of existing skin injury treatments urged us to further explore a harmless, feasible, and cost-effective formulation for use in skin injury repairs. An effective therapy would ideally decrease the bacterial load, establish stringent inflammatory control, and concomitantly increase wound tensile strength [4].

Curcumin is the dynamic component of the nutritional spice turmeric, and has demonstrated bactericidal, anti-inflammatory, and antioxidant effects [6]. Curcumin have been revealed to meaningfully affect skin wound repair. It acts on different steps of the normal skin repair process, in order to accelerate skin repair. Consistently, management of wounds in animal models, using curcumin, improves granulation tissue formation, collagen secretion, tissue remodeling, and wound closure [7]. However, according to the literature, there are conflicting results regarding the effect of administration of curcumin on skin wound repair [8–11]. In some studies, the positive effect of curcumin has been reported [8–10]. In these studies, wound closure, gross and histological measurements, and some molecular techniques have been used. On the other hand, very recently, Solimani et al. have analyzed the effects of pulsed wave photobiomodulation (PBM) and administration of the oral gavage of 40 mg/kg body weight of curcumin on the bacterial flora, and wound strength, in healthy (unpublished data, manuscript under review) and type one diabetic rats, in experimental animal models with excisional wound [11]. Solimani et al. observed that only PBM significantly increased wound strength of the healthy and diabetic healing wounds [11].

PBM has been dynamically applied to decrease pain, inflammation, and edema, in order to biostimulate wounds [12]. Many researchers have reported positive effects of a variety of PBM protocols on skin wound repair, in experimental healthy animals [13–17].

The beneficial use of a mixture of healing agents and medicines looks to be supportive, because in many illnesses, it could increase the effectiveness of treatments and is now the subject of extensive studies [18–21].

Nanotechnology tends to work at the atomic, molecular, and supra-molecular levels for making use of materials, and structures with new properties and functions, in biology and medicine, due to their small structure [22].

Here, we have designed curcumin-loaded superparamagnetic iron oxide (Fe_3O_4) (SPIONs) nanoparticles (curcumin), in order to improve the healing property. Thus, in this scientific approach, we hypothesized that the combined administration of pulse wave laser with curcumin, to an experimental model of acute skin wound, may accelerate wound closure rate, increase the wound strength, and decrease bacterial flora.

Materials and methods

Animals and study design

Forty Naval Medical Research Institute (NMRI) male adult mice, 3.5 months old, weighing ~30 g, were obtained from Pasteur Institute of Iran, the Production and Research Complex, Tehran, Iran. Mice were kept separately in standard cages and received standard mice and rat food pellets (Behparvar Co. Tehran, Iran). They were arbitrarily allocated into five groups, harboring six mice each. Group 1 was the control group. Group 2 was considered as a placebo group, and received distilled water, as a carrier of curcumin. Group 3 received laser. Group 4 received curcumin. Finally, group 5 received laser + curcumin. For the entire animals, one full-thickness excisional wound was introduced on their backs. On days 0, 4, 7, and 14, bacterial evaluations, wound area measurement, and tensile strength test were performed. Mice were housed individually in standard cages.

Surgery

Mice were anesthetized by injection of ketamine (50 mg/kg, i.m.) and diazepam (5 mg/kg, i.m.). One full-thickness of eight millimeter in diameter and round excisional skin wound was induced on the proximal part of the mice back with a standard punch, under disinfected conditions (Fig. 1).

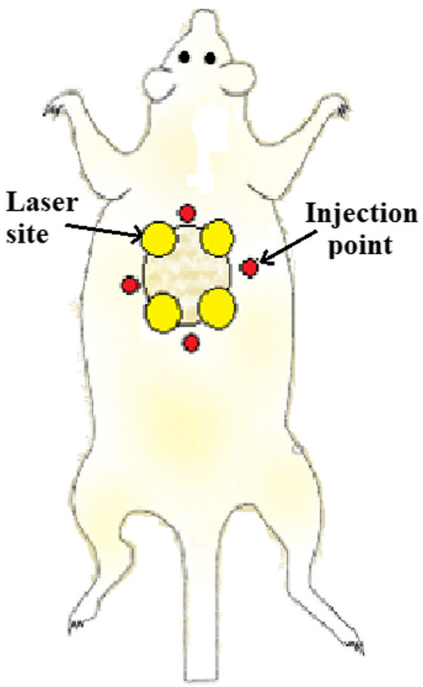

Fig. 1 Schematic depiction of the wound and curcumin nanoparticle-loaded injections and laser-treated points are shown

PBM

The wounds in the laser groups received laser (MUSTANG 2000, LO7 probe; Technica Co., Russia) with the following parameters: infrared, power density: 1.08 mW/cm^2, peak power output: 75 W, average power: 1.08 mW, spot size: 1 cm^2, pulse rate: 80 Hz, wavelength: 890 nm, pulsed duration: 180 ns, energy density: 0.2 J/cm^2, and duration of exposure of each point: 200 s.

The laser was started immediately, after inducing the wound. Laser was applied in four defined regions of the wound, and surrounding skin. Laser was applied once a day, 6 days a week, for the total period of 14 days [23].

Nanoparticle synthesis

Nanoparticles were donated by Dr. Simchi from the Department of Materials Science and Engineering, Sharif University of Technology, Tehran, Iran [24, 25].

Drug loading and release

Curcumin was integrated in the modified NPs by thermosensitive protocols, at the elevated temperature. Briefly, curcumin (0.01 g) was melted in acetone (10 mL), and gradually released into the precooled modified NPs solution in distilled water (DW) (4 °C). Subsequently, the temperature of the solution was elevated up to 37 °C to encapsulate curcumin in the NPs. The volume of integrated curcumin was defined by calculating the absorbance of the supernatant, using a UV-Vis spectrophotometer at 416 nm, after separating SPIONs by magnetic field and centrifugation. The drug loading efficiency of nanoparticles was 75%, calculated by the following equation:

$$\text{Loading efficiency } (\%) = \frac{\text{Total amount of curcumin} - \text{free curcumin}}{\text{Total curcumin}} \times 100$$

For determining the release of curcumin, drug-loaded nanoparticles solution (1 mg/mL) were transferred into a dialysis container, to be dialyzed against 150 mL DW (pH 7.4), containing Tween-80 (0.5% *w*/*w*) at 37 and 45 °C, with continuous stirring. Fe_3O_4 Mag-curcumin nanoparticles were suspended in DW. The drug release of curcumin profile was measured for 60 h at 37 and 45 °C. Transmission electron microscope (TEM) examination was performed on curcumin nanoparticles. At regular time intervals, the incubation medium was substituted with new media. The volume of the released medication, in the incubation medium, was measured by UV-Vis, using a spectrophotometer, and the remedy release percentage was computed by the following equation [23, 24]:

$$\text{Release } (\%) = \frac{\text{released curcumin}}{\text{total curcumin}} \times 100$$

Curcumin-loaded superparamagnetic iron oxide nanoparticles administration

A stock solution was made by suspending 5.4 mg curcumin nanoparticle in 100,000 μL DW, and then 100 μL of stock was suspended in 140 μL DW (totally 240 μL), was divided equally into four parts, and injected subdermally close to wound bed (5 mm distance), at four injection sites (by four injections). Injections sites had equal distances from each other. The solutions were shaken during administration (Fig. 1). Curcumin nanoparticles administration was performed on days 0, 4, 7, and 10, in groups 4 and 5.

Biodistribution of CUM-NPs

In four mice with skin wounds, 0.0054 mg of curcumin nanoparticles was administrated, similar to the mice of groups 4 and 5. Another four mice received the same amount of curcumin nanoparticles without any wounds. Skin samples were harvested after 24 and 72 h exposure with curcumin nanoparticles and digested. Briefly, 0.05 g of each skin sample was digested, using a 10 mg/mL elemental analysis. The sample was digested again in ultrapure nitric acid. Then, H_2O_2 was added and the mixture was heated in a high-pressure condition, until sample was completely digested. Finally, nitric acid was added. Data are expressed as nanograms per gram of fresh tissue [26].

Measurements of wound closure

Measurement of the total wound surface area was performed on days 0, 4, 7, and 14. Photos of the wounds were taken with a digital camera, and the surface area was computed, using ImageJ-NIH (USA). A ruler in the photo frame permits ImageJ calibration. The wound border was defined, using a digital pad, and the ImageJ software calculated the wound area in square millimeters (mm^2). The wound area of study groups was compared between the study groups each day.

Microbiological test

The microbiological test was performed, according to our previous study [11]. Samples were obtained from the animals' wounds on days 0, 7, and 14. Swabs were taken from the wounds for detecting *Pseudomonas aeruginosa*, as the Gram-negative bacteria and *Staphylococcus aureus* as the

Gram-positive bacteria. The number of microbes per sample was counted as colony-forming units (CFUs) [11].

Tensile strength test

Tensile strength test was performed according to our previous study [11]. Surgery was performed, and after 15 days, six rats from each group were randomly selected; rats were euthanized by inhalation of CO_2 and cervical dislocation. After careful dissection from underlying deep fascia, standardized rectangular skin specimens (3 mm × 30 mm strips) were harvested across the wound, using a double-blade cutting instrument. The samples' thickness was measured with a digital caliper. During the test, specimens were kept moistened in 0.9% NaCl solution. Specimens were mounted in a material testing machine (SANTAM, ENG. DESIGN CO LTD. IRAN). Two clamps were used with a rough surface, whereby the wound was placed in the free space. The distance between the edges of the clamps was 30 mm.

Deformation rate was kept constant at 10 mm/min. Specimens were loaded uniaxially; therefore, the failure and complete load–deformation curve could be recorded. From the load–deformation curve, the following biomechanical properties were automatically calculated. Bending stiffness (MPa) was calculated by dividing the maximum force by displacement of rupture. The maximum force (N) was measured directly from the load–deformation curve and represented the maximum tensile force applied, to rupture the specimen. Stress high load (N/mm^2) was calculated at the maximum force, divided by the cross-sectional area of the specimen. Energy absorption (J) was the area under the load–deformation curve [11].

Statistical analysis

Data were presented as mean ± SD of the mean. The student *t* test, one-way and two-way analysis of variance (ANOVA), and the least significant difference (LSD) tests were used for data analysis. The one-way ANOVA was used for analyzing tensile strength test. The two-way ANOVA was used for analyzing wound closure, and microbiological examination. The p value of < 0.05 was considered statistically significant.

Results

Physical properties of particles: size and morphology

The drug release of curcumin profile at 37 and 45 °C was shown in Fig. 2a. TEM image of SPIONs nanoparticles is shown in Fig. 2b. The average size of the nanoparticles was about 5 nm.

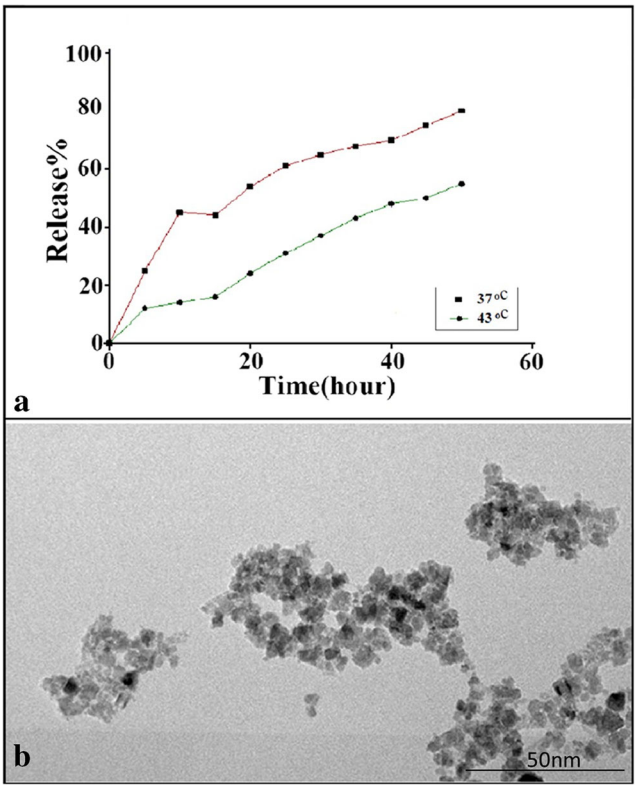

Fig. 2 **a** The drug release of curcumin profiles at 37 and 45 °C. **b** TEM image of SPIONs nanoparticles

Biodistribution of CUM-NPs

The highest value of curcumin nanoparticles (nanograms per gram of fresh tissue) was observed in injured skin samples, with 72 h exposure to curcumin nanoparticles, compared to the other groups (LSD test, all, $p = 0.000$). Injured skin samples, exposed to curcumin nanoparticles for 24 h, showed a better biodistribution compared to the healthy skin with 24 and 72 h exposure with curcumin nanoparticles (LSD test, both $p = 0.000$) (Table 1).

Clinical observations

No wound exudate was found in any wounds, in any study group. No significant differences were found in body weights, between study groups on days 0 and 14 post-surgery (Table 2).

Wound closure (mm^2)

Wound closure on day 0 is shown in Fig. 3. All mean ± SD of study groups on days 4, 7, and 14 are shown in Table 3.

No significant difference was found in wound area between study groups on day 0.

Table 1 Mean ± SD of CUM-NPs (nanograms per gram fresh tissue) of treated and non-treated skin samples at 24 and 72 h after exposure with curcumin nanoparticle, using the one-way ANOVA. The highest value of CUM-NPs was observed after treatment with curcumin nanoparticle for 72 h, compared to other groups.

Groups	Mean	Std. deviation
Healthy skin, 24 h	1.1450	.05686
Injured skin, 24 h	2.2250	.03873
Healthy skin, 72 h	1.1650	.03109
Injured skin, 72 h	3.1550	.04203

Injured skin samples, exposed to curcumin nanoparticle for 72 h, showed a better biodistribution than the healthy skin with 24 and 72 h exposure with curcumin nanoparticle (LSD test, both $p = 0.000$)

Wound closure, day 4

Laser (27 ± 6.4) and laser + curcumin (12 ± 2.6) groups have significantly enhanced wound closure, compared to the control group (46 ± 6.3) (LSD, $p = 0.002$ and $p = 0.000$, respectively), and for comparison with a carrier group (42 ± 8.9) (LSD, $p = 0.011$ and $p = 0.000$, respectively). Laser + curcumin group (12 ± 2.6) significantly enhanced wound closure, compared to the laser (27 ± 6.4) and curcumin (38 ± 9.1) groups (LSD, $p = 0.021$, and $p = 0.001$, respectively).

Wound closure, day 7

Laser (18.5 ± 5.6) and laser + curcumin groups (8 ± 1) have significantly enhanced wound closure compared to the control group (40 ± 10.9) (LSD, $p = 0.012$ and $p = 0.001$, respectively); and laser + curcumin (LSD for comparison with a carrier group) (26.5 ± 11.7) (LSD, $p = 0.005$). Laser + curcumin group (8 ± 1) significantly enhanced wound closure, compared to the curcumin (30.5 ± 10) group (LSD, $p = 0.011$).

Table 2 Mean ± SD of mice body weights on days 0 and day 14, using the one-way ANOVA. No significant differences were found in body weight on days 0 and 14, among the study groups

Groups	Weight	Day 0	Day 14
Control	Mean	38.7143	31.2857
	SD	1.25357	1.11270
Carrier	Mean	38.5000	33.7500
	SD	3.70328	3.10530
Laser	Mean	37.3333	33.6667
	SD	2.50333	2.50333
Laser + curcumin	Mean	38.6667	34.5000
	SD	1.86190	1.22474
Curcumin	Mean	37.7500	34.2500
	SD	2.25198	2.54951

Wound closure, day 14

The wound closure was significantly enhanced in laser (3.3 ± 1) and laser + curcumin (1.5 ± 1.3) groups compared to the control group (12.5 ± 1.3) (LSD, for comparison with control group, both $p = 0.000$), carrier group (17 ± 2.5) (LSD, for comparison with carrier group both p = 0.000), and curcumin (11 ± 1.9) (LSD for comparison with curcumin group, both $p = 0.000$). In curcumin group, the wound closure was significantly enhanced compared to the carrier group (LSD, $p = 0.001$) (Fig. 3a).

Two-way ANOVA for wound closure

The two-way ANOVA gives F statistics = 128 with sig = 0.000 for the factor after surgery time points (3); F statistics = 22.5 with sig = 0.000 for the factor groups (5); and F statistics 2.28 with sig = 0.019 for the interaction effect of after surgery time points × study groups (Fig. 3) We found significant differences in wound closure, among different time points. Significant differences also were found between control, carrier and curcumin groups, and laser and laser + curcumin groups. The two-way ANOVA shows that in the case of wound closure, wound area of entire groups decreased from day 0 to day 14. However, the decrease in laser + curcumin and laser groups was statistically more significant than the other groups. In addition, the two-way ANOVA for the wound area indicates that, in general, the laser + curcumin group has been more effective in wound healing, over the course of the experiment (Fig. 3).

Microbiological analysis

S. aureus and *Staphylococcus epidermidis*, as Gram-positive bacteria, have been found in wounds of study groups. The one-way ANOVA test demonstrated significant differences in the bacterial count, among the study groups. All mean ± SD of study groups on days4, 7, and 14 are shown in the Table 4. The microbiological tests also showed that the wounds were negative for *P. aeruginosa* in all study groups.

S. aureus count (in CFU)

Comparing the laser (1935 ± 120) and laser + curcumin (1787 ± 229), the *S. aureus* counts on day 7 was significantly decreased compared to the control (3662 ± 159) (LSD test, $p = 0.001$and $p = 0.000$, respectively) and to the carrier (3805 ± 176) groups (LSD test, both $p = 0.000$) (Fig. 4). Laser and laser + curcumin groups show a significant decrease in *S.*

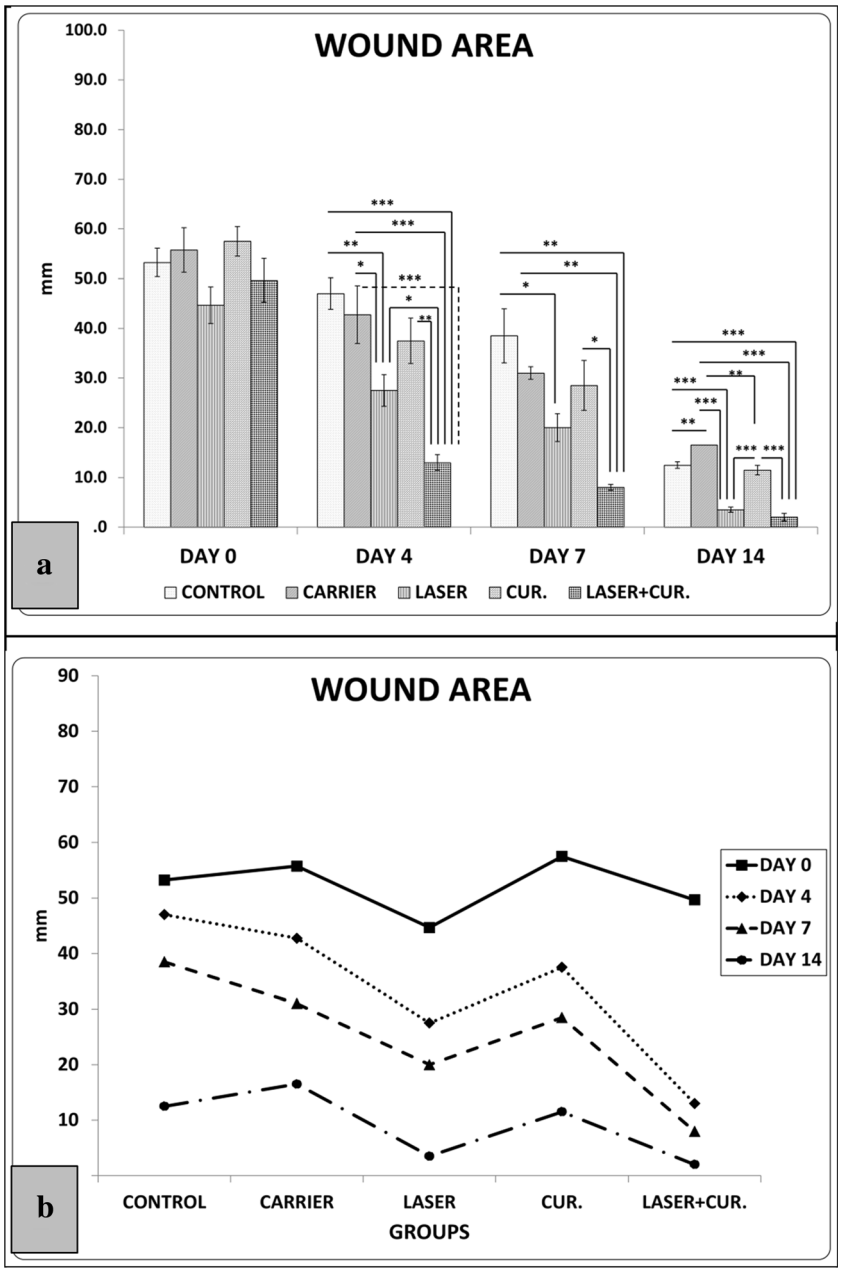

Fig. 3 **a** The comparison of mean ± SD of wound closure of study groups on days 0, 4, 7, and 14 using the one-way ANOVA, and the least significant tests. **b** The two-way ANOVA shows the wound area of entire groups decreased from day 0 toward day 14. However, results of laser + curcumin and laser groups were statistically more significant and better than the other groups. In addition, the two-way ANOVA for the wound area indicates that, in general, the laser + curcumin group has been more effective in wound healing over the days of the experiment; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

aureus count compared to the curcumin (3795 ± 346) (LSD test, both $p = 0.000$). On day 14, the laser (142.5 ± 24.7) and laser + curcumin (170 ± 21.2) show a significant decrease in count compared to the control (331.5 ± 26.1) (LSD test, $p = 0.002$ and $p = 0.004$, respectively) and to the carrier (330 ± 14.1) (LSD test, $p = 0.002$ and $p = 0.004$, respectively). Laser and laser + curcumin nanoparticles groups show a significant decrease in count compared to the curcumin (340 ± 56) (LSD test, $p = 0.002$ and $p = 0.003$) (Fig. 4a). In the case of *S. aureus*, the two-way ANOVA shows F statistics = 51.8 with sig = 0.000 for the factor group (5); F statistics = 15.1 with sig = 0.000 for the factor of after surgery time points (2); and F statistics = 35 with sig = 0.000 for the interaction effect of after surgery time points × study groups. We found significant differences in the bacterial count between control, carrier, and curcumin nanoparticles groups, with laser and laser + curcumin groups. Significant differences have been found between days 7 and 14 in the bacterial count of each study group (Fig. 4b).

S. epidermidis

The laser (1375 ± 176 CFU) and laser + curcumin (1676 ± 247 CFU) show a significant decrease in *S. epidermidis* counts on day 7 compared to the control (3250 ± 353 CFU) (LSD test, $p = 0.001$ and $p = 0.002$, respectively) and to the carrier

Table 3 Mean ± SD of wound closure in study groups on days 4, 7, and 14

Day	Groups	Mean ± SD
0	Control	55.5 ± 5.6
	Carrier	54.0 ± 6.4
	Laser	42.0 ± 6.4
	Curcumin	57.0 ± 5.9
	Laser + curcumin	48.0 ± 7.6
4	Control	46.0 ± 6.3
	Carrier	42.0 ± 8.9
	Laser	27.0 ± 6.4
	Curcumin	38.0 ± 9.1
	Laser + curcumin	12.0 ± 2.6
7	Control	40.0 ± 10.8
	Carrier	26.5 ± 11.6
	Laser	18.5 ± 5.5
	Curcumin	30.5 ± 10.0
	Laser + curcumin	8.0 ± 1.0
14	Control	12.5 ± 1.2
	Carrier	17.0 ± 2.5
	Laser	3.25 ± 1.0
	Curcumin	11.0 ± 1.9
	Laser + curcumin	1.50 ± 1.3

Table 4 Mean ± SD of bacterial count in study groups on days 7 and 14

Name of bacteria	Day	Groups	Mean ± SD
Staphylococcus aureus	7	Control	3250 ± 353
		Carrier	3200 ± 282
		Laser	1375 ± 176
		Laser + curcumin	1675 ± 247
		Curcumin	3720 ± 311
Staphylococcus aureus	14	Control	3675 ± 176
		Carrier	3750 ± 212
		Laser	1925 ± 106
		Laser + curcumin	1887 ± 229
		Curcumin	4475 ± 176
Staphylococcus epidermidis	7	Control	3662 ± 159
		Carrier	3805 ± 176
		Laser	1935 ± 120
		Laser + curcumin	1787 ± 229
		Curcumin	3795 ± 346
Staphylococcus epidermidis	14	Control	331 ± 26.1
		Carier	330 ± 14.1
		Laser	142 ± 24.7
		Laser + curcumin	170 ± 21.2
		Curcumin	340 ± 56.5

(3200 ± 282) groups (LSD test, $p = 0.001$ and $p = 0.003$, respectively) (Fig. 5).

Laser and laser + curcumin groups demonstrate a significant decrease in count compared to the curcumin (3720 ± 311) (LSD test, $p = 0.000$ and $p = 0.001$, respectively). On day 14, the laser (1925 ± 106) and laser + nanoparticles (1887 ± 229) show a significant decrease in count compared to the control (3675 ± 176 CFU) (LSD test, both $p = 0.000$) and to the carrier groups (3750 ± 212) (LSD test, both $p = 0.000$) (Fig. 5a). Laser and laser + curcumin groups show a significant decrease in the bacterial count compared to the curcumin (4475 ± 176) (LSD test, both $p = 0.000$).

In the case of *S. epidermidis*, the two-way ANOVA shows F statistics = 86.5 with sig = 0.000 for the factor group [5]; F statistics = 21.9 with sig = 0.001 for the factor of after surgery time points [2]; and F statistics = 0.699 with sig = 0.610 for the interaction effect of after surgery time points × study groups. We found significant differences in the bacterial count between control, carrier, and curcumin groups, with laser and laser + curcumin groups. However, no significant differences were found between days 7 and 14, in the bacterial count of each study groups (Fig. 5).

In terms of microbiology, our data suggest that laser and laser + curcumin groups statistically have a lower bacterial count, compared to the CUM-NPs, control, and carrier groups. In addition, these results showed that *S. aureus* counts were significantly decreased for all groups on day 14, compared to the day 7. However, the two-way ANOVA showed a significant increase in the bacterial count for *S. epidermidis* on day 14 for all groups, but this increase was lower for laser and laser + curcumin groups compared to the other groups.

Tensile strength test

The outcomes in term of tensile strength are shown in Fig. 6. All mean ± SD of study groups on days 4, 7, and 14 are shown in Table 5.

BS (MPa)

Laser + curcumin (120 ± 4.8), laser (107.7 ± 5.2), and curcumin (93.9 ± 12.7) show a significant increase in BS compared to the control (39 ± 2.8) and carrier groups (45.5 ± 3.37) (LSD test, all $p = 0.000$); and laser + curcumin and laser show a significant increase in BS compared to the curcumin group (LSD test, $p = 0.001$ and $p = 0.042$, respectively) (Fig. 6a).

MF (N)

Laser + curcumin (2.83 ± 0.65), laser (1.76 ± 0.04), and curcumin (1.52 ± 0.38) show a significant increase in MF compared to the control (0.52 ± 0.035) (LSD test, $p = 0.000$, $p = 0.004$, and $p = 0.014$, respectively) and carrier groups

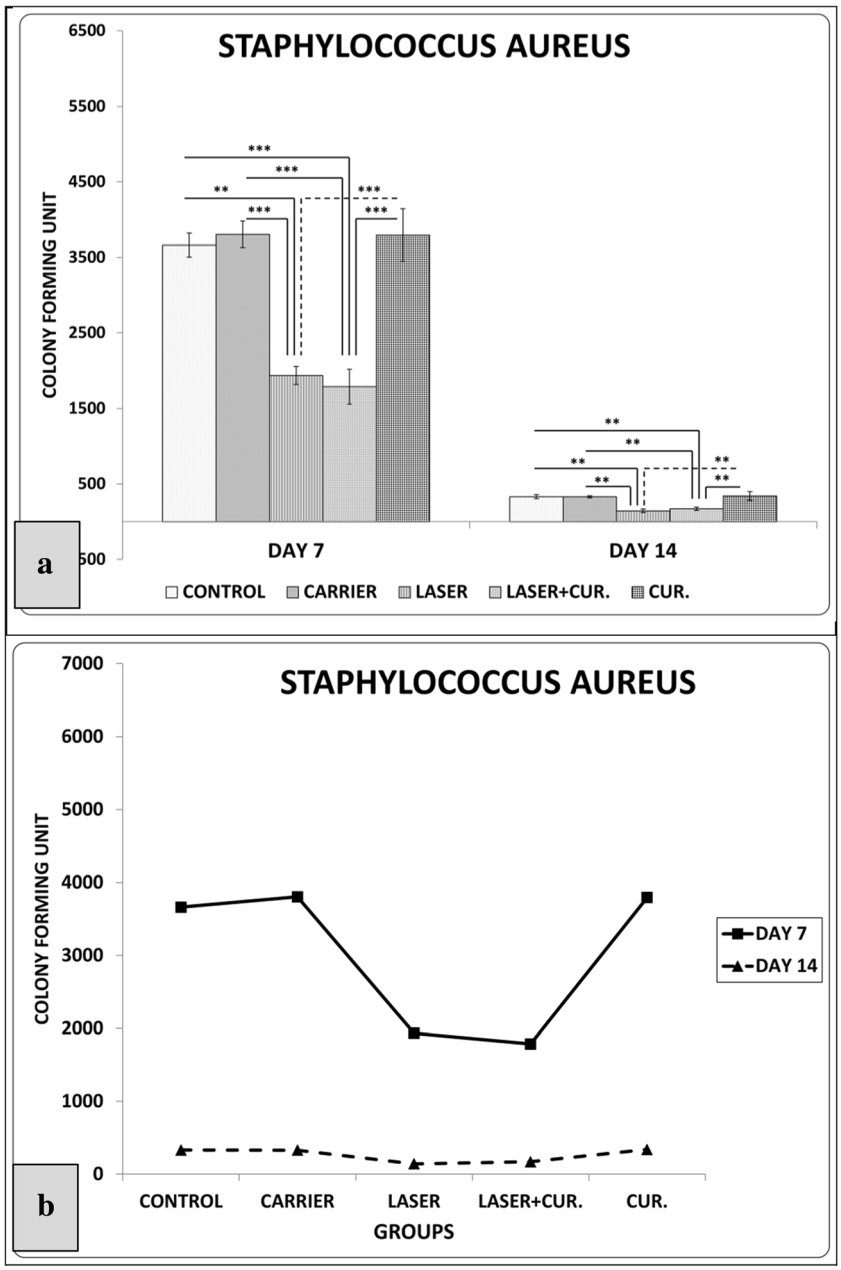

Fig. 4 **a** The comparison of mean ± SD of the colony forming units of *Staphylococcus aureus* in study groups on days 0, 4, 7, and 14 using the one-way ANOVA, and the least significant tests; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. The two-way ANOVA revealed that significant differences were found in CFUs among control, carrier, and curcumin groups with laser and laser + curcumin groups. Significant differences were found between days 7 and 14 in CFU of each study group (**b**)

(0.60 ± 0.05) (LSD test, $p = 0.000$, $p = 0.003$, and $p = 0.012$, respectively). Laser + curcumin group shows a significant increase in MF compared to the curcumin group (LSD test, $p = 0.002$) (Fig. 6b).

SHL (N/mm^2)

Laser + curcumin (0.94 ± 0.21), laser (0.58 ± 0.01), and curcumin (0.50 ± 0.12) show a significant increase in SHL compared to the control (0.175 ± 0.011) (LSD test, $p = 0.003$, $p = 0.000$, and $p = 0.012$, respectively) and carrier groups (0.20 ± 0.01) (LSD test, $p = 0.000$, $p = 0.003$, and $p = 0.012$, respectively). Laser + curcumin show a significant increase in SHL compared than to the laser and curcumin groups (LSD test, $p = 0.002$ and $p = 0.005$, respectively) (Fig. 6c).

EA (J)

Laser + curcumin (3.45 ± 0.42), laser (1.96 ± 0.29), and curcumin (2.16 ± 0.38) demonstrate a significant increase in EA compared to the control (0.915 ± 0.02) (LSD test, $p = 0.000$, $p = 0.005$, and $p = 0.002$, respectively) and carrier groups (0.54 ± 0.10) (LSD, all $p = 0.000$). Laser + curcumin show a significant increase in EA compared

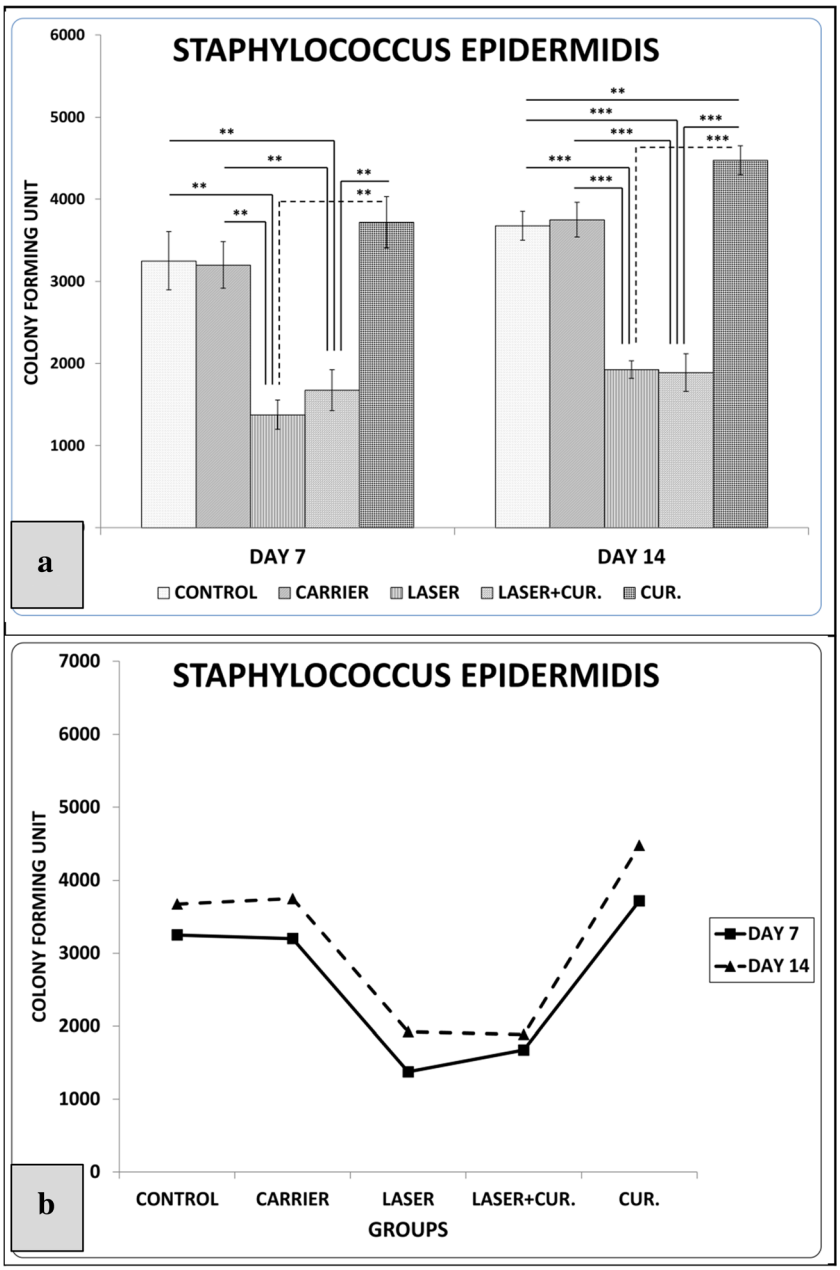

Fig. 5 **a** The comparison of mean ± SD of the colony forming units of *Staphylococcus epidermidis* in study groups on days 0, 4, 7, and 14 using the one-way ANOVA, and the least significant tests; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. The two-way ANOVA showed a significant increase in the bacterial count for *S. epidermidis* on day 14 for all groups, but this increase was lower for curcumin and laser + curcumin groups, compared to the other groups (**b**)

Table 5 Mean ± SD of tensile strength parameters in study groups on day 14

Mean ± SD→ Parameters Groups↓	Bending stiffness (MPa)	Maximum force (N)	Stress high load (N/mm^2)	Energy absorption (J)
Control	39 ± 2.8	0.52 ± 0.035	0.17 ± 0.11	0.95 ± 0.02
Carrier	45.5 ± 3.37	0.60 ± 0.05	0.20 ± 0.01	0.54 ± 0.10
Laser	107 ± 5.2	1.76 ± 0.04	0.58 ± 0.01	1.96 ± 0.029
Curcumin	93.9 ± 12.7	1.52 ± 0.38	0.50 ± 0.12	2.16 ± 0.38
Laser + curcumin	120 ± 4.8	2.83 ± 0.65	0.94 ± 0.21	3.45 ± 0.42

to the laser and curcumin groups (LSD test, $p = 0.000$ and $p = 0.00$, respectively) (Fig. 6).

Discussion

The current study addresses the complications encountered by patients and introduces the laser + curcumin as a potential solution for accelerating wound healing. The experimental aim was to create a novel management method for wound healing in patients. In the case of wound closure, the two-way ANOVA shows wound surface area of entire groups decreased from day 0 to day 14. However, data from laser + curcumin and laser groups, and especially laser + curcumin group, were statistically more significant compared to the other groups. In terms of microbiology, the two-way ANOVA showed that laser and laser + curcumin groups were statistically lower in bacterial count compared to the curcumin, control, and carrier groups. Finally, the one-way ANOVA showed that laser + curcumin, laser, and curcumin have significantly higher wound strength compared to the control and carrier groups; furthermore, laser + curcumin show a significant increase in wound strength compared to the other treatment groups.

Investigations of the factors influencing and hastening skin injury repairs are rapidly progressing. Since skin repair is a regulated process with many parameters involved, stimulation or inhibition by one agent alone cannot be expected to make any significant progress in accelerating the process and its efficacy [27].

Thus, in the current study, we have evaluated the combined effect of laser + curcumin on an excisional wound healing model in mice, in order to find a better therapeutic outcome. Curcumin was found to possess a wide range of beneficial and pharmacological properties, including anti-inflammatory, antioxidant, and anti-bacterial characteristics [6]. Therefore, significant attention has been paid in order to bring curcumin to the clinic for the improvement of wound healing over the recent years, such as the huge number of clinical trials, articles, and books [10]. Curcumin has also been demonstrated to modify skin injury repairs [7].

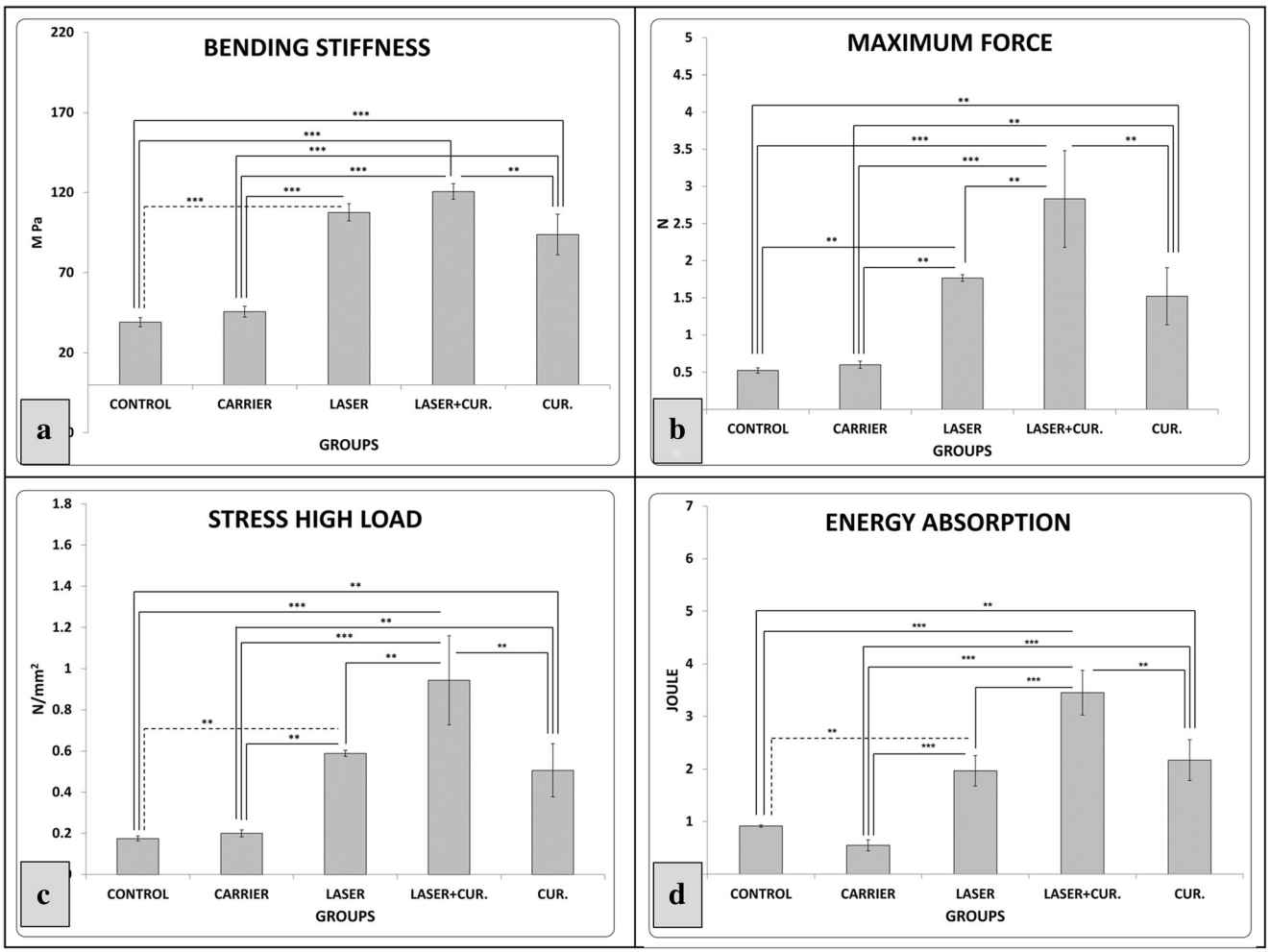

Fig. 6 The comparison of mean ± SD of the bending stiffness (**a**), the maximum force (**b**), high stress load (**c**), and energy absorption (**d**) of study groups on the day 14, using the one-way ANOVA, and the least significant tests; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

However, advances in this field and the medical application have so far been hindered due to its hydrophobicity, instability, weak absorption, and rapid general removal from the blood circulation and body [28]. Consistently, Solimani et al. demonstrated that curcumin, administrated by stomach gavage, did not significantly increase tensile strength properties of the healthy (unpublished data) and diabetic repairing skin wounds [11]. The tensile strength data demonstrated meaningful enhancements in the repairing process, after the combined administration of laser and curcumin, in an acute skin wound model in mice. The statistical analysis of our data, regarding the tensile strength examination, demonstrates that the repairing system in acute wounds in the laser + curcumin, curcumin, and curcumin (especially laser + curcumin) group were significantly tougher in SHL than the control wound group.

The elasticity of the skin is a very important function in a healthy individual, particularly in places where skin overlies the joints. In the current study, the use of laser + curcumin, laser, and curcumin (and especially laser + curcumin) as a potential curing modality have fast-forwarded the skin injury repairs and healing process [29]. Our findings show that laser + curcumin, laser, and curcumin (and especially laser + curcumin) can facilitate joint movements by increasing the elasticity of the repaired skin wound that overlies the joints [30].

Tensile strength test of skin injuries is important, since they represent wound dehiscence. Frequently, medical difficulties arise in patients with a diabetic foot ulcer, not due to a decline of the wound to cure primarily, but the site of the ulcer frequently reopens even with the minimum movement. Thus, the patient exerts pressure over a healed wound site, and it reopens, requiring additional and extended care. Much improvement in the tensile strength parameters was observed at the breaking point, following our treatment protocols [11, 17], proposing better medical outcomes for patients [31].

Fortunately, instability and poor absorption of curcumin can be addressed by applying an effective delivery unit. Active scientific research is undergoing to enhance curcumin's pharmacokinetics, systemic bioavailability, and biological activity by encapsulating or by loading curcumin into nanoformulations [32]. In this context, very recently, Naserzadeh et al. have reported positive neuroprotective effects for curcumin on schizophrenic rats mitochondria. They showed that effective treatment with nanoparticle (Fe_3O_4) loaded curcumin modulated mitochondrial dysfunction in the brains of rats with schizophrenia. Naserzadeh et al. concluded that using curcumin can be extended to preclinical and clinical use, and may have importance in schizophrenia and other diseases in the future [33].

Thus, regarding the vital role of mitochondria in wound healing [34], and positive effects of curcumin [33] and PBM [35] on mitochondria, we hypothesized that a combined administration of curcumin nanoparticles and laser could synergistically improve skin wound healing in an excisional wound healing mouse model, by improving the mitochondrial function. We have found curcumin nanoparticles and laser combined or alone significantly increased wound strength, compared to the control group. In addition, a synergistic effect of laser and curcumin nanoparticles has been observed. Our results are consistent with those of a previous report, showing that the application of gold nanoparticle plus PBM (808 nm diode laser, 100 mW, 50 s, 5 J/cm^2) has the ability to hasten skin injury repairs, because of enhanced epithelialization, collagen deposition, and fast vascularization [36]. Lau et al. concluded that using gold nanoparticle and PBM in the skin injury repairs showed that both treatments have beneficial effects on wound healing, compared to the control group.

Therefore, Lau et al. propose that the gold nanoparticle application plus PBM was more effective than the gold nanoparticle or PBM alone, in wound closure and histological examination. Lau et al. suggested that further studies on the combined effect of nanoparticles + PBM are needed to further explore the exact mechanisms of action [36]. Similarly, Shahnawaz Khan et al. suggested a novel method using gold nanorods to support the Nd-YAG laser (1064 nm, 240, and 300 s) for photothermal killing of *P. aeruginosa*, in order to directly heal the infected wound in the mice [37].

Dai et al. present that curcumin/gelatin-blended nanofibrous mats (NMs) effectively enhance the bioavailability of the curcumin for wound repair. Curcumin was positively formulated as amorphous nanosolid dispersion, and favorably released from gelatin-based biomimetic NMs, which can be simply used topically for experimental wounds. It has been stated that the beneficial impact of curcumin-loaded nanoparticle in hastening wound repair might be related to the synergistic signaling by the released curcumin during the healing process [10].

It is noteworthy that the temperature transformation region of modified magnetic nanoparticles is around body temperature, which makes them suitable for human biomedical applications, such as controlled drug delivery.

Our data analysis, using the two-way ANOVA, shows, in the case of wound closure, that wound surface area of entire groups decreased from day 0 toward day 14. However, laser + curcumin and laser groups and especially laser + curcumin group show statistically more promising result, compared to the other groups. In terms of microbiology, data analysis using the two-way ANOVA showed that laser and laser + curcumin groups statistically have a lower bacterial count than curcumin, control, and carrier groups. Finally, data analysis using the one-way ANOVA showed that laser + curcumin, laser, and curcumin significantly increased wound strength, compared to the control and carrier groups; furthermore, laser + curcumin significantly increased wound strength compared to the other treatment groups.

In conclusion, curcumin nanoparticles, pulse wave laser, and pulse wave laser + curcumin nanoparticles accelerate wound healing by increasing the wound closure rate, as well as wound strength, and decreased *S. aureus* bacterial counts. Furthermore, our statistical data analysis suggested that the combined treatment of pulse wave laser + curcumin nanoparticles enhanced the wound closure rate and wound strength, compared to the laser and curcumin nanoparticles alone. Here, we suggest the potential application of pulse wave laser + curcumin nanoparticles for diabetic and infected wounds in other preclinical models, in order to reduce infection and promote healing. Further studies are needed to explore the cellular and molecular mechanisms, concerning the combined and synergistic effects of pulse wave laser + curcumin nanoparticles on the healing process of acute cutaneous wounds, in animal models.

Acknowledgments The authors declare no financial support for this paper. Supported in part by NIH grant DK105692.

Role of funding source The authors declare no financial support for this paper.

Compliance with ethical standards

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

Ethical approval All methods were accepted by the Medical Ethics Committee of Shahid Behehti University of Medical Sciences (IRB approval number: IR.SBMU.MSP/REC.1395.9124).

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