Evaluating of the effect of low-level laser therapy on wound healing in rabbits

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Abstract

Background: A large number of studies have demonstrated the wound-healing effects of LLLT in vitro, in animal models, and in clinical practice. However, there are also differences in the study results, which are dose and wavelength dependent of LLLT. **Objective***:* Evaluation of the wound healing process in experimental animals treated with low- level laser therapy in clinical and histopathology. **Subjects and Methods:** Prospective study on 30 rabbits, each rabbit created two full thickness of 2R = 4 cm wounds on both sides of the back: wound A (treated with LLLT, 780 nm, 3 J/cm^2 with 72 s irradiation time, 1 time per day), wound B (control: no laser). Wounds are bandaged and laser irradiated once a day according to the procedure until the lesion is completely epithelialized. Wound biopsy was taken at: before treatment (D0), after 7 days (D7), after 14 days (D14) of treatment. Monitor and evaluate progress at the local wound. **Results***:* The area and speed of wound narrowing on the side of the laser site narrowed faster than the control side ($p < 0.05$). The results of rabbit skin histopathology showed that the number of inflammatory cells on the laser side decreased significantly compared with the non-laser side ($p_{(D14)} < 0.05$), while the number of neovascular and fibroblasts increased rapidly on the LLLT side when compared with the control side ($p_{(D7)} < 0.05$). **Conclusions***:* LLLT (780 nm, dose 3J/cm^2) increased wound healing in experimental rabbit model. LLLT promotes wound narrowing, reduces inflammation, stimulates angiogenesis, and increases collagen synthesis fibroblasts.

Keywords: *low-level laser therapy, wound healing, experimental animals, histopathology.*

1. INTRODUCTION

Since the 1960s, the efficacy of low-level laser therapy (LLLT) in wound treatment has been reported. Adre Mester, a Hungarian physicist, was the first to study the biological effectiveness of LLLT. Numerous reports have been published over the past few decades, demonstrating the positive effects of low-level laser therapy (LLLT) in in vitro, in vivo, and clinical studies [1,2,3]. The results of these studies have varied. The wavelength mainly used for phototherapy is from the red to near-infrared region corresponding to the optical window of 600 nm - 1000 nm, with energy density ranging from 1 to several hundred mW/cm^2 [4]. Contrary to the thermal effects created by high-power laser beams used in cosmetic and surgical procedures to destroy tissue, the low-power semiconductor laser therapy effect is a photobiomodulation effect. When the light source comes into contact with the skin, it enables photon energy to penetrate the tissue and interact with different intracellular biomolecules, thereby restoring cell function and improving the body's healing process [5,6,7]. The reason for the difference in research results was pointed out to be due to inconsistency, subjectivity in methods, and lack of research standards. The parameters (most importantly wavelength and dose) that are appropriate when using LLLT will determine the biological effectiveness on the wound healing process. In fact, to date there are not many standard research protocols on LLLT for wound treatment. The purpose of this study was to evaluate the wound healing process in animals experimentally treated with low-energy laser in clinical and histopathological settings. The results are systematically and methodically evaluated for future clinical application research.

2. SUBJECTS AND METHODS

2.1. Subjects

30 New Zealand white rabbits - Vietnam, both breeds, meeting experimental standards, healthy, agile, smooth white fur, no skin and gastrointestinal diseases, weight 2.2 - 2.7 kg. The animals were kept separately.

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2.2. Materials for research

- The LLLT device is made at the Institute of Materials Science - Vietnam Academy of Science and Technology (4-channel output, corresponding to 4 wavelengths of 670 nm, 780 nm, 805 nm and 980 nm), Optical power is adjustable in the range: 0 - 300 mW, power supply: 100V - 240 V, 50/60 Hz.

- Sterile scalpel, Leica DM1000 optical microscope, Optica Proview imaging software.

- Dressing rations (dressing equipment, cotton, bandages, gauze), 1 x 1 cm reticle plastic sheet, to determine the wound area, physiological saline NaCl 0.9%, Betadine solution 10%, vaseline of Le Huu Trac National Burn Hospital.

2.3. Method: Self-controlled experimental study.

2.3.1. Experimental wounding method on animals

The rabbit was shaved clean and cleaned the back area, then fixed onto a specialized laboratory table. On the back of the same rabbit, we marked the area creating a circular wound with a radius of 2R = 4 cm at two symmetrical positions on both sides of the spine column. The rabbit was given general anesthesia through the ear vein using Ketamine solution and the surgical area was sterilized with iodine alcohol. Afterward, the technician used a scalpel to create an incision corresponding to the designed area, removed the entire thickness of the rabbit's back skin, stopped bleeding, and covered the wound tightly with vaseline gauze.

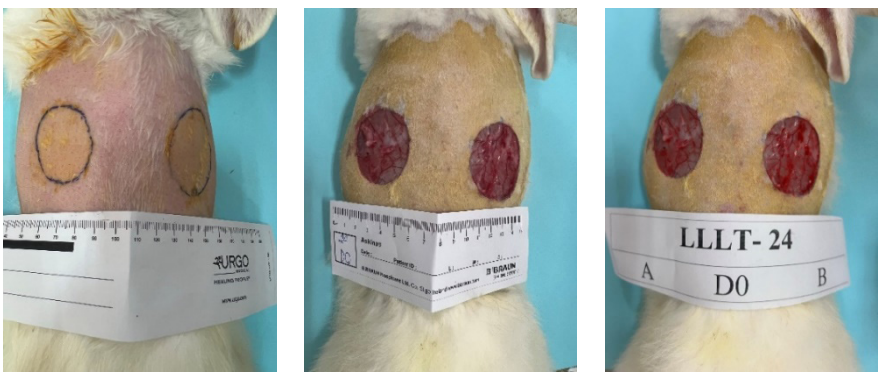


Figure 1. Image of the location where the wound was created on the rabbit's back

2.3.2. The process of laser irradiation for experimental wound treatment

Each rabbit had 2 experimental wounds, wound A (on the left) was treated with LLLT, and wound B (on the right) was not treated with laser (control). Both wounds were bandaged once a day according to the protocol until the tissue damage was completely healed. Follow-up and evaluation of the wound site's progress were conducted, and photographic evidence was taken.

The laser irradiation protocol for group A was as follows: The research rabbit was fixed on the table. The laser head was kept perpendicular to the wound surface and positioned 1cm away from the surface of the wound. The laser device was set to achieve a photon energy density of 3 J/cm^2 with a laser wavelength of 780 nm. The corresponding settings on the device were: Voltage: 10 V, continuous radiation mode, irradiation time t = 72s, once a day until the tissue damage was completely healed.

2.3.3. Evaluation of treatment effectiveness

At the site of the wound

- Daily monitoring of the following developments: Inflammation, bleeding, exudate, allergy, appearance of granulation tissue, wound area, tissue healing process, and healing rate.

The wound area in cm^2 is calculated by using the grid method. A transparent glass grid (with each square measuring 1cm^2) is placed on the wound and the wound area is traced onto the grid using a pen. The rate of wound contraction is calculated using the formula:

$$V = \frac{S1 - S2}{t}$$

Where:

V: The rate of wound contraction is measured in cm^2/day

S1, S2: The wound area at the studied time points (cm^2).

t: Number of study days.

Histological examination of rabbit skin wounds

+ Biopsy the wound tissue using a biopsy tool

+ Location of sampling: at the edge of the wound (including the remaining epidermal skin and the wound area)

+ The biopsy specimen is immediately fixed in 10% formalin solution for about 4 hours. Next, the specimen is processed using an automatic system. The sample is then embedded in paraffin for sectioning and stained with Hematoxylin – Eosin

+ Read histological lesions: observe under a light microscope at 10X magnification, observe and evaluate the overall image of the lesion, and measure the thickness of the necrosis. At 40X magnification, observe and count the inflammatory cells, new blood vessels, and necrotic cells. Use the Proview image capture software from Optica to measure the thickness of the necrosis and count the cells.

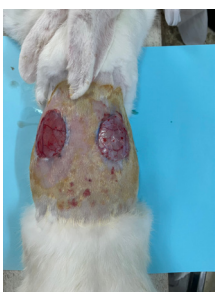
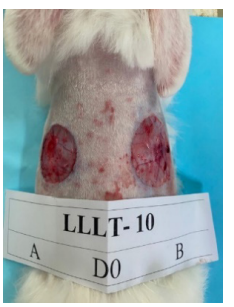
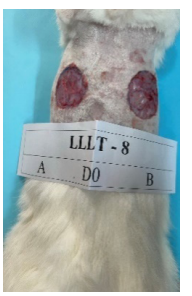
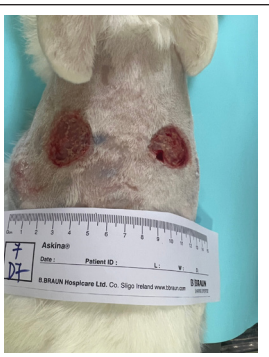
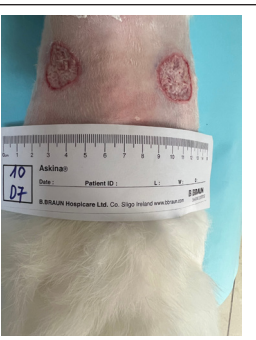
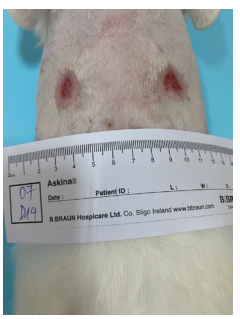
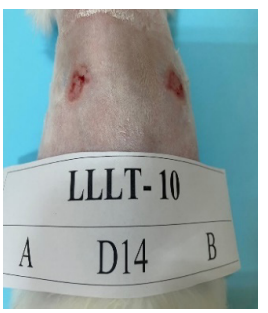
The sampling time: Before treatment (D0), after 7 days (D7), after 14 days (D14) of treatment.

2.3.4. Data analysis:

The research results were processed using SPSS 22 statistical software. The data were presented as mean values (±) standard deviation. T-test, non-parametric Mann-Whitney test, and Wilcoxon test were used. The p-value was considered significant when $p < 0.05$.

3. RESEARCH RESULTS

3.1. Impact of LLLT on wound healing in rabbits

	Image of rabbit's wound		
Time	**Rabbit 07**	**Rabbit 10**	**Rabbit 08**
D0			
D7			
D14			

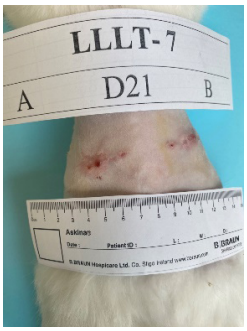
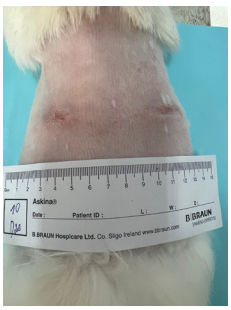
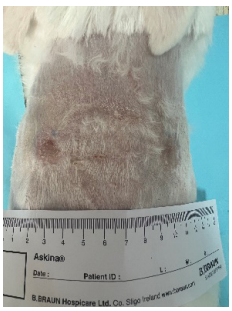
D21			

Figure 2. Experimental wound at different study time points.

Table 1. Changes in wound area on rabbits (χ ± SD) (*Mann-Whitney, **Wilcoxon test)

Time	Wound area (cm^2)		p*
	Position A (laser-irradiated group: LLLT) (n = 30)	Position B (non-irradiated group) (n = 30)	
D0	11.2 ± 0.09	11.2 ± 0.08	$p_{A-B} = 0.548$
D7	4.87 ± 1.24	5.87 ± 1.48	$p_{A-B} = 0.014$
D14	0.66 ± 0.45	1.02 ± 0.42	$p_{A-B} = 0.002$
p**	$p_{0-7}, p_{7-14}, p_{0-14} < 0.01$	$p_{0-7}, p_{7-14}, p_{0-14} < 0.01$	

Comment: The wound areas all decreased significantly after 14 days. At D7 and D14, the wound area at position A was smaller than at position B, with a statistically significant difference ($p < 0.05$).

Table 2. Wound healing rate in rabbits (χ ± SD) (T test)

Time	The contraction rate of the wound in rabbits (cm^2/day)		p
	Position A (laser-irradiated group: LLLT) (n = 30)	Position B (non-irradiated group) (n = 30)	
D0-D7	0.9 ± 0.17	0.76 ± 0.21	$p_{A-B} = 0.007$
D7-D14	0.6 ± 0.16	0.69 ± 0.21	$p_{A-B} = 0.06$
D0-D14	0.75 ± 0.33	0.73 ± 0.03	$p_{A-B} = 0.004$

Comment: The contraction rate of the wound at position A was faster than position B in the first 7 days and after 14 days, with statistically significant difference ($p < 0.01$)

3.2. The effect of LLLT on histological changes

Table 3. Changes in the number of capillaries at the wound site in rabbits. (χ ± SD) (*Mann-Whitney, **Wilcoxon test)

Time	Position A (laser-irradiated group: LLLT) (n = 17)	Position B (non-irradiated group) (n = 17)	p*
D0	2.41 ± 1	2.18 ± 0.73	$p_{A-B} = 0.558$
D7	9.29 ± 3,93	6.82 ± 2.45	$p_{A-B} = 0.046$
D14	7 ± 4.92	4.06 ± 2.045	$p_{A-B} = 0.068$
p**	$p_{0-7}, p_{0-14} < 0.01$ $p_{7-14} > 0.05$	$p_{0-7}, p_{7-14}, p_{0-14} < 0.01$	

Comment: The number of new vessels increased at both positions after 14 days. At time point D7: the number of new vessels at position A was significantly higher than position B, with a significant difference ($p < 0.05$). At time point D14: the number of new vessels at position A also increased more than position B, however, the difference was not statistically significant with $p > 0.05$.

Table 4. Changes in the number of fibroblast cell at the wound site in rabbits. (χ ± SD)
(*Mann-Whitney, **Wilcoxon test)

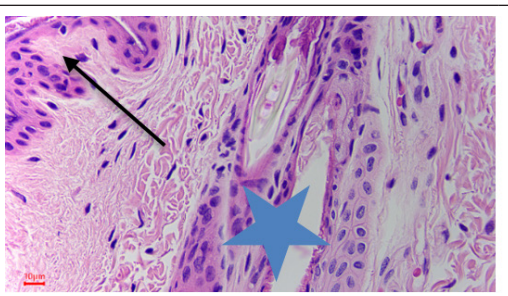
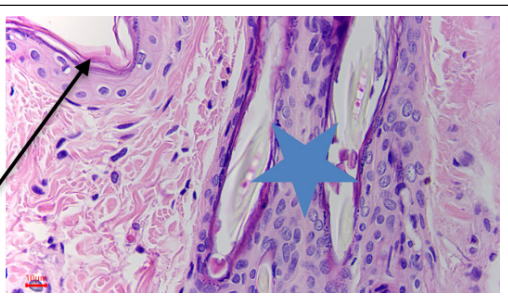
Time	Position A (laser-irradiated group: LLLT) (n = 17)	Position B (non-irradiated group) (n = 17)	p*
D0	0	0	
D7	15.06 ± 6.3	10 ± 5.73	p_{A-B}= 0.006
D14	7.47 ± 6.53	8.29 ± 6.66	p_{A-B}= 0.64
p**	p_{0-7}, p_{7-14}, p_{0-14} < 0.01	p_{0-7}, p_{7-14}, p_{0-14} < 0.01	

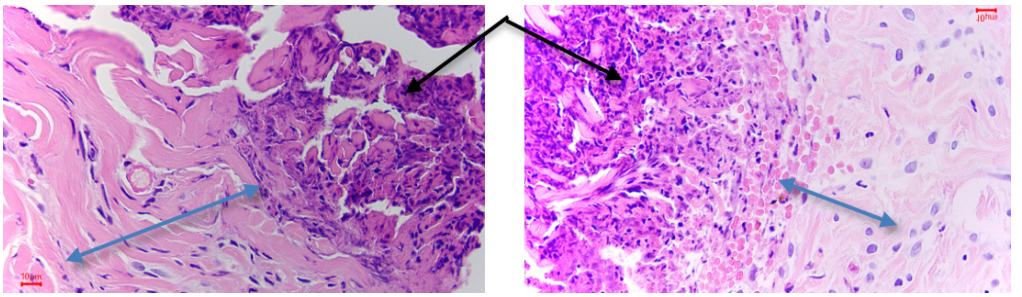
Comment: The number of fibroblast cell in both areas increased at D7, D14; with the highest increase at D7. At D7: fibroblast cell at position A increased significantly more than position B, with a significant difference with p = 0.006. At D14: There was no significant difference in the number of fibroblast cell between the two areas with p > 0.05.

Table 5. Changes in the number of inflammatory cells at the wound site in rabbits. (χ ± SD)
(*Mann-Whitney, **Wilcoxon test)

Time	Position A (laser-irradiated group: LLLT) (n = 17)	Position B (non-irradiated group) (n = 17)	p*
D0	5.53 ± 6.02	2.47 ± 2.26	p_{A-B}= 0.312
D7	29.82 ± 19.16	52.88 ± 42.39	p_{A-B}= 0.076
D14	11 ± 9.79	21.24 ± 14.99	p_{A-B}= 0.03
p**	p_{0-7}, p_{7-14} < 0.01 p_{0-14} = 0.135	p_{0-7}, p_{7-14}, p_{0-14} < 0.01	

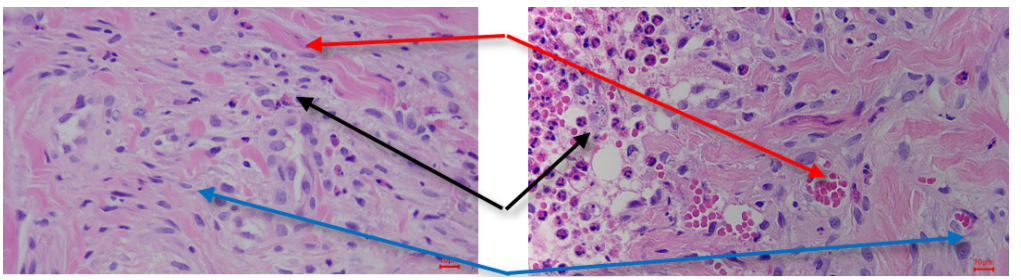
Comment: The number of inflammatory cells in both areas was quite low at the time of wound creation, then increased at D7 and decreased rapidly at D14. The number of inflammatory cells at position A was lower than position B at both D7 and D14, with a significant difference at D14 (p < 0.05)

Time	Wound	
	Position A (laser-irradiated group: LLLT) (n = 17))	Position B (non-irradiated group) (n = 17)
D0		
	The skin epidermis (indicated by the arrow) shows distinct layers of cells. The hind leg has many hair follicles (star shape). H&E staining, 40X magnification.	

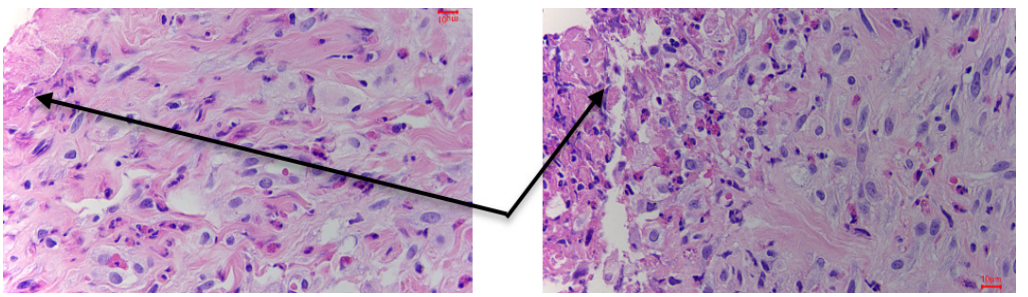


D7

Surface necrosis (one-way arrow) and thin granular tissue layer (two-way arrow). H&E staining, 40X magnification.

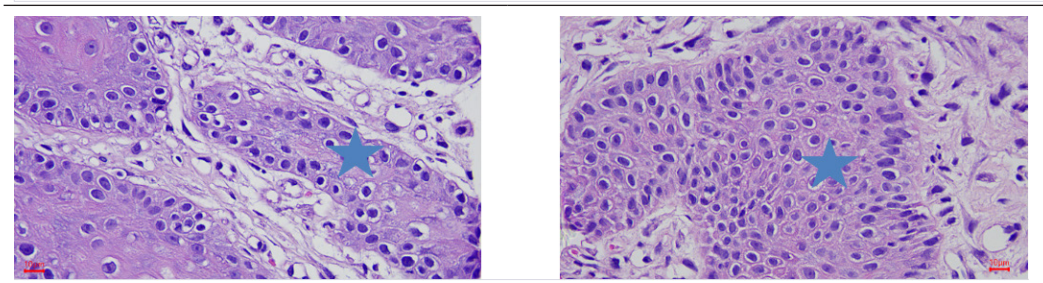


Infected inflammatory cells (black arrow), neovascularization (red arrow), and fibroblast (blue arrow). H&E staining, 40X magnification.



D14

There is surface necrosis (arrow) and a granular tissue layer. H&E staining, 40X magnification.



There is an image of the basement membrane (star shape). H&E staining, 40X magnification.

Figure 3. Images of the histology at the wound site of rabbit No.3 at different time points.

4. DISCUSSION

In this study, we used LLLT with a wavelength of 780 nm and a dose of 3 J/cm^2, which falls within the wavelength range (630 - 790 nm) that Chaves and colleagues [8] reported as optimal. In a review article on cell culture by AlGhamdi, K.M., A. Kumar and colleagues [9], it was shown that LLLT doses ranging from 0.5 to 4.0 J/cm^2 enhance cell proliferation without causing any cytotoxic effects.

The research results show that Laser has a positive effect on the wound healing process. Specifically, Table 3.1 indicates that the area of the wound on the laser-irradiated side is smaller than the non-irradiated side ($p < 0.05$), and the rate of wound contraction (Table 2) after 7 days in the laser-irradiated group (0.9 ± 0.17 cm^2/day) is higher than the non-irradiated group ((0.76 ± 0.21 cm^2/day) with $p < 0.01$. The research results are consistent with the study by Hodjati et al. [10] who concluded that the group of wounds on rabbits treated with LLLT (808 nm, 4 J) had significantly reduced wound area compared to the non-laser-irradiated group ($p = 0.003$) and faster tissue regeneration rate. Microscopically, looking at Table 3 and Table 4, we can see that the number of capillaries and fibroblast cell at both locations increased compared to the time of wound creation, indicating that the wound was healing favorably. And the number of capillaries and fibroblast cell at D7 increased more than at D14, indicating that the wound healing process was strongest in the first 7 days. At D7 in Table 3, the number of capillaries in the laser-irradiated group (9.29 ± 3.93/UOA (unit of area)) was higher than the non-irradiated group (6.82 ± 2.45/UOA), the difference was significant with $p < 0.05$. On the 14th day, we observed that the number of capillaries at location A was still higher than location B, but the difference was not statistically significant ($p > 0.05$). In Table 4, it shows that the number of fibroblast cell at D7 at location A (laser-irradiated) (15.06 ± 6.3) was higher than at location B (non-irradiated) (10 ± 5.73), the difference was significant with $p=0.006$, at D14 there was no difference between the two locations. Table 5 shows that the average inflammation score in both groups increased at D7, then decreased at D14, and the inflammation score at the laser-irradiated location was always lower than the non-irradiated location, the difference was significant at D14 ($p = 0.03$). These results are consistent with Chaves et al.'s [8] findings after collecting and analyzing 68 studies on LLLT in vitro and animal models. Laser has a biological effect of reducing inflammatory cells, increasing fibroblast cell formation, stimulating angiogenesis, granulation tissue formation, and collagen synthesis. Hussein et al.'s [11] study on diseased tissue samples between two groups of wounds created on rabbits with laser irradiation and non-laser irradiation on days 3, 7, and 14 showed that LLLT demonstrated better regenerative ability and faster recovery of structural and functional integrity compared to the control group. The study by Gupta, T. Dai, and Hamblin [12,13] on the effects of LLLT on mouse wounds at different wavelengths (635, 730, 810, and 980 nm) with a dose of (4 J/cm^2) showed that LLLT promotes wound healing, but depends on the wavelength. Specifically, the 810 nm wavelength showed optimal effectiveness, significantly reducing wound area, increasing cell proliferation, and enhancing tissue regeneration ($p < 0.05$) compared to other wavelengths. The study by Demidova-Rice and colleagues [14] showed that the 820 nm wavelength is optimal among the three wavelengths (635, 670, and 720 nm) in stimulating wound contraction in mouse skin after a single dose of LLLT following 30 minutes of creating the wound compared to the group without laser irradiation. Moreover, LLLT increased the number of smooth muscle actin (SMA) cells and stimulated wound contraction by promoting fibroblast cell differentiation into myofibroblast cells [15]. While differences in results from various studies can be attributed to factors such as wavelength, dose, and study protocols, the overall consensus underscores the potential of LLLT as a non-invasive and promising approach to expedite wound healing.

Further investigations could explore the optimal parameters of LLLT, such as different wavelengths and doses, and their effects on wound healing in diverse animal models and human subjects. Additionally, long-term follow-up studies could provide insights into the sustainability and long-lasting effects of LLLT-induced wound healing. The current findings shed light on the potential of LLLT as a valuable therapeutic tool in wound management, offering a non-pharmacological and non-invasive option to accelerate the healing process and improve patient outcomes.

5. CONCLUSION

In conclusion, this study evaluated the effects of low-level laser therapy (LLLT) on wound healing in a rabbit model. The results of this study demonstrate that LLLT, particularly using

a wavelength of 780 nm and a dose of 3 J/cm^2, significantly enhances the wound healing process. The treated wounds exhibited accelerated wound contraction, reduced inflammation, increased angiogenesis, and enhanced fibroblast activity. The wound area on the laser-irradiated side decreased faster, and the wound contraction rate was higher than the non-irradiated side. Histopathological analysis revealed increased neovascularization and fibroblast formation on the laser-irradiated side, along with a decrease in inflammatory cells. These findings support the positive impact of LLLT on wound healing processes in rabbits. This research contributes to our understanding of the cellular and molecular mechanisms underlying LLLT-induced wound healing and emphasizes its potential clinical applications.

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