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Research Paper: Histopathological Evaluation of the Effects of Low-level Laser Therapy on the Healing of Experimental Cryosurgical Wound in Normal Rat Skin

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ABSTRACT

Introduction: This research aimed to study the healing process of cryosurgical wounds after topical application of low-level laser and to evaluate its effects for the prevention of healing complications.

Materials and Methods: A total of 50 healthy male Wistar rats with an average weight of 200-250 g were selected and randomly divided into two main groups. In control group (N=25), cryosurgery was done without using of low-level laser. In experimental group (N=25), cryosurgery was done and the low-level laser was used for 10 days post-surgery. Each main group was divided into five subgroups (n=5) according to histopathological evaluation date (3, 7, 14, 21 and 28 days post-surgical) and healing process was evaluated on the wound specimens harvested at above-mentioned days. The full- thickness wounds were harvested in 40 mm in length and 20 mm in width and the specimens were stained using hematoxylin-eosin and Masson's Trichrome staining. The healing process was evaluated and compared in groups by observation of granulation tissue and collagen fibers amounts, epithelial gap size, the number of inflammatory cells, and the rate of angiogenesis. Data were collected and statistically analyzed and compared between the experimental and control groups on specific days using the Kruskal-Wallis and Dan post hoc statistical tests.

Results: The obtained data were compared between the experimental and control groups on different days using the Kruskal-Wallis and Dan post hoc statistical tests. The statistical results revealed significant differences between groups ($P<0.05$) in all mentioned variables. It revealed that the healing process in the experimental group took place at least one week earlier than the control group.

Conclusion: The results of this animal study showed that the low-level laser therapy on an experimental cryosurgical wound not only accelerates the healing process but also can be effective in the prevention of healing complications. The authors suggest further research to extend the results to human applications.

Keywords: Cryosurgery, Low-level Laser, Rat, Skin, Healing

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1. Introduction

Cryosurgery is one of the treatment modalities for hyperplastic, neoplastic, and granulomatous lesions of the skin and mucosa. In this method, controlled destruction of unwanted tissues will perform by a strong cryogenic substance such as liquid nitrogen or nitrous oxide gas, using a tool or device called "Cryo applicator." The healing of cryosurgical wounds is as open wounds healing and this kind of wound healing are frequently seen with scar tissue formation [1].

Cold injury to the skin can lead to complications such as hypertrophic scarring, hypopigmentation, and wet wounds, or superficial nerve damage. The mechanism of cryosurgery is based on the formation of ice crystals inside and outside the cell. By creating these crystals, the vital organelles of the cell are destroyed, and the nature of cell wall proteins and matrix change, and finally cause death by impaired blood flow. The duration of cryosurgery wound healing depends on the type of tissue, freezing temperature and time, the interval between freezing and defrosting, and the number of freeze-defrost times [2].

There are many references in the surgical literature to lack of scarring after cryosurgery. This finding has been investigated experimentally by comparing the effects on the skin of four freeze-thaw schedules commonly used in clinical practice with those of maximal cryosurgical injury. The absence of damage to collagen has been reported, and it is proposed that this is an essential factor in the often-cited lack of scarring. Changes in skin thickness over six months have been reported. Also, noticeable scarring after severe injury and long-term dermis thinning after less severe injury are reported. Evidence indicates that epidermal cells and fibroblasts differ in their susceptibility to low-temperature damage [3].

Laser has four unique properties that are not found in any other light source. These four characteristics are monochromatic, coherent, low divergence, and high intensity [4, 5]. Laser light has photobiostimulation effects due to its unique wavelength. On the other hand, each of the cellular components absorbs a specific wavelength of the laser. Light energy absorption by tissues leads to photobiomodulation effects, such as decreasing inflammatory response and collagen synthesis. Increasing alterations in protein synthesis will occur so that the cellular metabolism will improve, and better soft-tissue healing will occur subsequently [6].

Stimulation of mitochondrial chromophores increases cellular respiratory chain activity, [7] enhances adenosine triphosphate (ATP) synthesis and facilitates the production and proliferation of messenger RNA (mRNA), which is responsible for generating code to make procollagen [8, 9].

Infrared lasers have been reported to dilate blood vessels, reduce tissue ischemia, increase perfusion, transport nutrients, and eliminate cellular lesions due to increased levels of histamine nitric oxide [10]. Infrared laser has also reduced prostaglandin E2 (PGE2) in people with Achilles tendonitis [11]. PGE2 is a chemical mediator that facilitates the inflammatory process [12, 13]. Animal studies have shown that the He-Ne laser reduces PGE2 synthesis following acute inflammation in mice [14].

Previous studies reported that low-level laser has a positive effect on the healing process by reducing the intensity of the inflammatory reaction and increasing the number of organized collagen tissues, causing faster scar healing after surgery and increasing blood flow [11, 15].

This study aims to evaluate the healing process of a cryosurgical wound after topical application of a low-level laser and to answer the question, whether the use of low-level laser could prevent healing complications after cryosurgery.

2. Materials and Methods

Study participants

In this study, 50 healthy male Wistar rats with an average weight of 200-250 g and an Mean±SD age of 2 months (7 days) were used. The rats were randomly divided into two main groups and five subgroups within each group. They were kept in 10 special cages with a plastic body and a separate metal rod roof, with an unlimited amount of temperature and access to special water and food.

After two weeks, with daily care and review of rat behavior, the research process commenced by observing hygiene and changing it for one day in their special litter box and ensuring that the animals got used to the new conditions.

Study instruments

The low-level laser used in this research was ISOLASER 110DS plus (Tavan Bakhsh Novin Co., Iran) with a supply voltage of 220V AC, supply frequency of 50 Hz,

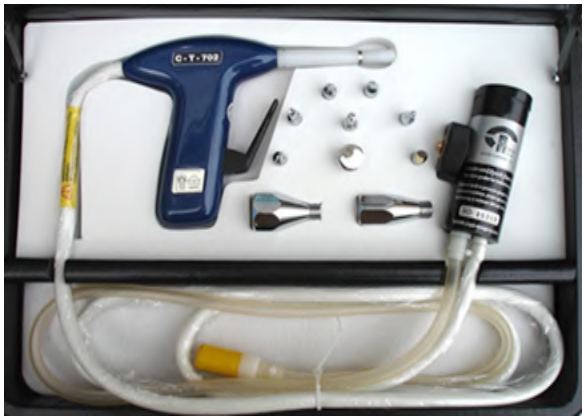

Figure 1. Cryo device model CT-702 JMR

Figure 2. Low power laser model ISOLASER100DS + JMR

a wavelength of 795-775 nm, a maximum output power of 50 mW, and beam size of 5 mm.

The cryosurgery device used in this study was a Joule-Thomson CT-702 device (Sarma Darman Co., Iran) with a closed oblique probe, and the cryogenic substance was N_2O (Nitrous Oxide) gas (Figure 1 and 2).

Interventions

Animal selection and maintenance

As previously mentioned, the animals were 50 male Wistar rats in approximately the same condition in terms of age (two months±7 days old), weight (200-250 g), and body condition. The total keeping period of the animals was 42 days: the animal got familiar with the environment in the first 14 days, and the wound healing process was assessed in the next 28 days. The rats were divided into groups of 5 and were kept in 8 special cages. Relevant information such as group type (control or test), date of surgery, time of histopathology sampling, etc., was written on each cage.

Methods of anesthesia, wound healing, and cryosurgery

At first, 10% ketamine at the rate of 100 mg/kg and 2% xylazine at the rate of 12.5 mg/kg [16] were injected intraperitoneally, which was followed by anesthesia for about 40-45 minutes.

After a few minutes, while checking the reflexes and using lubricant gel, the skin of the animal's back area was frozen to -40°C for 90 seconds by cryogenic probe 5 mm GD-13 using cryogenic nitrous oxide (N_2O). The tissue was then allowed to defrost to a temperature of 15°C (180 seconds), and a non-contact infrared thermometer in the range of -50°C to +350°C was used to control the temperature. Then the freeze-defrost steps were repeated. The above procedure was repeated up to 3 times.

After the cryosurgery stage, all rats were monitored until they regained consciousness and were transferred to a suitable environment for storage (Figure 3 and 4).

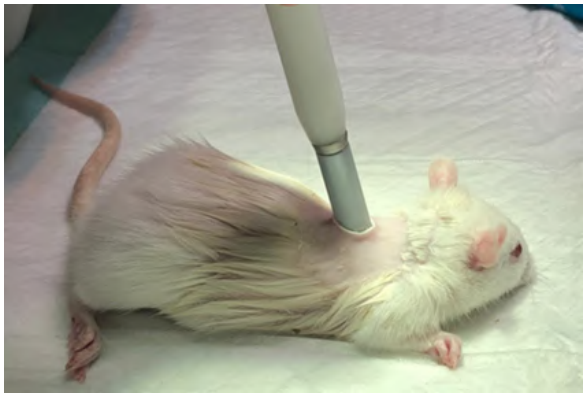

Figure 3. Sample freezing step by cryosurgery probe JMR

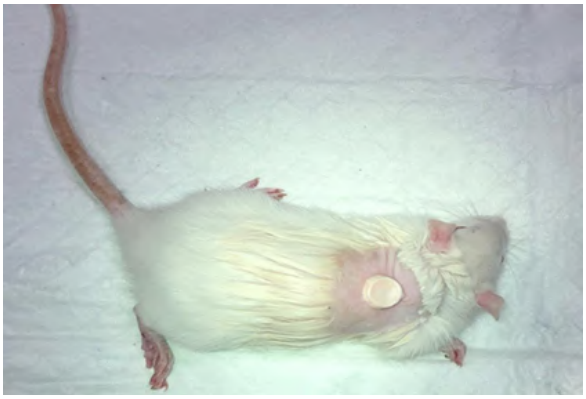

Figure 4. Defrost stage after freezing JMR

Laser therapy

In each wound caused by cryosurgery, a continuous mode of low-power laser with a frequency of 1000 Hz was applied in 8 points for 3 seconds at a dose of 6 j/cm^2. This procedure was performed for 10 days on the wound with an area of 2 cm^2 and in 25 rats as the experimental group (Figure 5).

It should be noted that the method and timing of laser treatment were based on the four characteristics of the acute or chronic lesion, having or not having pigment, the depth of the target tissue, and the purpose of treatment.

The laser probe was a pen-type one with a peak power of 50mW, spot size 5 mm, and a wavelength 775-795 nm, which was used directly on the wound with an approximate distance of 50 mm.

The sampling of skin tissue

At the specified times, i.e., 3, 7, 14, 21, and 28 days after the surgery, all subgroups were transferred to the histopathology laboratory. First, according to the mentioned drug doses, ketamine and xylazine were injected intraperitoneally with insulin syringes, and after a few minutes, the animal's reflexes were examined, and disinfection was performed around the wound. Next, the wound was removed along with a portion of healthy skin and placed in 10% buffer formalin solution (900 mL of water+100 mL of formalin 37%+4 g of monobasic sodium phosphate+6.5 g of sodium dibasic phosphate).

JMR

Figure 5. Laser treatment after cryosurgery on necrotic skin

Steps of preparing microscopic sections

Consolidation: At this stage, the tissues were cut to a maximum diameter of 0.5 cm and then placed in buffered formalin solution for 24 hours to fix the tissue (Figure 6).

Decomposition: Ascending alcohol was used to remove all water from the tissue. The tissue was immersed in alcohol with a concentration of 70%, 90%, and then absolute alcohol to remove tissue water completely.

Clarification: At this stage, xylol enters the cells and replaces alcohol. On the other hand, dissolving fats and modifying the refractive index of light made the tissue transparent. Xylol is a solvent of paraffin and can dissolve paraffin and enters the cells. Xylol is an intermediate solvent between alcohol and paraffin because xylol can be mixed with alcohol and paraffin.

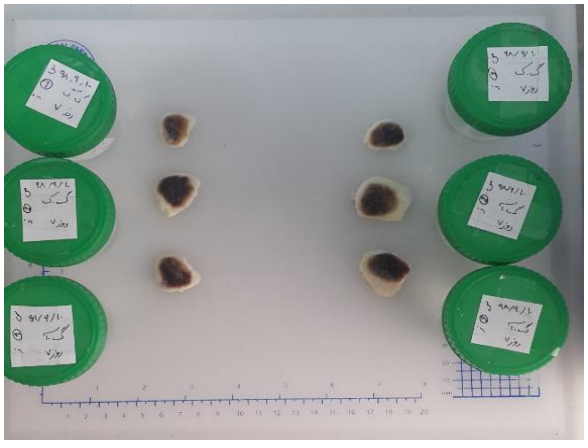

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Figure 6. Image of skin sample taken with 10% formalin container

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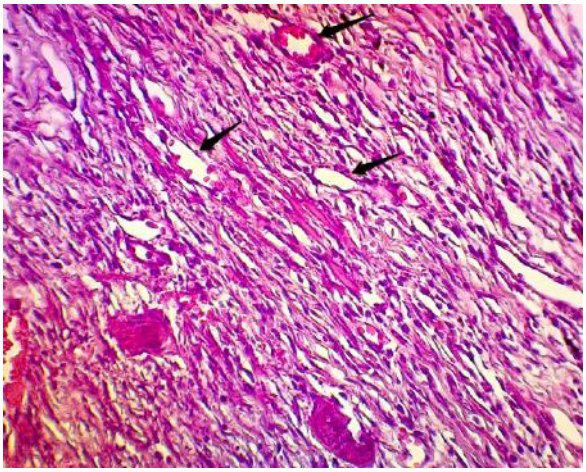
Figure 7. Image of slides prepared in two stains of hematoxylin-eosin and Masson's Trichrome



JMR

Figure 8. Three examples of the vessels counted seen in the pictures above (Eosin hematoxylin staining and 400xmagnification)

Filling with paraffin: The gradual penetration of molten paraffin into the pores and tissue cells changes the natural consistency of the tissue to the paraffin consistency, which makes it possible to have a good cut of tissue when molding with paraffin and finally to cut.

Molding: At this stage, the tissue filled with paraffin was placed in metal molds, and paraffin was poured on it, and after cooling, the paraffin mold and tissue were separated from the metal container.

Cutting and slicing: At this stage, 5-µ thick slices were taken from the mold with a microtome, and after placing them on the flute tissue and removing wrinkles, the tissue was removed from the surface of the water with the help of a slide, and the tissue was ready to be stained

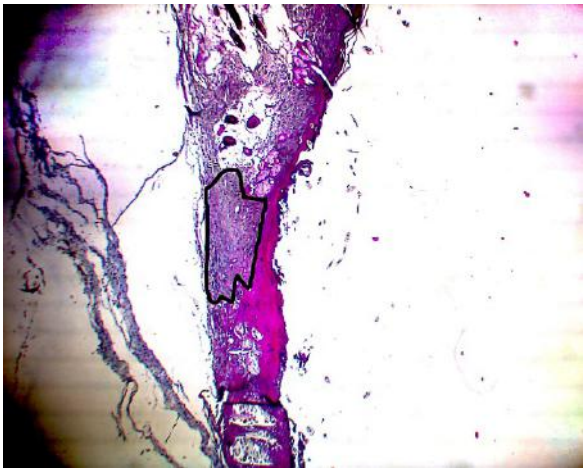


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Figure 10. An example of measuring the area of granular tissue (trichrome staining and 100xmagnification)

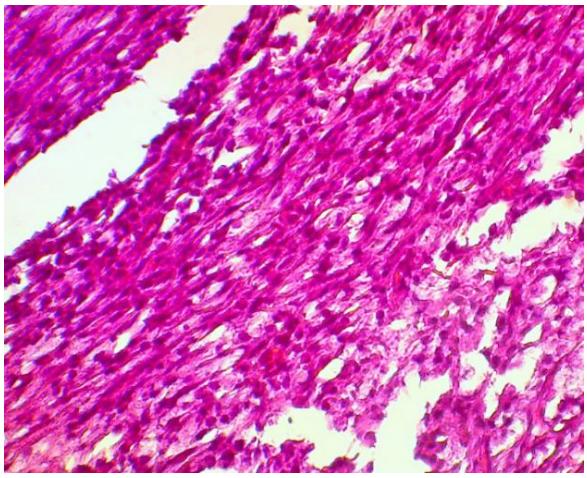

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Figure 9. A large number of inflammatory cells seen as dark spots (hematoxylin and eosin staining and 400xmagnification)

(Figure 7). In this study, two methods of hematoxylin and eosin and Masson's Trichrome staining were used:

Hematoxylin and eosin staining

To perform this staining, at first, paraffin was removed from the inside and around the tissue. Then the tissue on the slide was immersed in solutions with alcohol decreasing concentrations from absolute concentration, 90% and 70%, so that the dehydration step was performed.

After hydration, the tissue was placed in hematoxylin, and the tissue nucleus was stained and then rinsed with running water. The tissue was lithium carbonate supposed to be indented and then rinsed with running water. The tissue was then immersed in rosin and washed with running water. After performing the eosin step, the tissue was dehydrated by increasing alcohol concentrations and

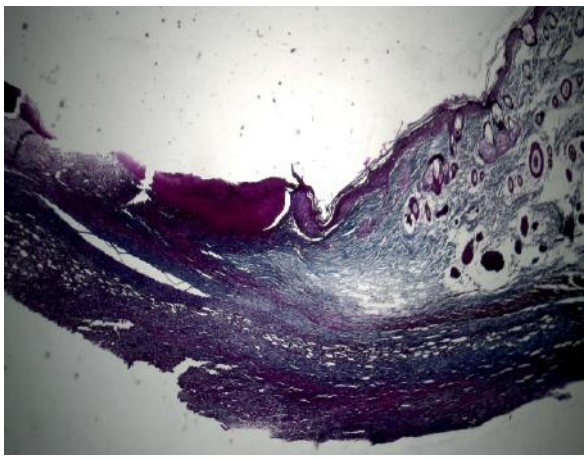

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Figure 11. Collagen in the image (Masson's Trichrome coloring and 100xmagnification)

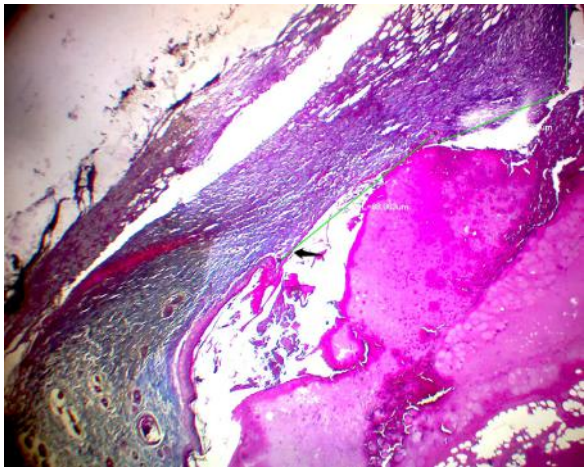

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Figure 12. The measurement of the epithelial gap in the above image seen from the arrow to the top of the image (Masson's Trichrome staining and 100x magnification)

clarified by xylol. As a result, the nuclei of the cells became dark blue to black, and the cytoplasm became pink.

Masson's Trichrome staining

To perform this staining, the inside and around the tissue were first paraffinized. Then, the tissue on the slide was immersed in solutions with decreasing alcohol concentrations of absolute concentration, 90%, and 70%, and the watering step was performed.

After hydration, the tissue was placed in hematoxylin, and the tissue nucleus was stained and then washed with running water. Excess dye was removed with the help of alcohol acid and then was washed with running water. The tissue was inserted into lithium carbonate for indentation and rinsed with running water. Then, the tissue was introduced into the fuchsin solution, then into the phosphomolybdic acid solution, and next, the tissue was placed in the methylene blue solution. As a result of this staining, the collagen fibers turned blue, and other tissue components turned red.

Angiogenesis

Hematoxylin and eosin staining was used for angiogenesis, and staining of the tissue formed under the wound was performed in all slides with 400x magnification and microscopic imaging. Veins with red blood cells or plain squamous tissue were observed and counted (Figure 8).

Inflammatory cell count

Hematoxylin and eosin staining was used to count the inflammatory cells, and all the tissues formed under the wound were seen under a microscope with 400x magnification and then observed and counted (Figure 9).

Granular tissue area

Masson's Trichrome stain was used to staining the tissue specimens and Scope Image 9.0 (H1C) software were used to measuring the granulation tissue area under the 400X magnification. Depending on the amount of granular tissue, microscopic imaging was performed on each slide. To ensure the correct diagnosis of this tissue, we used 100x magnification and 400x magnification. Afterward, the granular tissue area was measured in terms of micrometers using the software (Figure 10).

Collagen ranking

Collagen ranking was done using Masson's Trichrome staining with 40x magnification microscopic imaging and, if necessary, with 100x and 400x magnification. Based on the density and color, the lowest density and lightest color was ranked 1, and the highest density and darkest color were ranked 5 (Figure 11).

Epithelial gap

To measure the length of the epithelial cleft, trichrome staining as well as Scopelmage 9.0 (H1C) software, 40x magnification was used. Depending on the length of the gap, microscopic imaging was performed on each slide, and 100x magnification was used to ensure the correct diagnosis of this tissue. Then, the gap between the epithelium was measured in micrometers using the software (Figure 12).

Statistical analysis

Statistical analysis of data was done using IBM SPSS version 24 under Windows operating system. The Mann-Whitney non-parametric test was used to compare the non-parametric dependent indices between the control and experimental groups on each study day. In each group, the results were compared on different days using the Kruskal-Wallis statistical test and Dan post hoc test with a significance level of 0.05.

Comparison of parametric dependent indices between the control and experimental groups was performed using t-test. In each group, the values obtained on different days were compared by 1-way ANOVA with a significance level of 0.05 and Tukey's post hoc test.

Table 1. Mean±SD of collagen content in control and experimental groups

Groups	Mean±SD				
	Day 3	Day 7	Day 14	Day 21	Day 28
Control	a 1.20±0.45	a 1.60±0.55	a 2.00±0.71	ab 2.80±0.84	b 4.60±0.55
Experimental	a 1.40±0.55	ab 1.80±0.84	abc 2.80±0.84	bc 4.20±0.84	c 5.00±0.00

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* In each group, the results on different days without statistically significant difference (horizontally) are displayed with the same English letters, and the letters that are common in two different groups, regardless of the presence or absence of other letters, show no statistically significant difference between the two groups (P<0.05)

3. Results

In this study, the repair time of the effect of low power laser on the wound caused by cryosurgery was considered as a quantitative independent indicator, and the application of low-level laser on wound healing (control and experimental groups) was considered a nominal independent indicator of the study.

The quantitatively dependent indicators of these studies included histopathological assessment of the wounds for collagen content (score between 1 and 5), granular tissue size (micrometers per square meter), number of inflammatory cells (cells/mm^2), angiogenesis (cells/cm^2), and the length of the wound epithelial cleft (µm).

Collagen content

Collagen level was considered a discontinuous index, and the Mann-Whitney non-parametric test was used to compare it between the control and experimental groups on each day of the study.

The amount of tissue collagen in both control and experimental groups increased, and in the experimental group on the 28th day, it had the highest amount (score 5), which was a sign of wound healing on this day. The mean and standard deviation of collagen levels in each group per day are shown in Table 1.

The difference between collagen levels per sampling day between the control group and the experimental group was compared using the Mann-Whitney test. Based on the results obtained during the study days, the rank of collagen in the control and experimental groups was not significantly different, and no significant difference was observed between the groups (P<0.05).

Also, in each group, the results of ranking collagen levels on different days were compared using the Kruskal-

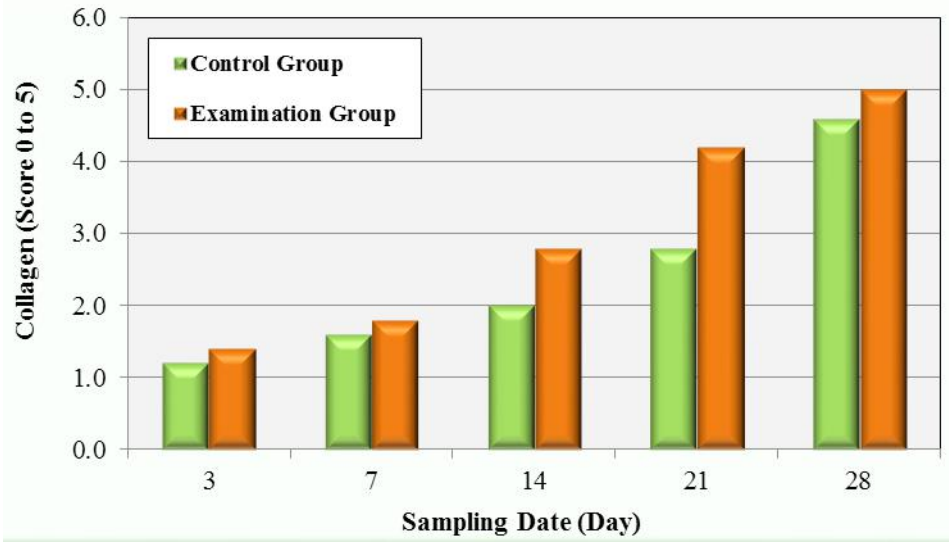

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Figure 14. Macroscopic comparison of sampled tissue in the control group on the 3rd day (a), the 7th day (b), the 14th day (c), the 21st day (d), and the 28th day (e) and in the experimental group on the 3rd day (f), the 7th day (g), the 14th day (h), the 21st day (i) and the 28th day (j)

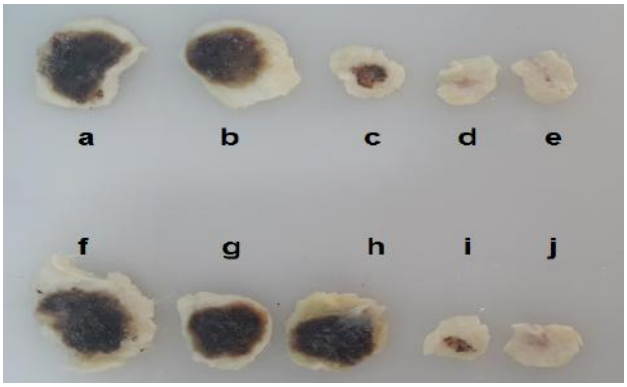

Figure 13. Average collagen in each group during the study days

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Wallis statistical test and Dan post hoc test with a significance level of 0.05. Based on these results, the amount of collagen in the control group on the 28th day is significantly different from that on the 3rd day. While in the experimental group, the amount of collagen from the 21st day is significantly different from the 3rd day (P<0.05). The process of changing the amount of collagen in each group is shown in Figure 13

Granular tissue area

Granular tissue area was considered a continuous (parametric) indicator. The comparison between the control and experimental groups for these two indicators was performed using the parametric t-test. Granular tissue also decreased in the control and experimental groups over time (Figure 14).

The difference between the granular tissue area in the control and experimental groups was compared by t-test. Based on the results, the control and experimental groups had significant differences in the area of granular tissue in all study days (P<0.05).

For each group, the values obtained from the granular tissue area on different days were compared together by using 1-way ANOVA with a significance level of 0.05 and Tukey post hoc test. Based on the results obtained in the control group on all days, a significant decrease in the area of granular tissue was observed, and the results of all days were statistically different (P<0.05). In the experimental group, a similar decrease was observed on all days (P<0.05). But no statistical difference was observed between days 21 and 28. The stages of granular tissue area change in each group are shown in Figure 15.

Inflammatory cells

The number of inflammatory cells was considered a parametric indicator. The number of these cells per square millimeter was counted, and it was found that their number decreased in all groups during the study days. The comparison between the number of inflammatory cells in the control and experimental groups was performed using the t-test. Based on the results, the number of inflammatory cells in the control and experimental groups was significantly different (P<0.05).

In each group, the obtained values from the number of inflammatory cells on different days were compared by 1-way ANOVA with 0.05 significance level and Tukey post hoc test. Based on the results obtained in both control and experimental groups, a significant decrease in inflammatory cells was observed on all days, and the results were statistically different (P<0.05). In both groups, there was no statistical difference between days 21 and 28. The amount of inflammatory cells in each group per day is shown in Tables 2 and 3.

Table 2. Area of granular tissue (μm²) in the groups

Groups	Day 3	Day 7	Day 14	Day 21	Day 28
Control	a 40157±2948	b 28802±3807	c 15640±700	d 9965±1012	e 4404±931
Experimental	a 32590±3771	b 23371±1839	c 12120±1157	d 4779±854	d 1996±711

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* In each group, the results without statistically significant difference on different days(horizontally) are displayed with the same English letters (P<0.05).

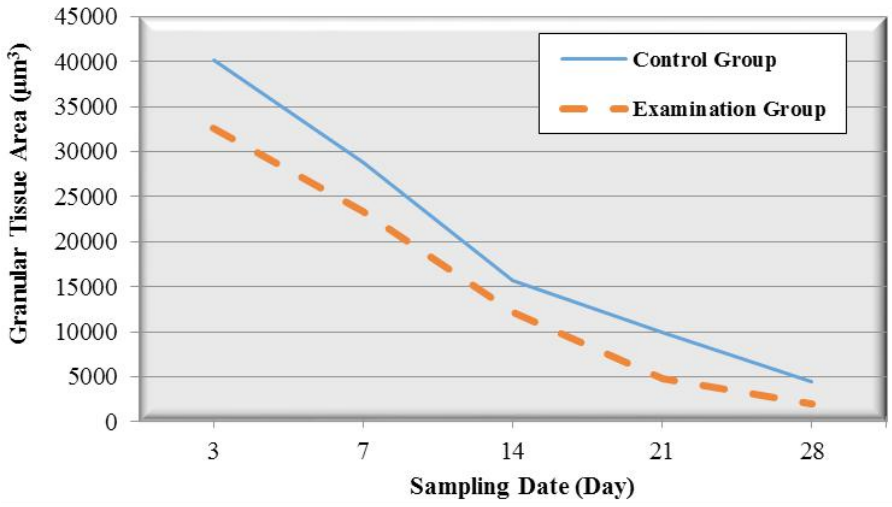

Figure 15. Stages of change in the area of granular tissue (µm²) in each group during the study days

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The process of change of inflammatory cells in each group is shown in Figure 16.

Angiogenesis

The rate of angiogenesis was calculated according to the number of vessels per square centimeter of wound tissue, and it was found that it increased in all groups. The average and standard deviation of angiogenesis in each group per day are shown in Table 4.

The difference between the rate of angiogenesis on each day of sampling in the control and experimental groups was compared by using t-test. Based on the results obtained during the 14th and 21st days, no significant difference was observed in the rate of angiogenesis in the control and experimental groups (P<0.05). However, on days 3, 7, and 28, the rate of angiogenesis in the experimental group was significantly higher than the control group (P<0.05).

For each group, the values obtained from the rate of angiogenesis on different days were compared using 1-way ANOVA with a significance limit of 0.05 and Tukey post hoc test. Based on the results obtained in both groups, a significant increase in angiogenesis was observed on all days, and the results were statistically different (P<0.05). There was no statistical difference between the third and seventh days only in the control group and between days 21 and 28 in both control and experimental groups (P<0.05). The process of angiogenic change in each group is shown in Figure 17.

Epithelial gap

The epithelial gap was calculated in micrometers, which decreased in all groups. Average and standard deviation of the epithelial cleft in each group per day are shown in Table 5.

The difference between epithelial gaps on each day of sampling between the control and experimental groups was compared using t-test. Based on the results obtained

Table 3. Mean±SD of inflammatory cells (cells/mm²) in the studied groups

Groups	Mean±SD				
	Day 3	Day 7	Day 14	Day 21	Day 28
Control	a 43778±2570	b 27917±3385	c 15149±865	d 3114±329	d 617±81
Experimental	a 22596±1083	b 19838±899	c 8998±627	d 1770±237	d 437±56

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* In each group, the results without statistically significant difference on different days (horizontally) are displayed with the same English letters (P<0.05).

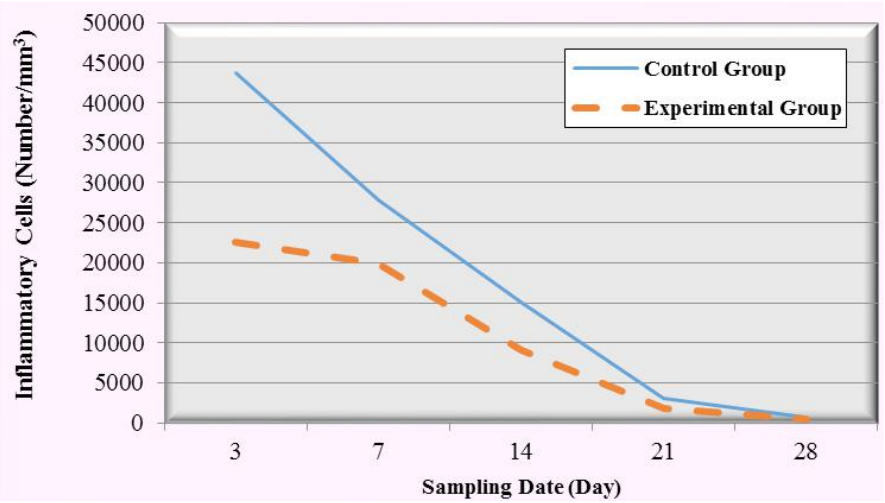

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Figure 16. The process of change of inflammatory cells (number of inflammatory cells per square millimeter) in each group

in the 3rd, 7th, 14th, and 21st days of the study, the epithelial gap was significantly different in the control and experimental groups and was significantly lower in the experimental group than the control group (P<0.05). On the 28th day, the epithelial gap was zero in both groups, and, therefore, no difference was observed between the two groups.

For each group, the values obtained from the epithelial gap on different days were compared using 1-way ANOVA with a significance level of 0.05 and Tukey post hoc test. Based on the results obtained in both groups, a decrease in the epithelial gap was observed on all days (P<0.05). In the control group, the results of the 7th and 14th days, as well as the 21st and 23rd days, were not significantly different from each other. But the results were significant on other days. In the experimental group, all days were significantly different, and a decrease in the epithelial gap was observed on all days. But there was no statistical difference between the 21st and 27th days (P<0.05) because the epithelial gap had reached zero on both days. The process of changing the length of the epithelial gap in each group is shown in Figure 18.

4. Discussion

This study aimed to find the answer to whether low-level laser prevents the healing effects of wounds caused by cryosurgery. There was no significant difference between the experimental and control groups on the same days. There was a significant difference in calculating the granular tissue area and counting the number of inflammatory cells on all days. In counting the rate of angiogenesis, this difference could be seen on days 7, 3, and 28. Also, regarding the variable of epithelial gap length, the difference could be seen on days 21, 14, 7, 3, and day 28; this variable reached zero in the two groups.

The experimental results comparing different days in each group showed a significant difference in the collagen ranking in the control group on day 28 and day 3, while this difference was visible in the experimental

Table 4. Mean±SD of angiogenesis (number of vessels per square centimeter of wound tissue) in the studied groups

Groups	Mean±SD				
	Day 3	Day 7	Day 14	Day 21	Day 28
Control	a 12928±1308	a 14976±968	b 20000±1949	c 6192±1477	c 5576±1398
Experimental	a 18376±1212	b 26128±1339	c 21928±1296	d 7920±1035	d 7808±652

JMR

* In each group, the results without a statistically significant difference on different days (horizontally) are displayed with the same English letters (P<0.05).

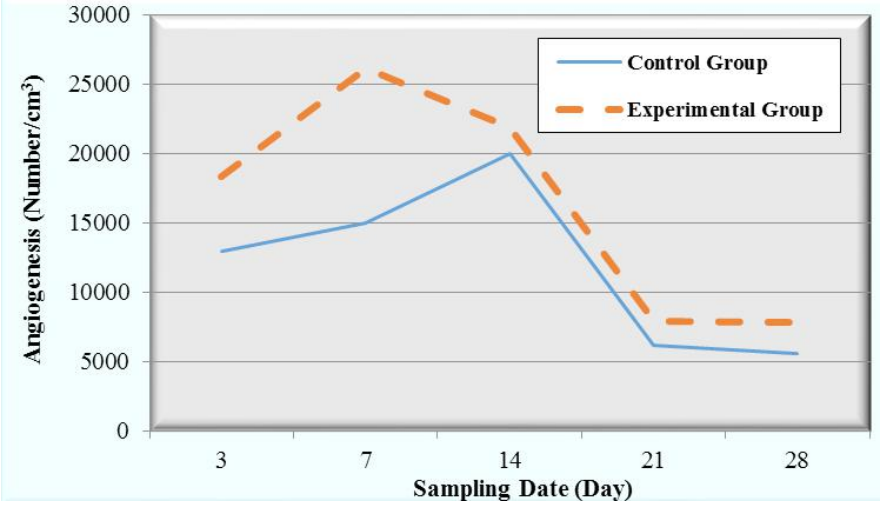

Figure 17. The process of angiogenesis change (number of vessels per square centimeter of wound tissue) in each group JMR

group from day 21 to day 3. There was a significant difference in calculating the area of granular tissue in the control group in all studied days, while in the experimental group, there was no significant difference between days 21 and 28.

There was a significant difference in the number of inflammatory cells in both study groups between days 21, 14, 7, 3, but there was no significant difference between days 21 and 28. In comparison, the healing process was similar in both groups, and the difference started from day 7 and continued until day 28 in all groups.

The difference between epithelial gaps on each day of sampling between the control and experimental groups based on the results obtained in the 3rd, 7th, 14th, and 21st days of the study, the epithelial gap was significantly different in the control and experimental groups and was significantly lower in the experimental group than the control group. On the 28th day, the epithelial gap was zero in both groups, and therefore no difference was observed between the two groups.

Numerous studies have been performed on low-level laser inside and outside the country; all showed the positive effect of lasers on wound healing.

Medrado et al. conducted a study in 2003 on the effect of low-level laser on wound healing and the biological effect of low-level laser on myofibroblasts in rats. They found that in the experimental group, compared to the control group, there was a significant decrease in swelling and inflammatory cells and a significant increase in collagen and elastin fibers. The reported results of this study were consistent with the results of our research, and the reduction of inflammatory cells and a significant increase in collagen were similar to our results [17].

Kazemi Kho et al. conducted a study in 2009 on the effect of low-level laser on diabetic foot ulcers and concluded that low-level laser could be an effective and safe treatment for foot ulcers caused by type 2 diabetes. The results of this study, like the results of our study, indicate the improvement of wound healing under the influence of low-level laser [18].

Table 5. Mean±SD of epithelial gap (µm) in the studied groups

Groups	Mean±SD				
	Day 3	Day 7	Day 14	Day 21	Day 28
Control	a 1333.0±274.4	b 758.2±84.2	b 674.8±58.2	c 90.4±10.4	c 0.0±0.0
Experimental	a 844.6±204.8	b 555.2±89.8	c 320.8±86.4	d 0.0±0.0	d 0.0±0.0

JMR

* In each group, the results without a statistically significant difference on different days (horizontally) are displayed with the same English letters (P<0.05).

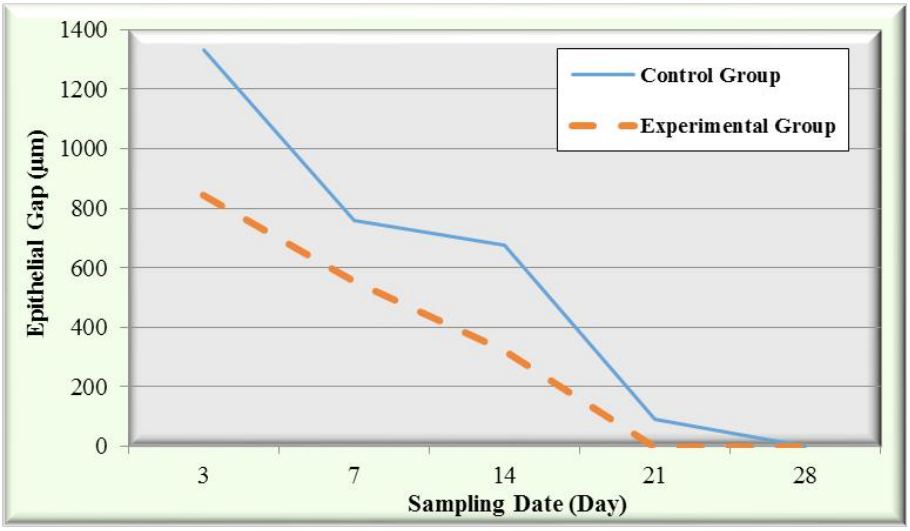

Figure 18. The process of changing the epithelial gap (micrometer) in each group during the study

JMR

In 2010, Collin et al. conducted a study to evaluate the role of a low-level laser on surgical wounds on 20 rabbits in two groups and found that the wound healing process was complete after 10 days. The healing process was such that the stitches were removed after 6 days. But in the control group, the stitches were not ready to be removed even after 7 days, and after 10 days, the wound did not heal completely. The reported results of this study are consistent with the results of our research [19].

In Brazil, Melo et al. conducted a study in 2011 to evaluate the effect of a low-level laser on surgical wounds on 40 rats in four groups. They concluded that low-level lasers reduce the intensity of the inflammatory response and affect active immune responses by altering the pattern of white blood cell penetration, as well as increasing the number of type 3 collagen fibers and, in addition, significantly increases the average number of newly formed vessels. The reported results of this study are consistent with the results of our research [20].

5. Conclusion

The results of this animal study showed that the low-level laser therapy on an experimental cryosurgical wound not only accelerates the healing process but can also be effective in the prevention of healing complications. We suggest authors conduct further research to extend the results to human applications.

Ethical Considerations

Compliance with ethical guidelines

The research project ethics was approved by Islamic Azad University Karaj Branch Research Council (Code: IR.IAU.K.REC.1398.049)

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

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