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### Original Article

## Comparative Study of Low Level Laser, Acupuncture and Liquid Stitch Effects on Surgical Wound Healing in Rat Model

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| ARTICLE INFO  | ABSTRACT  |
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| <p><i>Article History:</i></p> <p>Received 15 February 2022<br/>           Revised 5 March 2022<br/>           Accepted 10 April 2022<br/>           Online 10 April 2022</p> | <p>The objective of this study was to evaluate the efficacy of low-level laser, acupuncture, and liquid stitch on surgical wound healing in a rat model. 40 male Wistar rats (200-300 gr) were used in this study. Under general anesthesia (ketamine/xylazine), surgical preparation on both sides of the spine was performed and a skin incision was made on each side (1.5 cm). 40 wounds were closed by 3-0 polypropylene suture material in a simple interrupted pattern and half with liquid stitch. 80 wounds were divided into eight groups of ten: in groups A and B wounds were closed with polypropylene suture material and liquid stitch with no other treatment. In groups C and D wounds closed with suture materials, and liquid stitch, respectively, received acupuncture. In groups E and F, wounds closed with suture materials, and liquid stitch, respectively, received a low-level laser. In groups G and H wounds were closed with suture materials and liquid stitch, respectively, and received both laser and acupuncture. Treatments were started the day after surgery and continued until day 10. All the groups were subdivided into two equal groups which were sacrificed on days five and ten after surgery. Skin samples were taken for histopathological evaluations (H &amp; E, Masson's trichrome). The results showed that the quality of wound healing in groups C, D, E, F, G, and H, were statistically superior to the groups A, and B. Groups G and H which received both treatments, had better results than groups C, D, E, and F, which received only one of the treatments. Low-level laser treatment showed better results in comparison with acupuncture treatment. There was no significant difference between groups A and B. It can be concluded that both acupuncture and low-level laser has pro-healing properties on surgical wounds.</p> |
| <p><i>Keywords:</i></p> <p>Wound healing<br/>           low-level laser<br/>           acupuncture<br/>           liquid stitch<br/>           rat</p>                        |   |

### Introduction

Wounds are abnormalities in the natural and functional structure of the skin and underlying soft tissue.<sup>1-9</sup> Maintaining the integrity of the skin is critical

to protect against dehydration, bleeding, and the entry of microorganisms.<sup>10</sup> Skin wounds are caused by various reasons such as physical, chemical and biological damage. Wound healing includes four stages: homeostasis, acute inflammation, proliferation and

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maturation. Different methods have been proposed to improve the quality and to accelerate the mechanism of wound healing.<sup>1,4</sup>

Low-level laser (LLL) is one of the almost recent applied methods to accelerate wound healing.<sup>3,5</sup> LLL treatment is a method that uses low intensity light radiation in the light range of 830-540 nm.<sup>2</sup> It seems that the therapeutic effects of this method are achieved by photochemical reactions that change the permeability of cell membranes and consequently increase mRNA synthesis and cell proliferation.<sup>11-16</sup> LLL exerts its effects on wound healing through shortening the stage of tissue inflammation and accelerating the onset of cell proliferation, antibacterial effect, effect on cells mitochondrial function, increasing tissue blood flow and the process of cell membrane potential changes.<sup>17,18</sup> Acupuncture as the most famous branch of Traditional Chinese Medicine (TCM); also, have been used as a pro-wound healing measure.<sup>8,12</sup> It exerts its effects through both local and central mechanisms.<sup>19</sup> In wound healing, mainly the local effects functions by vasodilation and increasing blood flow, stimulating nerve endings, and activation of mast cells. Besides, through releasing neuropeptides it can reduce the local pain.<sup>20,21</sup> Liquid stitches have been used recently to substitute the ordinary skin suture materials in order to provide a fast, comfortable, safe, painless method with minimal infection and best cosmetic results.<sup>13,14</sup> It creates a thin elastic waterproof film upon applying over the wound edges, which, adheres well to the skin and withstands tension well. This film acts as a barrier against common bacteria. Liquid stitches are made primarily of cyanoacrylate.<sup>6,7</sup>

In the present study, wound closure with ordinary skin suture material is compared with liquid stitch, under the influence of Low-level laser, acupuncture and the combination of them on incisions wounds.<sup>9,10</sup>

## Materials and Methods

### Animals

40 male Wistar rats weighing 200-300 were purchased from the Neuroscience Research Center, Kerman University of Medical Sciences and kept in standard condition (temperature: 22 °C, humidity: 60%, dark/light cycle: 12 h, and water and food *ad lib*).

### Wound Creation and Grouping the Animals

Following anesthesia (ketamine, 90 mg/kg + xylazine, 10 mg/kg, intramuscularly), surgical preparation of both sides of spine in all the animals was performed

and a 1.5 cm full thickness skin incision was made on either side, using surgical scalpel (total of 80 wounds). Half of the wounds (40 wounds) were closed by 3-0 polypropylene suture material in a simple interrupted pattern and half (40 other wounds) with a liquid stitch. 80 wounds were divided into eight groups of ten: (Table 1)

Group A (Control): Wounds were closed in 3-0 polypropylene suture material in a simple interrupted pattern.

Group B: Wounds were closed with liquid stitch.

Group C: Wound closure was performed like group A, and the wounds received acupuncture at six points around the wounds, from the day after the surgery until 24 h. before scarification.

Group D: Wound closure was performed like group B, and post-wounding treatment was done like group C.

Group E: Wound closure was performed like group A, and the wounds received Low level laser from the day after the surgery until 24 h. before scarification.

Group F: Wound closure was performed like group B, and post-wounding treatment was done like group E.

Group G: Wound closure was performed like group A, and both acupuncture and Low level laser, were used over the wounds in the same fashion as groups C, and E.

Group H: Wound closure was performed like group B, and post-wounding treatment was done like group G.

All the groups were subdivided into two equal groups which were sacrificed at day five and ten after surgery, respectively.

**Table 1.** Experimental groups.

| Groups | Sutured (Polypropylene, simple interrupted) | Closed by liquid stitch | Acupuncture | Low level laser |
|--------|---|-------------------------|-------------|-----------------|
| A      | *   |                         |             |                 |
| B      |   | *                       |             |                 |
| C      | *   |                         | *           |                 |
| D      |   | *                       | *           |                 |
| E      | *   |                         |             | *               |
| F      |   | *                       |             | *               |
| G      | *   |                         | *           | *               |
| H      |   | *                       | *           | *               |

### Histopathological Study

Full-thickness skin samples were taken from wounds with a margin of 1 cm from healthy skin and immediately placed in 10% buffered formalin for at least 48 hours to be fixed. Following routine

preparation of tissues, serial sections of paraffin embedded tissues of 5 µm thicknesses were cut with a microtome and stained with hematoxylin-eosin (H & E) and Masson's trichrome (MTC) studied under light microscope. In histopathological examination, new epithelial thickness, angiogenesis, collagen amount (5th day), collagen maturation and organization (10th day),

edema and inflammation were evaluated and each parameter was scored according to the Table 2. Wounds size was microscopically measured with a digital camera (Eclipse 50i; Nikon, Tokyo, Japan). For this purpose, in each sample, 3-5 different areas were measured and its mean was considered as the size of each wound.

**Table 2.** Histopathologic scoring system for wound repair.<sup>1,9</sup>

| Number* | Histopathological Parameters   |
|---------|--|
| 1       | Re-epithelialization (0 = partial, 1 = complete but thinner the adjacent intact epidermis, 2 = irregular hyperplastic, 3 = regular hyperplastic, 4 = similar to normal epithelium) |
| 2       | Angiogenesis (0 = none, 1 = up to five vessels per HPF*, 2 = 6 to 10 vessels per HPF, 3 = more than 10 vessels per HPF)  |
| 3       | Polymorphonuclear cells (0 = none, 1 = scant, 2 = moderate, 3 = abundant)  |
| 4       | Edema (0 = none, 1 = mild, 2 = moderate, 3 = severe)   |
| 5       | Mononuclear cells (0 = none, 1 = scant, 2 = moderate, 3 = abundant)  |
| 6       | Collagen organization (0 = none, 1 = scant, 2 = moderate, 3 = fair, 4 = similar to normal area)  |
| 7       | Collagen deposition (0 = none, 1 = scant, 2 = moderate, 3 = relatively abundant, 4 = abundant)   |
| 8       | Collagen maturation (0 = none, 1 = scant, 2 = moderate, 3 = fair, 4 = similar to normal area)  |

\*Number 1-5: H & E, Numbers 7 and 8: Masson's's trichrome stain; old collagen fibers take deep green color and the new collagen fibers stain light green.

### Statistical Analysis

Pathologic evaluations of the results were analyzed semi-quantitatively by SPSS software version 23 and using Mann-Whitney statistical test. Obtained data from macroscopic and microscopic evaluation of wounds were presented as mean ± SE. *p*-value < 0.05 was considered as significant differences.

## Results

### Macroscopic Evaluation

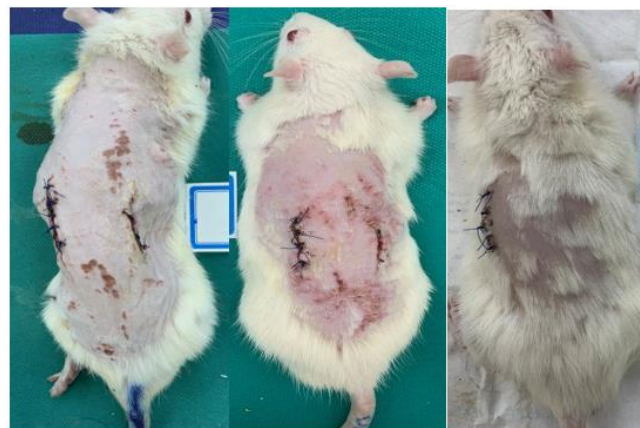
In macroscopic results, reduction in hyperemia and edema and wound size of treated wound with laser, acupuncture and combination of laser and acupuncture was observed in comparison with the control groups. Closed wound with liquid stitches had better appearance, compared with the sutured wounds and the wound disappeared on 10th day of experiment. Hair growth around the treated wounds were faster than the control groups (Figures 1-4).

### Histopathological Findings 5 Days after Wounding

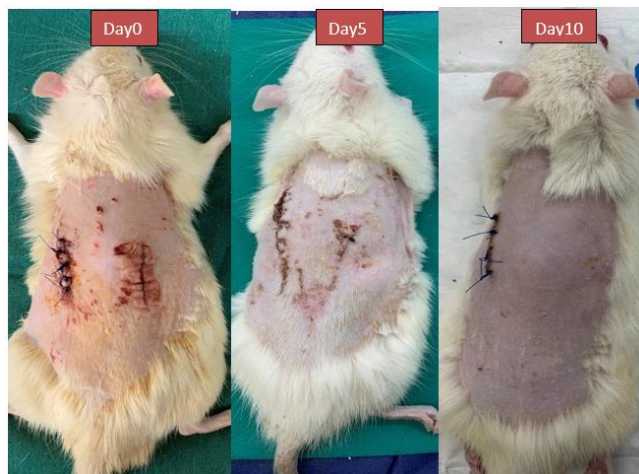
Five days after wounding, the formation of new epithelial tissue (re-epithelialization) was completed in all animals in the acupuncture and laser treatment groups. New-epithelium from the basement membrane to the surface was included the basal layer, stratum spinosum, granulosum and corneum layers respectively. In the control groups (suture (A) and liquid stitches (B) and combination-treatment groups (acupuncture + laser- G and H groups), the new-

epithelium in two out of the five samples, was formed only at the margin of the wound and there was a purulent exudate in the wound center.

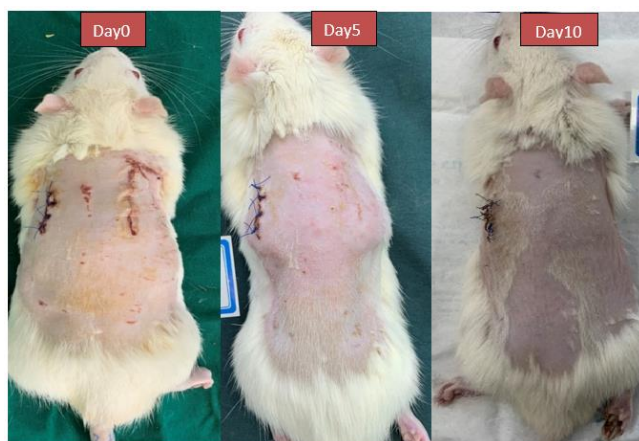
The best quality of re-epithelialization was observed in the acupuncture- liquid stitch treated group (group D) with a mean score of (3 ± 0.44) that was hyperplastic with regular pattern. In all groups, the repair site was filled with granulation tissue containing fine and irregular collagen, newly formed vessels (angiogenesis), fibroblasts and fibrocytes. The lowest number of neovascularization in the wound area associated with their maturation was seen in the acupuncture- liquid stitch (group D) and then in the laser- liquid stitch group (group F).



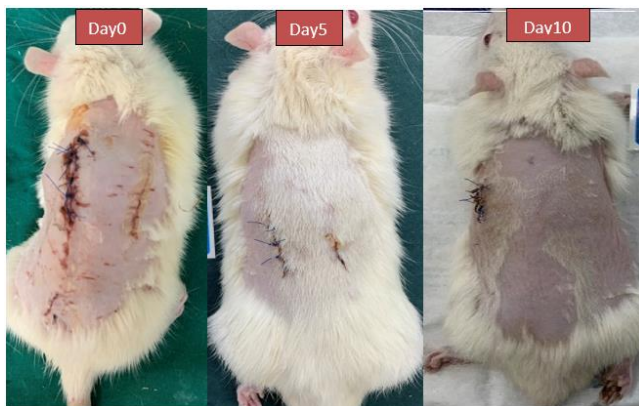
**Figure 1.** Macroscopic appearance of wounds in the control groups of sutures (left) and liquid stitches (right) over a period of 10 days (groups A & B).



**Figure 2.** Macroscopic appearance of wounds in the acupuncture- suture (left) and acupuncture-liquid stitches (right) over a period of 10 days (groups C & D).



**Figure 3.** Macroscopic appearance of wounds in the of laser-suture (left) and laser- liquid stitches (right) groups over a period of 10 days (groups E & F).



**Figure 4.** Macroscopic appearance of wounds in the of combination groups: suture (left) - liquid stitches (right) over a period of 10 days (group G & H).

The highest number of neovascularization was in the liquid stitch (B) and sutures (A) control groups. Lymphocyte inflammatory cells with mild (+1) to moderate (+2) scores were present in all control and treatment wounds and there was no statistically

significant difference between treatment and control groups. Granulomatous reactions involving epithelioid and giant cells were seen in some sutured samples. The purulent exudate was seen on the surface of some wounds and penetrated to underlying layer. The lowest number of neutrophils in the wounds was related to the liquid stitch treatment groups with acupuncture (group D) and then laser (group F), although there was no statistically significant difference between them (Figures 5-8).

In this study, after 5 days, the amount of deposited collagen fibers in the repair area was investigated with Masson's trichrome staining. The better results were observed in the acupuncture- liquid stitch group (D) ( $2.8 \pm 0.83$ ) in comparison with the other groups (Figure 9).

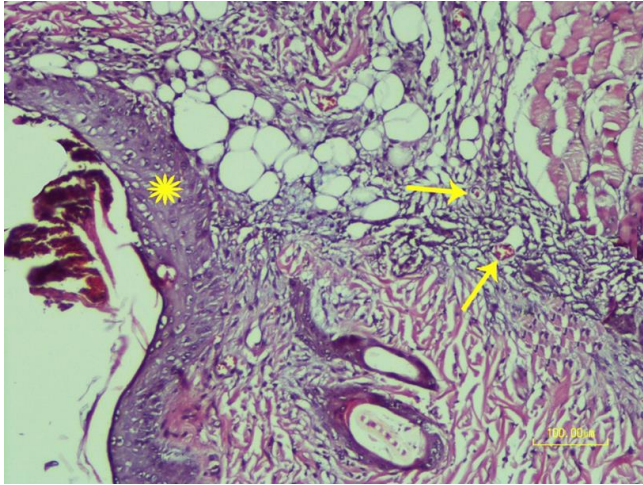
In the microscopic study and according to the results of statistical tests, some histopathologic parameters such as collagen formation, edema, neutrophils and mononuclear inflammatory cells in the wounds of all control and treated groups had no significant difference. Angiogenesis was significantly decreased in the wounds closed with liquid stitch that treated with laser and also acupuncture separately than with other control and treatments group ( $p < 0.05$ ). Also, the highest thickness of new-epithelium happened in liquid stitch- acupuncture group (group D) ( $p < 0.05$ ).

#### *Histopathological Findings 10 Days after Wounding*

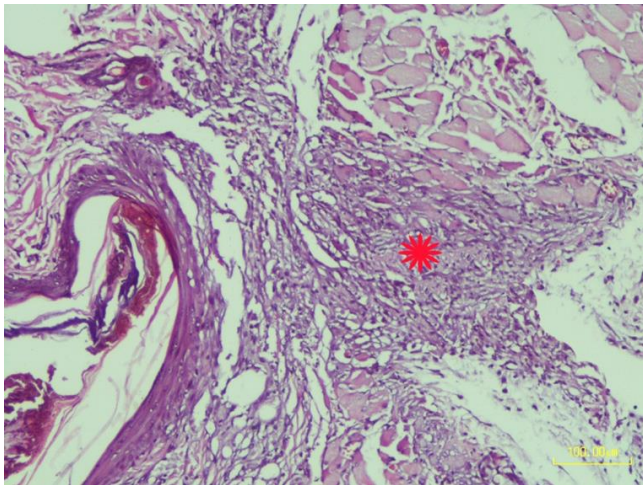
10 days after wound creation, regeneration of new-epithelium was completed in all treatment and control groups. Wound maturation was better in the acupuncture and laser treated liquid stitch groups (groups D, and F, respectively) which were more similar to the surrounding intact epithelium, though no significant difference was found. In granulation tissue, collagen density and number of fibrocytes increased. Better organization and arrangement was observed in the liquid stitch-acupuncture group (group D). In all groups, the number of vessels was reduced without obvious differences among them. Mild infiltration of mononuclear inflammatory cells including lymphocytes and macrophages, were occurred in the wound site in all groups. Presence of neutrophils was not seen in all closed wounds with liquid stitch, but in few samples of the control suture and combined-treatment groups (Figures 10 and 11).

#### **Discussion**

Wound healing has three main phases: inflammation,



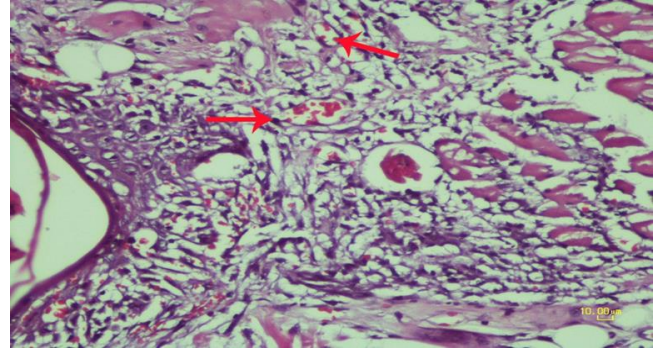
**Figure 5.** Liquid stitch-acupuncture group five days after wounding. A little amount of granulation tissue, decrease in the number of new vessels (+2) (arrow) and infiltration of lymphocyte cells (+1) are well-evident. The new epithelial tissue (asterisk) is hyperplastic and regular (3+) (H & E staining, Bar = 100  $\mu$ m).



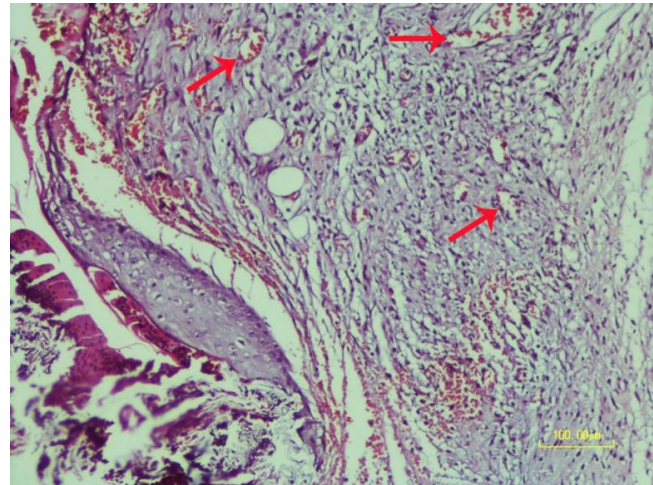
**Figure 6.** Suture- laser group five days after wound creation. The hyperplastic and irregular new epithelium (2+) and abundant collagen (+ 3) (asterisk) is deposited in the wound site (H & E staining, Bar = 100  $\mu$ m).

proliferation, and remodeling.<sup>22,23</sup> Until the healing process takes place, the inflammatory phase is gradually replaced by the proliferative phase, which is characterized by the extensive migration of fibroblasts to the wound area.<sup>24,25</sup> This cellular subset is responsible for the synthesis, deposition, and regeneration of collagen fibers required for wound healing after tissue damage.<sup>26</sup> In healthy tissues, collagen fibers create strength, integrity and structure, therefore, when tissues are damaged following injury, collagen deposition is necessary to replace lost tissue and restore anatomical structure and function.

Some drugs and surgical methods are used to accelerate the healing of wounds. In addition to the use



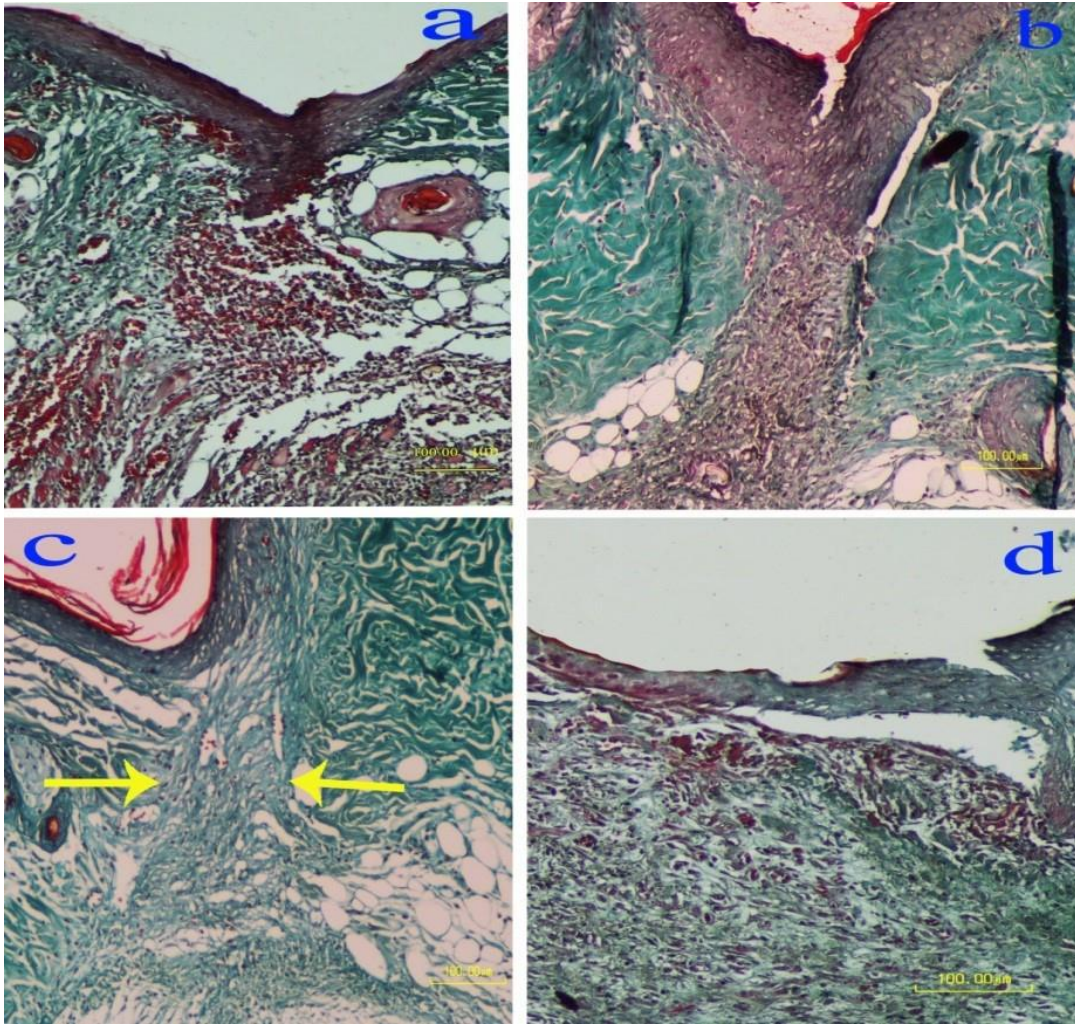
**Figure 7.** Suture-combination group (laser + acupuncture) 5 days after wounding. The new epithelium is relatively hyperplastic and irregular (2+). Deposition of thin collagen fibers (+1), presence of neovessels (2+) (arrows) and infiltration of lymphocytes (+2) are characteristics of granulation tissue (H & E staining, Bar = 100  $\mu$ m).



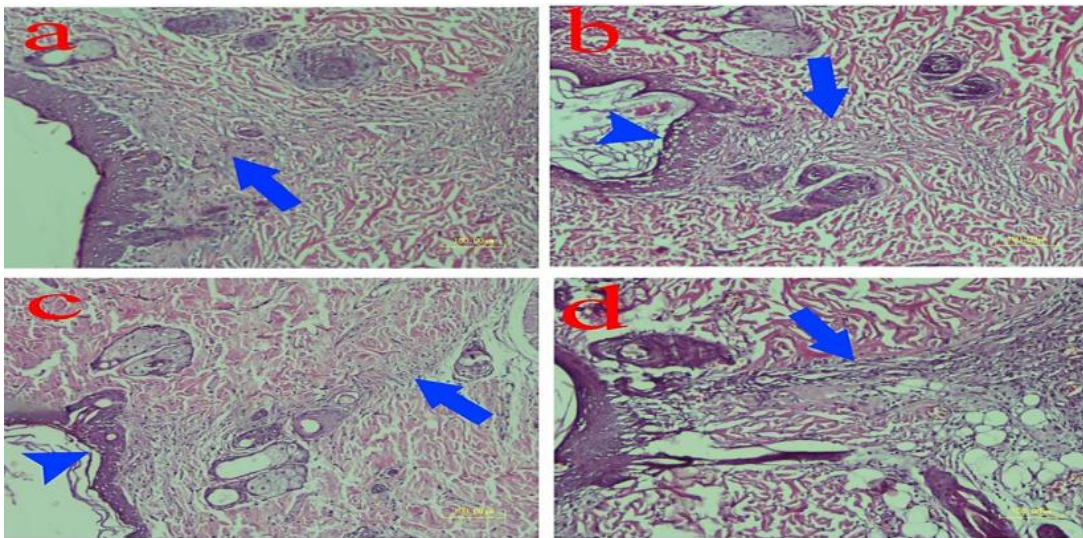
**Figure 8.** Suture control group five days after wound creation. Epithelialization is completed. Granulation tissue is edematous with high score (3+) of angiogenesis (arrows) (H & E staining, Bar = 100  $\mu$ m).

of drugs and various chemical and herbal substances for wound healing, in recent years, the effect of low-level lasers on activating wound healing is shown.<sup>27,28</sup> In recent decades, significant efforts have been made for using of laser systems immediately after surgery in order to make positive changes in the physiological process of wound healing without scarring and improve the appearance of surgical scars.<sup>29,30</sup>

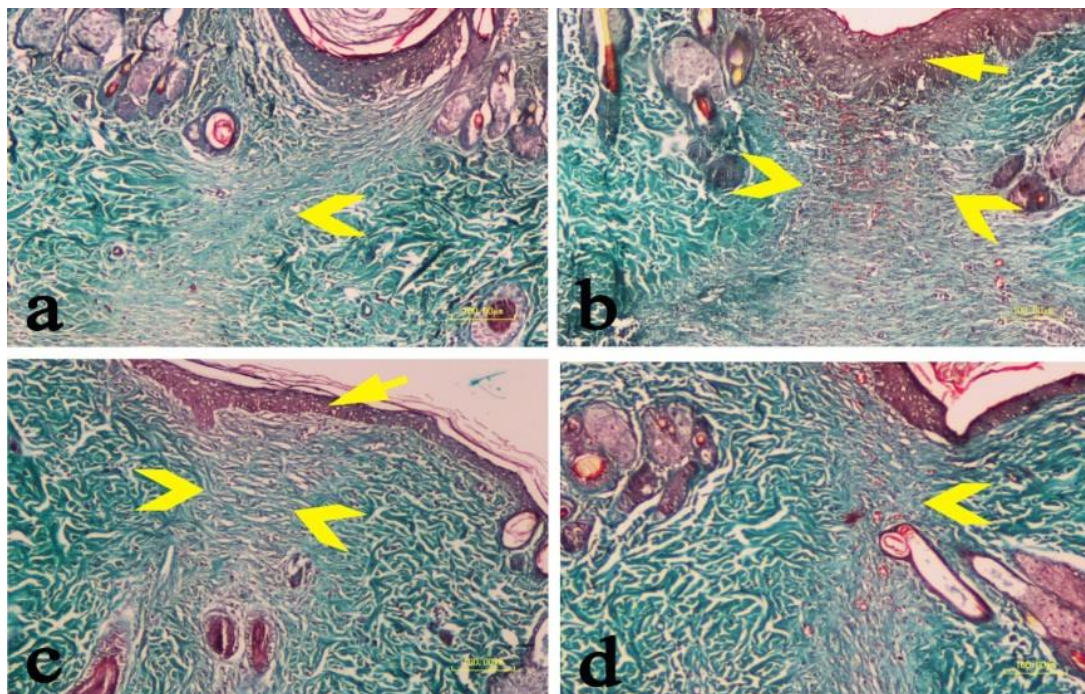
Low-level lasers are absorbed without heat by molecules in the cell called photoreceptors and certain wavelengths can activate these molecules.<sup>26,31</sup> Laser with the following mechanisms accelerates wound healing: 1. Increase the production of DNA, RNA and proteins, 2. Increase collagen production, 3. Angiogenesis, 4. Faster re-epithelialization, 5. Anti-inflammatory effect with effect on leukocytes and macrophages, 6. Activation of the immune system by acting on IgG, IgM and complement, 7. Increased blood



**Figure 9.** Evaluation of collagen with Masson's trichrome staining 5 days after creating wounds shows better maturity of this fiber in the group (D: acupuncture- liquid stitch group (Figure c) in comparison with the liquid stitch control group (group B) (Figure a), laser- liquid stitch group (or group F) (Figure b) and liquid stitch-combination group (Figure d). In this image, the new-epithelium in the group D (Figure c) is more similar to the intact epithelium. The arrows in this image show the wound area (Bar = 100 µm).



**Figure 10.** 10 days After wounding, the new-epithelium (arrowhead) in the acupuncture- liquid stitch group (group D) (Figure c) is more similar to healthy epithelium, while in liquid stitch control group (group B) (a), laser- liquid stitch (group F) (b) and the combined-treatment liquid stitch group (group H) (d) is still hyperplastic. The arrows in this image show the wound area, which in group D (Figure c) shows a small remnant of scar tissue at the wound site with better maturity (HE staining, Bar = 100 µm).



**Figure 11.** 10 days after wounding, in the acupuncture- liquid stitch group (group D) (figure c) the thickness of the new epithelium (arrow) is reduced and is more similar to the intact epithelium, while in suture -acupuncture group (group C) (figure a), control - suture (group A) (figure b), and Control - liquid stitch (group B) (figure c) is hyperplastic. The arrows in this image show the wound area. In group B (figure c), the collagen fibers at the wound site are thicker than in the other groups and show better maturity and organization (Masson's trichrome staining, Bar = 100  $\mu$ m).

**Table 3.** Results of semi-quantitative evaluation of various parameters of healing 5 days after wounding (mean  $\pm$  SE).

| Histopathologic parameters | Groups                       |                             |                             |                              |                           |                             |                              |                              |
|----------------------------|------------------------------|-----------------------------|-----------------------------|------------------------------|---------------------------|-----------------------------|------------------------------|------------------------------|
|                            | H                            | G                           | F                           | E                            | D                         | C                           | B                            | A                            |
| The amount of collagen     | 2.0 $\pm$ 0.31               | 61 $\pm$ 0.24               | 2.2 $\pm$ 0.37              | 2.4 $\pm$ 0.73               | 2.8 $\pm$ 0.58            | 2.4 $\pm$ 0.47              | 1.4 $\pm$ 0.24               | 1.8 $\pm$ 0.44               |
| edema                      | 1 $\pm$ 0.31                 | 2 $\pm$ 0.54                | 0.8 $\pm$ 0.2               | 0.8 $\pm$ 0.48               | 0.6 $\pm$ 0.24            | 1.2 $\pm$ 0.37              | 2 $\pm$ 0.44                 | 1.4 $\pm$ 0.4                |
| Neutrophils                | 1 $\pm$ 0.63                 | 0.6 $\pm$ 0.24              | 0.4 $\pm$ 0.24              | 0.6 $\pm$ 0.24               | 0.2 $\pm$ 0.2             | 1.4 $\pm$ 0.5               | 1 $\pm$ 0.54                 | 0.6 $\pm$ 0.4                |
| Mononuclear inflammation   | 1.6 $\pm$ 0.24               | 1.6 $\pm$ 0.4               | 1.4 $\pm$ 0.24              | 1.2 $\pm$ 0.2                | 1.4 $\pm$ 0.24            | 1.4 $\pm$ 0.24              | 1.4 $\pm$ 0.24               | 1.2 $\pm$ 0.2                |
| <i>p</i> _Value            | ( <i>p</i> > 0.05)           |                             |                             |                              |                           |                             |                              |                              |
| Angiogenesis               | 1.2 $\pm$ 0.37 <sup>ab</sup> | 2 $\pm$ 0.44 <sup>ab</sup>  | 1.2 $\pm$ 0.2 <sup>b</sup>  | 1.6 $\pm$ 0.24 <sup>ab</sup> | 1 $\pm$ 0 <sup>b</sup>    | a 2.4 $\pm$ 0.4             | 1.8 $\pm$ 0.37 <sup>ab</sup> | 2.2 $\pm$ 0.37 <sup>ab</sup> |
| Thickness of epithelium    | 2 $\pm$ 0.31 <sup>ab</sup>   | 1.4 $\pm$ 0.6 <sup>ab</sup> | 2.2 $\pm$ 0.2 <sup>ab</sup> | 1.8 $\pm$ 0.58 <sup>ab</sup> | 3 $\pm$ 0.44 <sup>b</sup> | 1.2 $\pm$ 0.48 <sup>a</sup> | 1.2 $\pm$ 0.37 <sup>ab</sup> | 1.2 $\pm$ 0.58 <sup>ab</sup> |
| <i>p</i> _Value            | ( <i>p</i> < 0.03)           |                             |                             |                              |                           |                             |                              |                              |

**Table 4.** Different repair parameters in treatment and control groups 10 days after wounding (mean  $\pm$  SE).

| Groups                  | H                  | G              | F              | E              | D              | C              | B              | A              |
|-------------------------|--------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Angiogenesis            | 1.2 $\pm$ 0.25     | 1.6 $\pm$ 0.4  | 1.4 $\pm$ 0.24 | 1.6 $\pm$ 0.24 | 1.4 $\pm$ 0.24 | 1.4 $\pm$ 0.24 | 1 $\pm$ 0      | 1.4 $\pm$ 0.24 |
| Collagen mature         | 2 $\pm$ 0          | 2.4 $\pm$ 0.4  | 2.6 $\pm$ 0.4  | 2.2 $\pm$ 0.37 | 2.6 $\pm$ 0.24 | 2.4 $\pm$ 0.24 | 2.6 $\pm$ 0.24 | 2.4 $\pm$ 0.24 |
| Collagen organization   | 2.2 $\pm$ 0.25     | 2.2 $\pm$ 0.37 | 2.2 $\pm$ 0.48 | 2.2 $\pm$ 0.37 | 2.6 $\pm$ 0.24 | 2.4 $\pm$ 0.24 | 2.4 $\pm$ 0.24 | 2 $\pm$ 0.31   |
| Neutrophils             | 0 $\pm$ 0          | 0.8 $\pm$ 0.37 | 0 $\pm$ 0      | 0 $\pm$ 0      | 0 $\pm$ 0      | 0 $\pm$ 0      | 0 $\pm$ 0      | 0.8 $\pm$ 0.58 |
| Thickness of epithelium | 2.7 $\pm$ 0.25     | 2 $\pm$ 0.54   | 3.4 $\pm$ 0.4  | 3 $\pm$ 0      | 3.4 $\pm$ 0.4  | 2.4 $\pm$ 0.4  | 2.8 $\pm$ 0.37 | 2.8 $\pm$ 0.37 |
| Inflammation            | 1.6 $\pm$ 0.24     | 1.6 $\pm$ 0.24 | 1.4 $\pm$ 0.40 | 1.2 $\pm$ 0.20 | 1.4 $\pm$ 0.24 | 1.4 $\pm$ 0.24 | 1.4 $\pm$ 0.24 | 1.2 $\pm$ 0.20 |
| <i>p</i> _Value         | ( <i>p</i> > 0.05) |                |                |                |                |                |                |                |

**Table 5.** Different size parameters in treatment and control groups 5 and 10 days after wounding.

| <b>Test Statistics<sup>b</sup></b>    |                   |                   |                   |                   |
|---------------------------------------|-------------------|-------------------|-------------------|-------------------|
| <b>DAY5</b>                           | A & B             | C & D             | E & F             | G & H             |
| <b>Mann-Whitney U</b>                 | 8.000             | 2.000             | 1.000             | 7.000             |
| <b>Wilcoxon W</b>                     | 18.000            | 8.000             | 11.000            | 22.000            |
| <b>Z</b>                              | -.490             | -1.091            | -2.205            | -.735             |
| <b>Asymp. Sig. (2-tailed)</b>         | .624              | .275              | .027              | .462              |
| <b>Exact Sig. [2*(1-tailed Sig.)]</b> | .730 <sup>a</sup> | .400 <sup>a</sup> | .032 <sup>a</sup> | .556 <sup>a</sup> |
| <b>DAY10</b>                          | A & B             | C & D             | E & F             | G & H             |
| <b>Mann-Whitney U</b>                 | 11.000            | 2.000             | 8.000             | 4.000             |
| <b>Wilcoxon W</b>                     | 26.000            | 17.000            | 23.000            | 14.000            |
| <b>Z</b>                              | -.313             | -1.640            | -.490             | -1.155            |
| <b>Asymp. Sig. (2-tailed)</b>         | .754              | .101              | .624              | .248              |
| <b>Exact Sig. [2*(1-tailed Sig.)]</b> | .841 <sup>a</sup> | .143 <sup>a</sup> | .730 <sup>a</sup> | .343 <sup>a</sup> |

a. Not corrected for ties. b. Grouping Variable: treat

circulation and lymphatic drainage, 8. Metabolisms shift to aerobic, 9. Reducing pain and secretion of pain mediators that have anti-restorative effect.<sup>32,33</sup>

In the present study, The results of angiogenesis and thickness of epithelial tissue in wounds treated on day 5 in different groups are as follows: The thickness of newly formed epithelium after 5 days of treatment was better in the liquid stitch -acupuncture group (group D) than the other groups and was significantly different from its control group (group B). Angiogenesis was seen in the healing tissue. After 5 days, the rate of angiogenesis in the acupuncture- liquid stitch treatment group (group D) and then the laser- liquid stitch group (group F) showed the greatest decrease compared to the other groups, and macroscopically less hyperemia was seen, which was significantly different from the control group ( $p < 0.05$ ). There was no significant difference between other treatment and control groups. It means that laser and acupuncture techniques accelerated the process of angiogenesis in comparison with the control groups. The results of the present study were in agreement with results of the previous studies: Valdinaldo Aragão de Melo et al.<sup>11</sup>, showed low-level laser therapy resulted in modulate of the inflammatory response, enhanced deposition of collagen fibers and increase in number of newly formed vessels. Ethne L. Nussbaum et al.in 2009, showed laser protocol improved wound healing during the first 8 days after wounding as indicated by wound area and histology.<sup>3</sup> Kelly Cristina Borges Tacon et al. in 2011, showed the low-level laser acted positively on the healing of skin wounds in rats. This finding suggests that the beneficial effects depended preferentially on

direct effects of the laser light on host tissue. Ryoichi Suzuki et al.in 2016 showed low-level laser therapy with a 660-nm diode laser with an output power density of 11.3 mW/cm<sup>2</sup> was found to enhance wound healing in incised tissues.

The results of collagen levels after 5 days of treatment in rats in different groups showed that laser treatment in both groups of sutures and liquid stitch (groups E and F, respectively) as well as acupuncture-liquid stitch (group D) was slightly better than the other groups, and the difference was significant. There was no significant difference between liquid stitch control (group B) and treatments, and suture control (group A) and its various treatments.

Mononuclear inflammatory cells in the liquid stitch group (group B) and the suture group (group A) were not significantly different in comparison with the other groups, though the laser- liquid stitch (group F) and acupuncture- liquid stitch (group D) showed lower neutrophil counts in the tissue.

After 5 days of treatment, wound size showed significant differences in all groups. In histopathologic results, the wound size of the laser, acupuncture and combination groups is much less than the control group. In this study, less inflammation was observed in closing wounds with liquid stitch than suture.

As a result of present study low-level laser therapy modulates the inflammatory response, increases collagen fiber deposition, and increases the average number of newly formed vessels and may cause a short-term acute inflammatory reaction in the early stages of wound healing and accelerate the inflammatory phase.<sup>4</sup>

The mechanism of action of acupuncture based on

new theories can be divided into two main parts: A) Local and segmental mechanisms: Anti-inflammatory effect on tissues, increased peripheral blood flow and B) Non-positional and systemic mechanisms. Acupuncture regulates the inflammatory process, increases blood circulation and growth factors and has an effect on inflammatory cytokines, proliferation and angiogenesis, and ultimately heals wounds by reducing inflammatory reactions and increasing angiogenesis and cell matrix regeneration. Acupuncture is a complex system of diagnosis, treatment and prevention of diseases that is based on the body's response to stimulation of specific points by the needle and exerts its therapeutic effect by affecting the internal organs and thus the body's function.<sup>12</sup>

Dwight et al., 2014, showed that acupuncture reduces edema and causes wound contraction and increased blood circulation, and angiogenesis, which was also in accordance with the result of the present study. Erja E Saarto et al., 2010, showed that the use of a single acupuncture treatment right after surgery in dogs did not appear to have any beneficial effects in surgical wound healing.<sup>12</sup> Sang In Park et al., 2012, showed acupuncture treatment around the edges of wounds, promotes wound healing through decreasing inflammatory cytokine release, increasing newly generated cells, and stimulating angiogenesis and granulation-tissue formation.<sup>15</sup>

When the acupuncture needle is inserted around the patient's wound, red areas are seen around the needle. In fact, this may be an axonal reflex. With stimulation of acupuncture, vasoactive neuropeptides such as CRGP (Calcitonin Gn Related Peptide) and substance P are released following a reflex. These substances are released by stimulating the C (C) - and A - delta filaments and lead to an increase in perfusion around the needle and possibly the tissues beneath it, and this effect remains even after removal of the needle. Biochemically, they also have growth promoting properties and, along with other factors, cause tissues to heal faster.<sup>8</sup>

Acupuncture has an anti-inflammatory and analgesic role in pain conditions. These produced substances inside the nerve cell are transported to the site of inflammation or pain along with oxoplasmic currents. With the accumulation of these substances at the site of inflammation and pain, anti-inflammatory and analgesic properties appear.<sup>8</sup>

According to the results of this study it can be concluded that liquid stitch can be an ideal method for

closing wounds. This method is fast, comfortable, safe, cheap, and painless with minimal infection. It polymerizes to form a thin, waterproof elastic layer that adheres well to the skin and tolerates stretch well. In this study, it was found that closing wounds with liquid stitch had less inflammation than closing wounds with sutures. Sandro Cilindro de Souza et al. in 2007, showed liquid stitch has been well-tolerated with several advantages and fewer complications rather than sutures. They described liquid stitch did not induce necrosis, allergic reactions or infections, and has lower costs. Afisu A.Oladega et al. in 2019, showed liquid stitch seems to have beneficial haemostatic effect on postoperative bleeding.<sup>6,7</sup> Also, the results showed that acupuncture and LLL, both have pro-healing effects on surgical wounds.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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