EFFECT OF LASER BIOSTIMULATION ON THE HEALING OF CUTANEOUS SURGICAL WOUNDS IN PIGS

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Abstract

The objective of this study was to investigate the impact of therapeutic laser irradiation on wound healing and skin regeneration in pigs as a supplementary treatment. The experiment was conducted on 32 young pigs divided into four groups: groups I and III served as control, group II comprised pigs with undamaged skin, and group IV consisted of pigs with cutaneous surgical wounds in the dorsal area. Groups II and IV were subjected to laser irradiation. Laser biostimulation was carried out using a CTL 1106 MX semiconductor laser in the continuous wave mode of operation at a wavelength of 810 nm and a maximum output of 100 mW. Following three weeks of observation and clinical skin trials, specimens for a histopathological analysis were collected. The reported results indicate that laser treatment shortens the wound healing process by speeding up the growth of granulation tissue and improving skin elasticity. Laser irradiation of the skin in pigs increases cellular infiltration of the corium and stimulates the proliferation of the stratum germinativum cells of the epidermis. Laser irradiation may be recommended as supplementary therapy in the treatment of surgical wounds.

Key words: swine, surgical wounds, laser therapy, biostimulation.

When introduced for medical use, lasers delivered a variety of new options in the treatment of diseases, which are resistant to other forms of therapy. Non-invasive laser irradiation was first used in 1969 by Master (10), who applied low-level laser irradiation to stimulate biological processes in difficult to heal wounds and ulcers. This event gave rise to the development of Low Level Laser Therapy (LLLT). Despite initial doubts concerning the efficacy of laser therapy, LLLT has come to occupy a prominent place in contemporary medicine. Research into the biostimulating effect of laser radiation determined its therapeutic indications, which have been officially recognised by the FDA in 1989 as valid therapeutic methods (1). The efficacy of laser therapy has been determined in view of the following findings:

- Low-level irradiation produces a positive effect at the cellular level,
- irradiation at an approximate wavelength of 1,000 nm is most readily absorbed by the tissue,
- energy is absorbed at the level of cellular chromophores and mitochondrial cytochromes,
- biostimulation involves the stimulation of electron transport in the respiratory chain, which leads to energy accumulation in ATP,
- low-level laser irradiation has few side effects,
- laser biostimulation should be regarded as supplementary rather than the main form of treatment.

Low-energy lasers are widely applied in tissue biostimulation. Research results have shown that LLLT involves non-specific stimulation of tissue regeneration and fibroblast proliferation, and increases the activity of selected metabolic processes at the cellular level. The positive clinical results of laser biostimulation in the treatment of skin diseases have not been sufficiently researched at the level of cellular responses that prevents an objective assessment of the efficacy of this therapeutic method.

Complications in the healing of surgical wounds in animals are frequently encountered in veterinary practice. Most of them result from wound infections contracted during or after surgical treatment or at the first stage of the healing process. In view of previous research investigating the therapeutic value of laser biostimulation (3, 7, 14, 9), therapeutic irradiation was applied to stimulate the regeneration of damaged skin in pigs as a supplementary treatment. Laser irradiation was an alternative to the required surgical treatment involving repeated excision of the wound as a result of skin adhesion complications. A histopathological analysis of cutaneous surgical wounds will verify the results of clinical observations.
Material and Methods

All surgery and treatment procedures in pigs were carried out with the consent of the Local Ethics Committee, in particular in view of the guidelines concerning the minimisation of stress during and after the procedure. The experiment was conducted on 32 farm-raised Polish Large White gilt-piglets weighing approximately 20 kg. The animals were kept under standard laboratory conditions, with free access to feed and water.

Anaesthetics were applied to ensure that the performed procedures were painless. The animals were immobilised with special emphasis on stress reduction, and they were premedicated through intramuscular injection to lower anxiety, minimise pain, and induce muscle relaxation (azaperone, xylazine, and ketamine) before general anaesthesia. The animals were anaesthetised by intravenous infusion of thiopental in fractionated doses. After surgical anaesthesia had been induced, the procedure involved a skin incision in the loin area, followed by the closure of the surgical wound.

The animals were divided into four equal groups. Group I was a control group. Group II comprised pigs, whose skin was subjected to laser biostimulation in the dorsal area. Group III consisted of pigs with cutaneous surgical wounds in the dorsal area, which were subjected to a routine procedure of surgical stitching and antibiotic treatment. Group IV comprised pigs with cutaneous surgical wounds, which were biostimulated with laser irradiation. The laser biostimulation was performed with the use of a CTL 1106 semiconductor laser in the continuous wave mode of operation at a wavelength of 810 nm and a maximum output of 100 mW. The transmission depth of radiation emitted by the laser was around 7 cm. Laser beam irradiation was applied at \( P = 50 \text{ mW} \) and \( E = 8 \text{ J/cm} \) from 1 min. Prior to laser treatment, the skin was shaved and degreased with 70% ethyl alcohol. The procedures were done daily for 21 days.

Since laser light initially causes blood vessels to contract, thus reducing the skin resistance, non-contact irradiation was administered for approximately 2-3 min at the beginning of every procedure. The laser head should be positioned at an adequate distance from the animal’s skin. In the proper part of the biostimulation procedure, the laser head was set perpendicularly to the skin in the dorsal area. The contact, point-irradiation was applied by pressing the wound area with the probe. The skin in the wound area (groups III and IV) was protected against potential bacterial infections by spraying with atomised aluminium. Antibiotic protection was applied two times in pigs of the group III in the form of amoxycilin injections with prolonged activity. After the animals had woken up from the anaesthesia, they were transferred to the post-op room with free access to water and feed. To eliminate unnecessary pain, animals of the groups III and IV were administered noraminophenzone injections two times: 9 and 16 h after the surgical procedure.

During three weeks of observation, cutaneous wounds were clinically examined in each investigated group to determine the rate of granulation tissue formation, the extensiveness, and resilience of the scars formed at the initial stages of the healing process, and, consequently, the effect of laser irradiation on the total time of wound healing. After three weeks, the animals were sacrificed through the administration of an overdose of sodium pentobarbital. Sections of wounded skin were sampled from all animals for a histopathological analysis. Tissue sections were briefly rinsed with buffered phosphate solution (0.1 M, \( \text{pH} = 7.4 \), at 4º C) and transferred to 18% phosphate-buffered saccharose where they were stored until dissection. Skin sections were fixed with 10% neutralised formalin (\( \text{pH} = 7.4 \)) and embedded in paraffin. Microtome sections were stained with haematoxylin and eosin.

Results

The results of the conducted clinical trials indicate that laser biostimulation shortens the time of wound healing by speeding up the growth of granulation tissue and cicatrisation. It improves skin elasticity and has a painkilling effect. The use of laser irradiation shortened the healing rate of skin wounds by about 10 d in the group IV in comparison with the group III, where skin adhesion was obtained without laser after 15 d. Changes in the epidermis and corium were observed in the histopathological sections. Table 1 shows the type and occurrence of these changes in each of the examined group.

The epidermis and corium of the skin from the group I (control) animals had a regular structure. The skin was covered by a thin, corneal layer of the epidermis. The investigated sections also showed regular skin glands and thick strands of collagen fibre. The epidermis of pigs from the group II was thin and comprised stratum germinativum cells stimulated to proliferate and a thin corneal layer flaking off in small scales. In the places treated with laser irradiation, the epidermis was thickened, stratum germinativum cells were stimulated to proliferate, and they formed finger-like cellular processes protruding into the corium. Small clusters of eosinophilic cells, lymphocytes, plasmocytes, neutrophilic leukocytes and - in one pig – fibroblasts, were found around blood vessels and focally under the epidermis. Newly formed fibrous connective tissue with thin strands of collagen fibres was determined around the foci of cellular infiltration. The epidermis of the group III pigs was thickened (Fig. 1), marked by hyperkeratosis and the presence of small intraepidermal cysts and cysts surrounding skin hair sheaths. Cell infiltration, locally abundant, comprised lymphocytes, plasmocytes, histiocytes, eosinophilic cells, and neutrophilic leukocytes. Fibroblasts and newly formed connective tissue (Fig. 2) with signs of cicatrisation in two animals were also observed. The epidermis of the group IV pigs was subject to excessive focal thickening. Stratum germinativum cells were stimulated to proliferate (Fig. 3), and hyperkeratosis of the corneal layer of the epidermis was noted. The infiltration of eosinophilic cells, lymphocytes, and fibroblast, was also observed. The newly formed connective tissue was built of thin collagen fibres undergoing cicatrisation (Fig. 4).
<table>
<thead>
<tr>
<th>Type of changes</th>
<th>Group I n=8</th>
<th>Group II n=8</th>
<th>Group III n=8</th>
<th>Group IV n=8</th>
</tr>
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<tbody>
<tr>
<td>No changes</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Thin epidermis – comprising two-three cellular layers</td>
<td>7</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Thickening of the epidermis – focal proliferation of cells and hyperkeratosis</td>
<td>8</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Excessive scaling of the corneal layer of the epidermis</td>
<td>8</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Proliferation of <em>stratum germinativum</em> cells with the presence of finger-shaped processes in the corium</td>
<td>3</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Intraepidermal cysts with cell acantholysis</td>
<td>8</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Cysts surrounding skin hair sheaths</td>
<td>5</td>
<td>2</td>
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<tr>
<td>Swelling of the papillary layer</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
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<tr>
<td>Infiltration of eosinophilic cells, lymphocytes and plasmocytes</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td></td>
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<tr>
<td>Infiltration of neutrophils and histiocytes</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td></td>
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<tr>
<td>Fibroblast proliferation</td>
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<td>5</td>
<td>4</td>
<td></td>
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<tr>
<td>Presence of newly formed fibrous connective tissue</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td></td>
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<tr>
<td>Cicatrisation of connective tissue</td>
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<td>2</td>
<td>1</td>
<td></td>
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<tr>
<td>Abscesses in the corneal layer of the epidermis</td>
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<td>1</td>
<td></td>
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<tr>
<td>Blood extravasation in the corium</td>
<td>2</td>
<td>1</td>
<td>1</td>
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Fig. 1. Thickening of the epidermis with finger-shaped processes protruding into the corium and the presence of small cysts (arrows) in a pig of group III. HE, 120x

Fig. 2. Proliferation of newly formed connective tissue covered with thickened epidermis in a pig of group III. HE, 120x

Fig. 3. *Stratum germinativum* cells stimulated to proliferate (white arrows) and minor inflammatory cell infiltration around blood vessels (black arrows) in the corium of a pig of group IV HE, 240x

Fig. 4. Newly formed connective tissue undergoing cicatrisation in a cutaneous wound treated with laser in a pig of group IV. HE, 240x
Discussion

The use of laser biostimulation in the treatment of difficult to heal wounds and ulcers offers additional therapeutic possibilities. The number of indications for this type of treatment is on the rise, in particular because laser therapy contraindications and adverse side effects are not observed when the procedure is conducted properly. Cases, which most frequently require laser therapy, involve all types of wounds that heal through the growth of granulation tissue. The above includes post-traumatic wounds with corium defects (post-traumatic wounds) and initially infected wounds that heal through the growth of granulation tissue. The number of laser therapy procedures and their duration are determined by the extensiveness of the wound, the presence of a local infection, and the observed treatment results. Visible improvement is usually reported after 21 d of treatment. The best results are observed when the irradiation treatment takes place on a daily basis.

As noted by many scientific publications, laser light stimulating effect on the regeneration is observed mostly in the connective tissue and in the epithelium. Laser biostimulation speeds up the healing process of wounds, burns, and bone fractures. The main mechanism, which supports the regeneration and accelerates cell proliferation, consists in the intensification of cellular metabolism. The systemic effect of laser therapy results from the immunosuppressive properties of irradiation (8).

According to recent research, low-energy laser irradiation has a positive biostimulating effect on skin wounds in rats. It shortens the inflammatory phase of the healing process, speeds up cell proliferation, and shortens the maturation stage of cutaneous adhesion (4, 11). The findings of other authors confirm that laser therapy of cutaneous wounds speeds up epithelialisation, stimulates fibroblast reactions and leukocyte permeability, and enhances neovascularisation (2). Some authors have noted that even a single laser irradiation procedure speeds up the healing of skin wounds (13). The findings of other researchers (12) have confirmed the Arndt-Schulz rule, which states that the magnitude of the applied stimulus influences its biological effect. The application of laser light with a shorter wavelength and high radiation power had a similar biostimulating effect to the treatment involving laser light with a longer wavelength and low radiation power.

The results of clinical observations of cutaneous surgical wounds in every experimental pig indicate that the time of granulation tissue growth, epidermisation, and complete wound healing was substantially shortened (by around 30%) in the group with biostimulation. This effect exerted by LLLT confirms the hypothesis that photons enhance the efficiency of granulocyte phagocytosis (6). Neoangiogenesis plays a vital role in the process of wound healing. The results of experimental studies (5) have shown that laser irradiation stimulates neoangiogenesis.

The results of this clinical trial confirm that laser irradiation of skin in pigs, increases cell infiltration in the corium, mainly around blood vessels. Laser biostimulation also enhances the proliferation of *stratum germinativum* cells and supports the formation of new connective tissue, which undergoes cicatrization.

The results of the conducted experiment support the conclusion that laser therapy has a biostimulating effect on the skin at the tissue and cellular level, and therefore it may be recommended as supplementary therapy in the treatment of surgical wounds.

References