Noninvasive Assessment of Low Power Laser Radiation Effect on Skin Wound Healing Using Infrared Thermography

M.A. Calin, S.V. Parasca, M.R. Calin, D. Savastru and D. Manea

Abstract**—**The goal of this paper is to examine the effects of laser radiation on the skin wound healing using infrared thermography as non-invasive method for the monitoring of the skin temperature changes during laser treatment. Thirty Wistar rats were used in this study. A skin lesion was performed at the leg on all rats. The animals were exposed to laser radiation (λ = 670 nm, P = 15 mW, DP = 16.31 mW/cm^2) for 600 s. Thermal images of wound were acquired before and after laser irradiation. The results have demonstrated that the tissue temperature decreases from 35.5±0.50°C in the first treatment day to 31.3±0.42°C after the third treatment day. This value is close to the normal value of the skin temperature and indicates the end of the skin repair process. In conclusion, the improvements in the wound healing following exposure to laser radiation have been revealed by infrared thermography.

Keywords**—**skin, wound, laser, thermal image

I. Introduction

WOUNDS are the most common injuries encountered in both humans and animals. Depending on the depth and the possibility of contamination, wounds may produce local or secondary phenomena, local or general, affecting the health status and patient behavior. In the case of simple and uncomplicated wounds, the healing process is conducted in the form of sequence of morphological changes which is named "sanatio per primam intentionem" and usually lasts between 8 and 12 days [1]. Complete healing may occur after 3 weeks. This healing period can be shortened by using the low power laser radiation.

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Effects of low power laser radiations on healing of wounds were demonstrated for the first time, by Mester et al. 1971 [2]. Since then, numerous studies have been focused on understanding and developing therapeutic methods based on low power laser radiations with applications in the treatment of various medical conditions.

The research conducted in this field have demonstrated that low power laser radiation action produces a local increase in regenerative processes [3], a better drainage of edema [4], pain relief [5] and an increase in defense processes [6]. At the cellular level these processes are recognizable by increasing of cell multiplication rate (multiplication of fibroblasts, keratinocytes, macrophages) and increased intracellular metabolic processes (stimulation of ATP synthesis, release of calcium ions from mitochondria, increased DNA and RNA synthesis). At the tissular level, there is a better nutrition of the tissues as a result of multiplication of vascular networks, a better lymph drainage due to increased number of lymphatic networks, installation of the local and general analgesia following endorphin and bradykinin synthesis, intensification of local defense processes by increasing the phagocytosis due to the activity of the macrophages and polymorphonuclears, and triggering B lymphocyte differentiation processes followed by the synthesis of antibodies by plasma cells. It also increases superoxide dismutase and free radicals which promote local bactericidal activity. Local synthesis of collagen, reticulin and elastin are increasing due to increased intake of nutrients which accelerates the regenerative process.

All these effects of low power laser radiation on wound healing have been highlighted by: clinical observations accompanied by thermometry and imaging techniques (acquisition, processing and image analysis), hematological examination (morphology determinations of blood leukocytes, biochemical examination of blood), biochemical examination of urine and histological examination (performed on biopsies from the wound at the end of irradiation).

Another way to investigate and to optimize the application of low power laser radiations on wound healing is infrared thermography.

Infrared imaging permits to map the surface thermal distribution of the body. Skin temperature distribution depends on the heat exchange processes between skin, inner tissue, local vasculature, and metabolic activity. At local level, a disease or a disorder can affect the heat balance or exchange processes resulting in modification of the skin temperature.

Infrared thermography has been widely used as diagnostic method in oncology [7], neurology [8], rheumatology [9], dermatology [10], sports medicine [11], etc. and less as a method of evaluating the effectiveness of medical treatments.

The role of infrared thermography for monitoring of the laser radiation effects on biological tissues was demonstrated by a small number of studies [12,13].

The aim of this paper is to evaluate the effects of low power laser radiation on the healing of rat skin lesions using infrared thermography for the visualization and quantification of the variations of the skin temperature. It is known that the wound from its inception to healing is warmer than its surroundings and we presume that temperature variations induced by wound exposure to low power laser radiation can provide useful information about the healing process.

II. Materials and Methods

A. Animals

In our experiments we used thirty adult Wistar healthy rats (10-12 weeks) weighting between 100-150g. The animals were kept in conditions stipulated by the law 305/2006 referring to European Agreement for protection of animals used in experiments and other scientific purpose (1986) and by the Convention Protocol amend (Strasbourg 1998); Romania adopted this law on the15th of February 2006.

The calf of the right hind leg of the animals was depilated and the shaved area was prepared with iodine alcohol. After performing local anesthesia using 1% xylocaine, a partial thickness wound with an area of approximately 100 mm^2 (a strong clip) was inflicted. This experimental protocol was approved by the Ethics Commission.

The animals, identically quartered and fed, were divided in two groups: experimental group (15 animals) and control group (15 animals).

B. Laser Irradiation

After acclimatization for 10 min, continuous irradiation with a laser system SCL-TR (INOE 2000, Romania) with P = 15 mW and wavelength λ = 670 nm ± 10 nm was applied using the contact method. The spot size on the tissue was 0.92 cm^2 with a power density of 16.31 mW/cm^2 and the time exposure was set at 600 s. Laser treatment was applied for three consecutive days.

C. Infrared Thermography

A noncontact ThermaCamB2 Infrared Camera (FLIR System: Field of view / min focus distance 34° x 25°/0.1m, thermal sensitivity <0.10°C at 25°C and spectral range 7.5 to 13 μm) was used for the temperature recordings. Analysis of the thermal images was performed with the Quick Report v1.0 software which allows measurements over a defined region of interest. The thermal images of the wounds were taken before and after each irradiation session

D. Statistical Analysis

Data are presented as means ±SD. The mean value and its standard deviation were calculated using Microsoft Excel and differences between control group data and the experimental group data were tested statistically with Student's t test. Statistical significance was assumed if $p < 0.05$.

III. Results

The temperature of normal and injured rat skin before laser treatment was 30.5°C respectively 35.2°C as it is shown in Fig. 1.

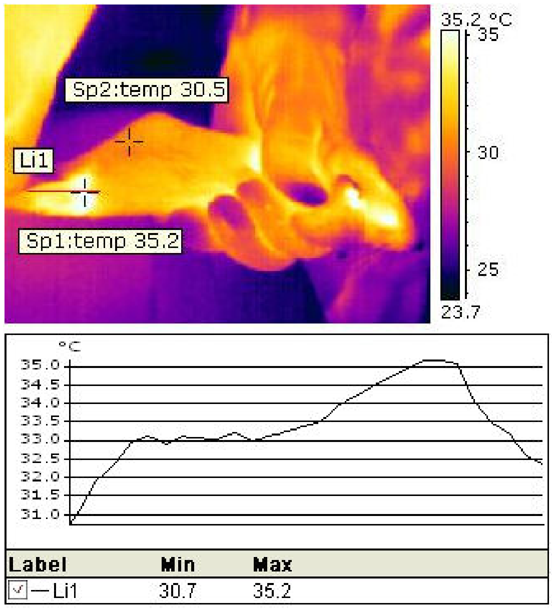

Fig. 1 Thermal images and profile of skin temperature across the line Li1 for animal no. 1 acquired before the laser treatment

The thermal images of wounds acquired before and after each session of irradiation reveal the variation of temperature due to both photochemical and thermal effects induced by laser radiation on biological tissue.

For example, animal 1 (experimental group) showed a decrease of the temperature of the wound from T_{1i} = 35.2°C to T_{1f} = 32.9°C during the irradiation process in the first day. This decrease of temperature appears because the treatment with low power laser radiation could have the effect to reduce the inflammatory process (photochemical effect) and increase evaporation at the surface of the wound (thermal effect).

Next day (24 hours after the first irradiation session) the temperature showed an increasing value from T_{1f} = 32.9°C to T_{2i} = 34.9°C. This increased value of temperature has revealed that the wound still affects the heat balance and a new laser treatment is necessary for the establishing the thermal equilibrium.

After the second irradiation session, the skin temperature has decreased to T_{2f} = 33.5°C and has reached almost normal values after the third treatment (T_{3f} = 32.4°C). This modification of wound skin temperature might be regarded as an enhancement of cell metabolism.

Distinct animals from experimental group exhibit different behavior after three days of treatment. The skin temperature decreases during the first laser radiation exposure from T_{1i} = 35.48±0.52°C to T_{1f} = 33.19 ±0.53°C (Fig. 2.a) and increases in the next 24 hours to T_{2i} = 34.40 ±0.39°C. During the second session of irradiation, the temperature decreases again reaching the value T_{2f} = 33.03 ±0.39°C (Fig. 2.b). After 3 days of treatment the skin temperature became T_{3f} = 31.35 ±0.42°C (Fig. 2.c). This value is near the normal value of the skin temperature and indicates the end of the skin repair process.

Animals from control group exhibit an increase of temperature from the first to the third day of experiment (Fig. 3).

In the first and the third day, the temperature of wound was T_1 = 35.46 ± 0.55°C and T_3 = 36.41 ± 0.48°C respectively. This increased temperature after three days is probably due to local inflamation. Local inflamation is believed to slow the healing process and decreasing the intensity of the inflamatory phase is one of the mechanisms that reduces healing time in wounds exposed to low power laser therapy.

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In the statistical analyses between the experimental and control groups, the following values were obtained: p = 5.11765E-10 after the first day of treatment, p = 2.4149E-9 after the second day of treatment and p = 5.70703E-14 after the third treatment day.

In all these cases the calculated p-value is below the chosen threshold ($p < 0.05$) and the alternative hypothesis is accepted (the difference between the mean temperature of the groups is Proven to be a fact).

IV. DISCUSSION

Low power laser radiations have been used in medicine for over three decades in many medical centers of the world to treat a wide variety of diseases (acute and chronic musculoskeletal aches and pains [14], edema [15], indolent ulcers [16], chronic inflammation [17] and autoimmune diseases [18].

In all these medical applications, clinical observation, imaging techniques, biochemical and histological examination were used for the evaluation of the effects of laser radiation on the biological tissue.

In this paper we have proposed the use of infrared thermography as a useful, rapid and non-invasive way to evaluate the effect of low power laser radiation on wound healing based on the changes in the temperature of the injured area.

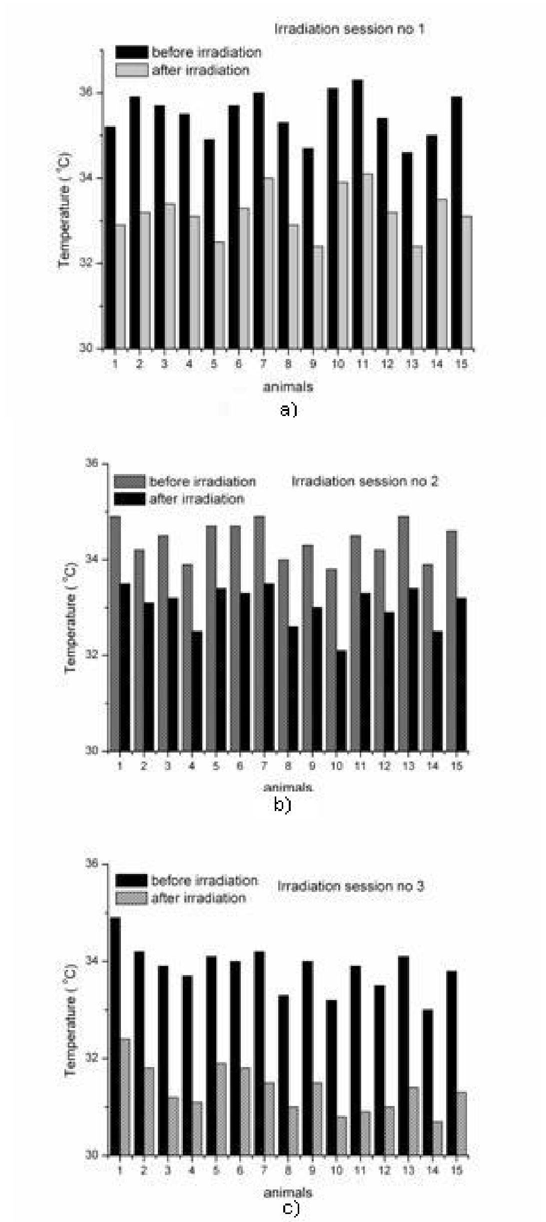

Fig. 2 The modification of skin temperature induced by exposure to low power laser radiation; a) first day; b) second day, c) third day

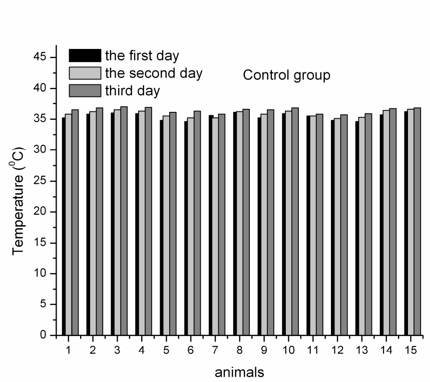

Fig 3 The variation of injured skin temperature of the rats from the control group

Before treatment, the temperature of the normal and injured skin was 30.5±0.2°C and 35.5±0.5°C respectively. In response to laser radiation exposure, the modification of wound temperature gives rise to characteristic pattern during the whole experiment, the common denominator being the fact that tissue temperature decreases from 35.5±0.50°C in the first treatment day to 31.3±0.42°C after the third day. This decrease of skin temperature to normal levels demonstrates the effect of low power laser radiation in accelerating the healing of the skin wound.

These results demonstrate that infrared thermography can be used for monitoring of the laser treatment, but it cannot be compared to other structural methods that give more information about wound healing process.

V. Conclusion

In conclusion, the thermal images have revealed the improvements in the wound healing following exposure to laser radiation.

We can state that use of the infrared thermography allows monitoring of the effects of low power laser radiations on the skin wound healing. Observation of temperature changes during the laser treatment allows selection of optimum parameters of laser work and would permit maximal treatment results with implications on both pacient health and cost of treatment.

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References

[1] G.S. Lazarus, D.M. Cooper, D.R. Knighton, D.J. Margolis, R.E. Pecoraro, G. Rodeheaver, „et al Definitions and guidelines for assessment of wounds and evaluation of healing,” *Arch Dermatol* vol. 130, no. 4, pp. 489-493, 1994.

[2] E. Mester, T. Spiry, B. Szende, J.G. Tota, “Effect of laser rays on wound healing,” *Am J Surg* vol. 122, pp. 532-535, 1971.

[3] D. Hawkins, N. Houreld, H. Abrahamse, “Low Level Laser Therapy (LLLT) as an Effective Therapeutic Modality for Delayed Wound Healing,” *Ann NY Acad Sci* vol. 1056, no.1, pp. 486-493, 2005.

[4] P.C. Lievens , “The effect of combined HeNe and IR laser treatment on the regeneration of the lymphatic system during the process of wound healing,” *Lasers Med Sci* , vol. 6, no 2, pp. 193-199, 1991.

[5] J.T. Hopkins, T.A. McLoda, J.G. Seegmiller, G.D. Baxter, “Low-Level Laser Therapy Facilitates Superficial Wound Healing in Humans: A Triple-Blind, Sham-Controlled Study,” *J Athl Train* vol. 39, no. 3, pp. 223-229, 2004.

[6] W. Posten, D.A. Wrone, J.S. Dover, K.A. Arndt, S. Silapunt, M. Alam, “Low-level laser therapy for wound healing: mechanism and efficacy,” *Dermatol Surg* vol. 31, no.3, pp. 334-340, 2005.

[7] E.Y.K. Ng, “A review of thermography as promising non-invasive detection modality for breast tumor,” *Int J Therm Sci vol.* 48, no.5, pp. 849-859, 2009.

[8] B. Kateb, V. Yamamoto, C. Yu, W. Grundfest, J.P. Gruen, “Infrared thermal imaging: a review of the literature and case report,” *Neuroimage, vol.* 47, 2009.

[9] G. Martini, K.J. Murray, K.J. Howell, J. Harper, D. Atherton, P. Woo, “et al Juvenile-onset localized scleroderma activity detection by infrared thermography,” *Rheumatology* vol 41, pp. 1178-182, 2002.

[10] S.S. Han, C.H. Jung, S.C. Lee, H.J Jung, Y.H Kim, “Does skin temperature difference as measured by infrared thermography within 6 months of acute herpes zoster infection correlate with pain level?” *Skin Research and Technolog,* vol. 16, no. 2, pp.198 – 201, 2010.

[11] C. Hildebrandt, C. Raschner, K. Ammer, “An overview of recent application of medical infrared thermography in sports medicine in Austria” *Sensors* vol. 10, pp. 4700-4715, 2010.

[12] A. Hakgüder, M. Birtane, S. Gürcan, S. Kikino, F.N Turan, “Efficacy of low level laser therapy in myofascial pain syndrome: An algometric and thermographic evaluation,” *Lasers Surg Med vol.* 33, no. 5, pp. 339 – 343, 2003.

[13] H. Fikácková, T. Dostálová, R. Vosická, V. Peterová, L. Navrátil, J. Lesák, „Arthralgia of the temporomandibular joint and low-level laser therapy,” *Photomed Laser Surg* vol. 24, no. 4, pp. 522-527, 2006.

[14] J. M. Bjordal, C. Couppè, R. Chow, J. Tunèr, A.E. Ljunggren, „A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders” *Aust J Physiother*, vol. 49, no. 2, pp. 107-116, 2003.

[15] A. Stergioulas, “Low –level laser treatment can reduce edema in second degree ankle sprains,” *J Clin Laser Med Surg* vol. 22, no. 2, pp. 125-128, 2003.

[16] A. Schindl, M. Schindl, H. Pernerstorfer-Schön, U. Mossbacher, L.Schindl, ”Low intensity laser irradiation in the treatment of recalcitrant radiation ulcers in patients with breast cancer – long-term results of 3 cases,” *Photoderm Photoimmun Photomed* vol. 16, no. 1, pp. 34-37, 2000.

[17] D.M. Ferreira, R.A. Zangaro, A.B. Villaverde, Y. Cury, L. Frigo, G. Picolo, “et al Analgesic effect of He - Ne (632.8 nm) low - level laser therapy on acute inflammatory pain,” *Photomed Laser Surg* vol. 23, no. 2, pp. 177-188, 2005.

[18] R.G. Oliveira, F.M. Aarestrup, C. Miranda, B.J. Vieira, A.P. Ferreira, L.C. Andrade, “Low-Level Laser Therapy Reduces Delayed Hypersensitivity Reaction to Ovalbumin in Balb/C Mice,” *Photomed Laser Surg* vol. 28, no. 6, pp. 773-777, 2010.