Low-Level Laser Therapy is More Effective Than Pulse Ultrasound Treatment on Wound Healing: Comperative Experimental Study

Düşük Enerji Seviyeli Laser Tedavisi Yara İyileşmesinde Kesikli Ultrason Tedavisinden Daha Etkindir: Karşılaştırmalı Deneysel Çalışma

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ABSTRACT Objective: To investigate and compare the effects of pulse ultrasound (US) and low-level laser therapy (LLLT) on wound healing. **Material and Methods:** Thirty-two rats were included in the study and two full-thickness skin wounds were made on dorsum area of the rats bilaterally, with a 17 mm hole-punch. The animals were divided into two groups. Pulsed US (with a power of 0.1 W/cm^2, a frequency of 1 MHz, 5 minutes daily) was applied to to the right sided wounds of Group A (n= 16) and Ga-As laser (830 nm wavelength, 0.5 J/ cm^2 dosage of 1 MHz frequency for 1 minute duration) was applied to right sided wounds of Group B (n= 16). Left sided wound were considered as controls and same procedures were applied without any current (sham). Biochemical and histopathological evaluations were performed in each group on 7th and 15th days. **Results:** Inflammatory cells tended to decrease in both treatment groups on the 7th day, however, this finding did not reach a statistical significance (p> 0.05). Fibroblasts and collagen were found to be significantly increased in the laser group when compared to the other group on the 7th day (p< 0.05). Angiogenesis was found to be significantly increased only in the laser group when compared to the other group on the 15th day (p< 0.05). There were no significant differences in tissue nitric oxide values between the groups although the values in the laser group tended to be higher on the 15th day (p=0.058) **Conclusion:** In this comparative study, LLLT was found to significantly accelerate mainly proliferative phase while pulse US had no effect on wound healing. Our results support the consideration that LLLT may constitute a beneficial treatment modality for wound healing.

Key Words: Laser therapy, low-level; ultrasonography; wound healing

ÖZET Amaç: Yara iyileşmesinde düşük enerjili lazer tedavisi (DELT) ve kesikli ultrason (US) tedavilerinin etkinliklerini incelemek ve karşılaştırmak. **Gereç ve Yöntemler:** Çalışmaya 32 rat dahil edildi ve deneklerin dorsal bölgesinde bilateral olarak tüm deri tabakalarını kapsayan 17 mm çapında iki yara oluşturuldu. İki gruba ayrılan deneklerin ilk grubunun (Grup A, n= 16) sağ taraftaki yaralarına kesikli US (1 MHz başlık ile 0.1 W/cm^2 şiddetinde, 5 dakika süreyle) uygulanırken ikinci gruba (Grup B, n= 16) DELT (830 nm dalga boyunda 0.5 j/cm^2 dozunda %50 şiddetinde, 1 dakika süreyle) uygulandı. Sol taraftaki yaralar kontrol olarak alındı ve tedaviler aynı şekillerde ancak ışın verilmeksizin uygulandı (sham). Yedinci ve 15. günlerde sakrifiye edilen deneklerde biyokimyasal ve histopatolojik değerlendirmeler yapıldı. **Bulgular:** Inflamatuvar hücrelerin yedinci günde her iki tedavi grubunda da azalmış olduğu, ancak istatiksel anlamlılığa ulaşmadığı gözlendi (p> 0.05). Bu dönemde yapılan histolojik incelemelerde fibroblast ve kollajen miktarının lazer grubunda, kontrol ve kesikli US tedavi grubuna göre anlamlı düzeylerde artmış olduğu gözlendi (p< 0.05). Onbeşinci günde yapılan histolojik incelemelerde ise yeni damar oluşumunun lazer grubunda diğer gruplara göre anlamlı düzeylerde yüksek olduğu saptandı (p< 0.05). Tüm gruplar arasında özellikle doku nitrik oksit değerleri açısından farklılık gözlenmedi ancak lazer grubunda 15. günde yükselmeye meyil olduğu gözlendi (p= 0.058). **Sonuç:** Bu karşılaştırmalı çalışmada, lazer tedavisinin yara iyileşmesinin proliferatif fazını belirgin olarak hızlandırdığı, bununla birlikte kesikli US tedavisinin yara iyileşmesi üzerine anlamlı etkisi olmadığı gözlenmiştir. Çalışmamızın sonuçları, yara iyileşmesinde lazer uygulamasının faydalı bir tedavi yöntemi olduğunu ileri süren görüşleri desteklemektedir.

Anahtar Kelimeler: Lazer terapisi, düşük-seviyeli; ultrasonografi; yara iyileşmesi

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Wound healing constitutes one of the major problems in treating the pressure or venous ulcers, and problems related with wound healing still play important roles for the increased morbidity in rehabilitation medicine.[1] Despite advances in understanding cellular and biochemical properties of the wound healing in the last few decades, wound care is still a challenging issue for patients and health professionals. In addition to conventional wound care methods, several physical modalities were introduced into the clinical practice to improve wound healing. In the last 30 years, a great number of studies have been conducted on the acceleration of wound healing with several physical modalities. Amongst them, therapeutic ultrasound (US) and low-level energy laser therapy (LLLT) are the two treatment modalities which are still under evaluation.

The beneficial effect of US treatment on wound healing was initially supposed by Paul et al., and then was supported by some others.[2-4] It is generally accepted that US affects the early proliferative phase of the wound healing.[5] In vitro studies and animal models have been demonstrated that ultrasound decreases inflammation, accelerates fibrinolysis and angiogenesis, increases fibroblasts and matrix synthesis and stimulates macrophage-derived fibroblast mitogenic factors.[6] Such findings form the basis for the use of ultrasound to promote and accelerate tissue healing. However, the literature contains many studies that could not be able to demonstrate any beneficial effect of US treatment on wound healing.[7-9]

LLLT was firstly introduced as a therapeutic modality in chronic soft tissue ulcers by Mester et al.[10] During the past 40 years, LLLT has been frequently used in clinical practice. However, at the present there is no general agreement on how exactly the low- energy laser influences the process of wound healing. Although the main mechanism of action of lasers on the skin is mediated by photothermal effects, LLLT typically causes low temperature changes. Although temperature does not increase noticeably, LLLT may induce biological consequences.[11] The stimulatory effects of low-level laser irradiation at the cellular level have been shown in many studies. It was found to increase the activity of cytochrome oxidase and adenosine triphosphatase, DNA synthesis, collagen and procollagen production, and cell proliferation.[12] Although several studies have investigated its therapeutic benefit on pressure ulcers, the clinical use of LLLT for wound healing is still under investigation because many investigators report conflicting findings in animal and human studies.[13-21]

In the present literature, the comparative data of both treatment modalities concerning wound healing is very limited.[22] Moreover, to the best of our knowledge, there is no data that compare the two treatment modalities in excisional wound healing in terms of both histopathologic and biochemical changes. So that, the present study was designed firstly to investigate and secondly to compare the individual effects of US and LLLT on the wound healing process in terms of microscopic and histo-pathological evaluation on the 7th and 15th days. Because nitric oxide (NO) has been proposed as a possible active agent for accelerating wound healing, measurements of tissue NO production were also taken into consideration in order to evaluate the biochemical changes in the cellular level.[23,24]

MATERIAL AND METHODS

Thirty two healthy, adult, male Winstar rats weighing between 250-300 g were included in the study. The animals were housed in a temperature and light controlled environment separately with ad libitum access to water and rat pellet. Experimental procedures were approved by our Institute's Ethical Committee, and manuplations of the rats were performed under the rules of the Institutional animal ethics committee of University of Celal Bayar, complying with European Community Council Directive (86/609/EEC).

SURGICAL PROCEDURE

Surgical procedures were performed under general anesthesia administered by intramuscular injection of ketamine hydrochloride (25 mg/kg) and xylasine hydrochloride (5 mg/kg). The animals were closely shaved on the dorsum. Two 17-mm diame-

ter full thickness skin defects were created on the left and right side of the dorsal midline in each rat. A lancet and a custom designed 17-mm diametered circular surgical blade were used to produce equal sized skin defects. Wounds were photographed immediately after the surgical procedures and left open without any dressing.

TREATMENT GROUPS AND PROCEDURES

The animals were randomly divided into two treatment groups: Group A (n= 16) for pulsed US and Group B (n= 16) for LLLT. Treatments were initiated two hours after the surgical procedure and were performed daily, until the study ended. Endolaser 476 (Enraf-Nonius Co.,The Netherlands) laser device and Sonopuls 434 (Enraf-Nonius Co., The Netherlands) ultrasound device were used for therapeutic applications.

Right sided wounds of the Group A were treated with pulsed ultrasound under general anesthesia to maintain immobilization of the animal. Wounds were filled with physiologic saline until the skin level and covered with a sterile surgical drape. An aquasonic gel was applied above the drape as a coupling agent. Pulsed ultrasound (2 ms on, 8 ms off, and 0.1 W/cm^2 intensity) treatment was performed using an 8-mm diameter, 1 MHz frequency probe with circular movements for five minutes daily. The same procedure was performed to the left-sided wounds using a transducer without any ultrasound output to serve as the control (Sham method).

Right-sided wounds of the Group B were treated with low-energy laser irradiation using a continuous Gallium-Arsenide-Aluminum (Ga-As-Al) laser beam (830 nm) with an energy output of 30 mW at 50% level. In each treatment session, a dosage of 0.5 J was applied for 1 cm^2 of wound surface. Laser probe was hold perpendicularly 5 mm above the wound surface. Laser irradiation was applied to the center of the wound for 33 seconds and to its periphery for 33 seconds with slow circular movements. The laser indicator light without any energy output was used to apply the same procedure to the left-sided wound to serve as the control (Sham method).

Each group was then divided into two subgroups with respect to the treatment period. Group A1(n= 8) and Group B1 (n= 8) received seven days of treatment. Group A2 (n= 8) and Group B2 (n= 8) received 14 days of treatment. All animals were sacrificed under general anesthesia with a blow to their heads. All wounds were photographed immediately after this procedure to assess the changes in the wound size.

HISTOPATHOLOGICAL AND BIOCHEMICAL ANALYSIS

The ulcer areas of the animals in all of the four groups were excised with surrounding normal skin. All samples were fixed in 10% formaline and embedded in paraffin. Five-micrometer thick sections were stained with hematoxylin and eosin and Masson's trichrome. Immunohistochemically, CD34 staining (clone QBEnd/10; diluted 1:100; Neomarkers, MS-363-P1, Lot:363P603B) was performed in each sample. The thickness of the fibrosis, polymorphonuclear leukocyte (PMNL) infiltration and chronic inflammatory cells, such as macrophages and eosinophils at the base of the wound were examined microscopically. Fibroblastic activity and collagen density were evaluated on Masson's trichrome stained slides. Capillary vessel proliferation (angiogenesis) was evaluated on CD34 stained slides by counting the capillary vessels at base of the ulcerated area.

Fibroblastic activity, density of chronic inflammatory cells and polymorphonuclear leukocytes were classified into four as minimal, mild, moderate and pronounced. Diameter of the wound and fibrosis at the base of the wound were measured at x100 magnification of the light microscope (Olympus E300). The mean capillary vessel number was determined by counting all capillary vessels on consecutive five high power areas of CD34 stained slides.

WOUND TISSUE NITRIC OXIDE ASSESSMENT

Biochemical assessments of stable NO oxidative metabolites, nitrite (NO2) and nitrate (NO3) levels, were analyzed to estimate the tissue NO production. Assessment of tissue nitrite and nitrate levels was based on Griess method which was previously

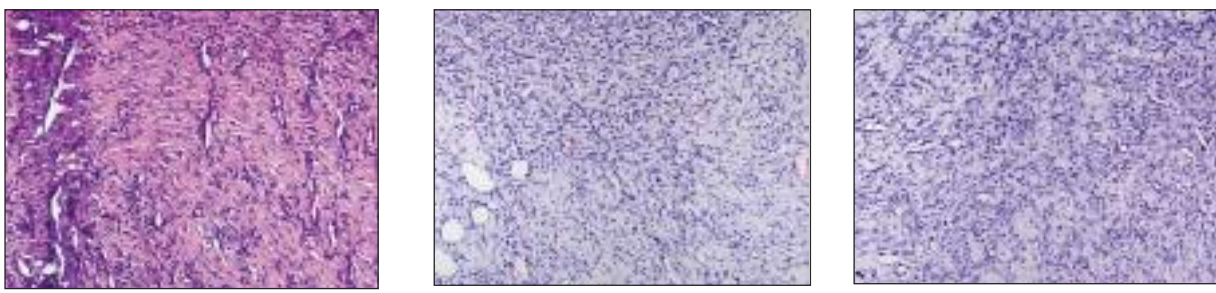
described elsewhere.[25] Samples were homogenized in ice-cold 50mMol/L Tris HCl buffer with pH 7.4. Samples were deproteinized with the Somogy reagent, and the supernatants were used. An aliquot of samples was taken for the nitrite assessment which was produced by diazotization of sulfanilamide and coupled to naphthylethylene diamine. Another aliquot of samples was taken for total nitrite and nitrate assessments which were reduced by Cu-coated Cadmium granules in glycine buffer at pH 9.7 and further produced by diazotization of sulfanilamide and coupled to naphthylethylene diamine. The nitrate level was determined as the difference between the two aliquots. The final absorbance of the reaction was measured at 545 nm with a spectrophotometer. A standard curve was obtained by concentrations of 2-10 micromol/L sodium nitrate. Data in this study presents the sum of nitrite and nitrate which are the NO metabolites and are expressed in micromol/gr wet tissue.

Statistical analysis

Data were analyzed using SPSS for Windows, version 10.0. Mann-Whitney U test was used for the analytic assessments. The difference was considered as statistically significant when the two-tailed p-value was less than 0.05.

RESULTS

No significant difference was found between the control groups in terms of histopathologic and biochemical data ($p > 0.05$). Biopsy specimens revealed that PMNL and chronic inflammatory cells tended to decrease in both treated sides compared to control sides, however, the difference was not significant on the 7th day ($p > 0.05$). Fibroblasts and collagen were found to be significantly increased in Laser treated group compared to its control group on the 7th day ($p = 0.015$ and $p = 0.02$, respectively), while no significant change was observed in US treated group (Figure 1) (Table 1). This signifi-



A B C

FIGURE 1: Fibroblastic proliferation in control (A), ultrasound (B), and laser groups (C) on the 8th day. Laser treatment (C) showed a significantly increased fibroblast and collagen proliferation when compared to control and ultrasound groups (HE,x100).

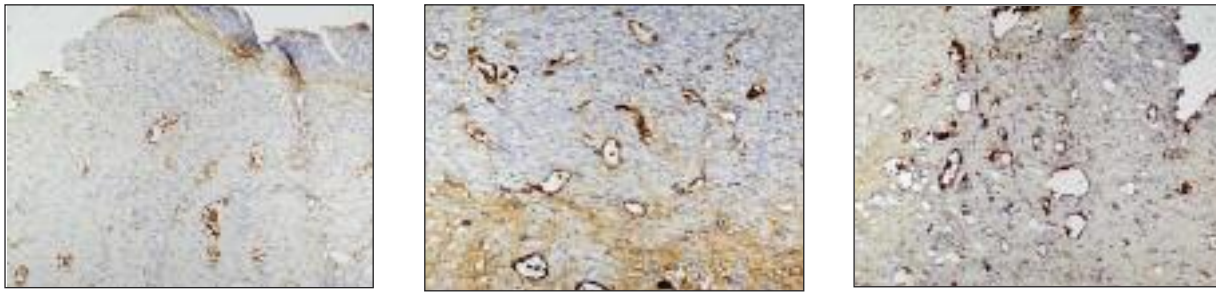
TABLE 1: Analysis of histopathological and biochemical results on 7th day.

Parameters	Ultrasound treatment (Group A1) n= 8	Ultrasound control (Group A1, Sham) n= 8	P	Laser treatment (Group B1) n= 8	Laser control (GrupB1, Sham) n= 8	P
PMNL*	2.6 ± 0.8	2.9 ± 0.9	0.58	3.3 ± 0.5	3.9 ± 0.9	0.2
Chronic inflammatory cells*	2.4 ± 0.5	2.7 ± 0.6	0.63	2.5 ± 0.5	2.8 ± 0.7	0.37
Fibroblasts*	1.6 ± 0.5	1.5 ± 0.5	0.91	2.3 ± 0.5	1.5 ± 0.4	0.015
Collagen*	2.1 ± 0.6	2.0 ± 0.6	0.67	2.8 ± 0.6	2.0 ± 0.5	0.02
Angiogenesis £	62.6 ± 11.6	53.8 ± 13.8	0.43	63.5 ± 13.15	61.8 ± 10.3	0.48
Nitric oxide (umol/mg)	0.071 ± 0.045	0.066 ± 0.03	0.12	0.058 ± 0.032	0.052 ± 0.038	0.75

PMNL; Polymorphonuclear leukocytes

£ mean capillary vessel number

'Average value of the cells calculated in microscopic area as; 1=minimal, 2=mild, 3=moderate, 4=pronounced



A B C

FIGURE 2: Angiogenesis in control (A), ultrasound (B) and laser groups (C) on the 15th day. Laser treatment (C) showed a significant increase in angiogenesis compared to control and ultrasound groups (CD34, x100).

TABLE 2: Analysis of histopathological and biochemical results on 15th day.

Parameters	Ultrasound treatment (Group A2) n= 8	Ultrasound control (Group A2, Sham) n= 8	P	Laser treatment (Group B2) n= 8	Laser control (Grup B2, Sham) n= 8	P
PMNL*	2.3 ± 0.6	1.8 ± 0.5	0.36	3.0 ± 0.5	2.8 ± 0.9	0.42
Chronic inflammatory cells*	2.0 ± 0.5	1.8 ± 0.6	0.62	2.4 ± 1.1	1.9 ± 0.8	0.58
Fibroblasts*	1.1 ± 0.4	1.3 ± 0.5	0.7	1.6 ± 0.5	1.5 ± 0.5	0.92
Collagen*	2.4 ± 0.5	2.9 ± 0.4	0.18	2.1 ± 0.5	2.0 ± 0.5	0.95
Angiogenesis £	58.1 ± 8.9	56.1 ± 7.9	0.78	73.8 ± 7.5	58.4 ± 5.7	0.003
Nitric oxide (μmol/mg)	0.089 ± 0.047	0.098 ± 0.06	0.61	0.12 ± 0.07	0.082 ± 0.06	0.058

Abbreviations are given in Table 1

cance was lost on the 15th day. Angiogenesis was similar on the 7th day in both treatment groups. However, a significant increase was observed on the 15th day in the laser treated group when compared to its controls (p= 0.003), while there was no significant change in the US treated group (Figure 2) (Table 2). Comparison of histopathologic and biochemical results of US and LLLT groups revealed that laser treatment had a significant effect on fibroblast and collagen levels on the 7th day when compared to US group (p= 0.029 and p= 0.013, respectively) (Table 3). On the 15th day, angiogenesis was found to be significantly more pronounced in the laser treated group when compared to US group (p=0.018) (Table 3). There were no significant differences in tissue nitric oxide levels between the groups although the values in the laser group tended to be higher (with 10% significancy)on the 15th day (p= 0.058).

TABLE 3: Comparison of histopathological and biochemical results of both treatment groups on 7th and 15th days.

Parameters	7th day Ultrasound treatment n= 8	7th day Laser treatment n= 8	P	15th day Ultrasound treatment n= 8	15th day Laser treatment n= 8	P
PMNL*	2.6 ± 0.4	3.3 ± 0.5	0.49	2.3 ± 0.6	3.0 ± 0.5	0.12
Chronic inflammatory cells*	2.4 ± 0.5	2.5 ± 0.5	0.92	2.0 ± 0.5	2.4 ± 1.1	0.39
Fibroblasts*	1.6 ± 0.5	2.3 ± 0.5	0.029	1.1 ± 0.4	1.6 ± 0.5	0.12
Collagen*	2.1 ± 0.6	2.8 ± 0.6	0.013	2.4 ± 0.5	2.1 ± 0.5	0.34
Angiogenesis £	62.6 ± 11.6	63.5 ± 13.15	0.91	58.1 ± 8.9	73.8 ± 7.5	0.018
Nitric oxide (μmol/mg)	0.071 ± 0.045	0.058 ± 0.032	0.12	0.089 ± 0.03	0.12 ± 0.07	0.29

Abbreviations were given in Table 1.

DISCUSSION

Difficulties of wound healing in immobilized patients or in patients with spinal cord injuries still constitute a significant problem in rehabilitation medicine, and lengthening of the wound healing period may cause an increase in morbidity and mortality.[1] For this reason, in the last 40 years interests were shifted to discover any therapeutic modality that may accelerate the wound healing and may help to shorten the treatment period. Among these, pulsed US is still under investigation. It has been previously suggested that US decreased inflammation, increased fibroblasts and collagen and matrix synthesis, accelerated angiogenesis, and increased tissue tensile strength.[6] These beneficial effects form the basis for the use of US to accelerate wound repair and healing. However, today there is no consensus for the use of US for wound healing. Although some investigators have shown that it was beneficial,[3,4] some others were failed to prove any acceleration on wound repair.[7-9] Probably, differences in study designs, as well as different length and dosage of applications are the main causes for these differences.[6] In animal studies, US application with an intensity of 0.1-0.5 W/cm^2 with a frequency of 1-3 MHz was shown to accelerate the inflammatory phase of repair.[3,26] For this reason, in the present study a similar treatment protocol was used (0.1 W/cm^2 with a frequency of 1 MHz, 5 minutes daily).

Since the rat skin contains three layers, it is frequently used to investigate the phases of wound healing: inflammation, proliferation and maturation. Although it not always easy to strictly separate these three phases from each other, the inflammatory phase mostly takes part during the first five days, however it was shown continue for 15 days.[27,28] The proliferatory phase generally occurs on 7th to 15th days and then the maturation phase takes place.[27,28] Our experimental model consisted of a circular wound inflicted bilaterally on the dorsum of the rats. When someone takes into consideration that pressure ulcers are generally circular rather than linear, it appears that our experimental model is more appropriate to investigate and to adapt the effects of different therapeutic modalities to clinical practice. Unlike the incisional wound model where wound limbs are close to each other to improve wound healing, excisional wounds may need more time to improve. From this point of view, we mainly focused on the late parts of the first two phases.

In the view of the histological changes, a decrease in PMNL and chronic inflammatory cells, and an increase in fibroblasts, collagen and angiogenesis on 7th and 15th days were found in US group when compared to their controls, however these beneficial effects could not reach to a statistical significance. As a result, we could not demonstrate any statistically significant beneficial anti-inflammatory or proliferatory effect in US group although there was a tendency in acceleration of wound healing. Many investigators agree that application of US has beneficial effects on the inflammatory phase of repair and this application can affect the onset of proliferative phase.[3,26] However, our findings and many others did not support this idea.[29,30] Briefly, our result concerning the lack of efficacy of US treatment on wound healing is in agreement with the others previously documented similar negative results[7-9] On the other hand, it must be kept in mind that we might have been missed the anti-inflammatory effect that had been taken place in the first 7 days.

Low-level laser therapy was firstly introduced by Mester et al. as a therapeutic modality in wound healing.[10] During the last 40 years, its efficacy in wound repair was investigated and supported with many animal[13-17] and human studies.[20,21] Today, in many countries except United States, LLLT is widely used for the treatment of a variety conditions including neurologic, dental and dermatologic disorders.[11] Due to the existence of some conflicting data for its efficacy in wound healing in animal[18,19,29] and human studies[20,21] there is still no consensus for the routine use of this therapeutic modality worldwide.

Laser parameters such as wavelength, time of exposure, and dosage used in clinical and in vivo studies to study the effect of LLLT on wound healing have not been standardized yet.[11] So, it is dif-

ficult to compare the results of different experiments that used different laser parameters. Most LLLT research studies, probably owing to cost or availability issues, have used helium neon (HeNe) lasers, but most of the newer studies are proffering the newer Gallium-Arsenide-Aluminum (Ga-As-Al) laser. A wide variation exists in recommendations for the optimal energy for different conditions; the usual ranges are from 0.1 to 10 J/cm^2.[31] A maximal increase in DNA synthesis was found at a dose of 0.5 J/cm^2 and maximal regeneration was observed with the use of a wavelength of 802 nm.[31] Therefore, in the present study, we used Ga As laser with a wavelength of 830 nm and at 0.5 J/cm^2.

Because fibroblasts play an important role in wound healing, effect of LLLT on fibroblasts is important. A significant increase in fibroblast-mediated procollagen production by LLLT was shown in many previous animal studies.[13,14,33] In a recent study, Gal et al. have shown that laser stimulation shortened the inflammatory phase and accelerated the proliferation and maturation phases.[27] These investigators also observed that LLLT positively stimulated the regeneration of injured epidermis and repairment of injured muscle and concluded that LLLT positively influences all phases of rat skin healing. In the present study, laser application significantly increased fibroblast and collagen composition on the 7th day. In a similar experimental study, Bisht et at. have shown that fibroblastic proliferation and collagen production was detected on the 5th day and was at maximum on the 9th day.[13] Similarly, in many other published papers this beneficial effect was observed between the 5th and 10th days.[22,27] Our finding is in agreement with Bisht et al. and others. In this period, angiogenesis was found to be slightly higher in laser group.[13]

Angiogenesis is one of the major factors related to tissue repair; it is involved from the beginning of the healing process since the vessels are responsible for re-establishing the supply of oxygen and nutrients, allowing an increase in metabolic rate and mitotic activity. Most of the recent studies on laser-induced angiogenesis have been directly or indirectly motivated by the seminal paper of Abergel et al.,[14] which indicated an angiogenic effect was induced by LLLT. In a very recent study, Corazza et al. showed that LLLT with doses of 5-20 j/cm^2 demonstrated expressive results in angiogenesis on 3rd, 7th and 14th days.[34] In our study, the significant increase in angiogenesis was observed on the 15th day. This delay may be due to lower laser dosage that was used in our study. In another recent study, transforming growth factor (TGF-B) was shown to play a central role in mediating the accelerated healing response.[35] In this study TGF-B expression was shown to be maximum on 14th day. Although the experimental model was different in this study, their finding may help to explain the significant increase in angiogenesis on the 14th day in our experiment.

Accumulating evidence indicates that NO plays an important role in wound healing and NO was shown to be directly correlated with collagen and fibroblast deposition and an increase in an-giogenesis.[36] In the present study, we also investigated cutaneous NO levels to during the 7th and 15th day of wound healing to clarify the supposed interaction. In both treatment groups NO levels were similar on the 7th day and were not different from the controls. However, the cutaneous NO level tended to increase in laser group on the 15th day in which angiogenesis was found to be significantly increased. This finding is in agreement with the consideration that NO plays a central role in angiogenesis.[24,37] In the present study, although the stable NO oxidative metabolites such as nitrite (NO2) and nitrate (NO3) levels were measured, it must be kept in mind that short half-life of the molecule may limit measuring the cellular levels of NO during wound healing, and this limitation constitutes an obvious problem in experimental studies.[36]

Many studies have assessed the individual effects of US and LLLT on wound repair, however, they have been rarely compared. In a literature search, we found only one research comparing the effects of US and LLLT on experimental wound healing.[22] In that study, Demir et al. used an incisional wound model in which the 4th and 10th days were taken in conside- ration to investigate the histopathological changes. Angiogenesis was not evaluated in their study. They concluded that both US

and LLLT had beneficial effects on both phases of wound repair, while LLLT was more effective than US in inflammation and proliferation phases. Our result regarding the effects of US on wound healing does not support their findings, because we could not demonstrate any significant effect with the use of US as many others.[79] However their conclusion for the effect of LLLT on wound repair is similar to our findings. The reasons for the different results may arise from the differences of the study designs. Firstly, we worked on an excisional (circular) wound model which was more similar to clinical pressure ulcers while their model was incisional. Secondly, we investigated the histopathological changes on the 7th and the 15th days while they were interested in the 4th and the 10th days. Thirdly, our treatment protocol included pulsed US with a dose of 0.1 W/cm^2 at a frequency of 1 MHz while their dose was 0.5 W/cm^2 at a frequency 1 MHz, and we applied GaAs laser with a wavelength of 830 nm and with power of 0.5J/cm^2, while their wavelength and power was 904 and 0.5J/cm^2, respectively.

In conclusion, in this comparative study, LLLT was found to accelerate significantly the proliferative phase while US had no effect on wound healing. Our results support the consideration that LLLT may constitute a beneficial treatment modality for treating wound healing.

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