Application of low level laser technologies for pain relief and wound healing: overview of scientific bases

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Background: Laser irradiation is characterized by several physical parameters, such as wavelength, spot size, power, power density, energy, energy density, duration of irradiation, frequency of irradiation, and interval between irradiations. The lack of definitive evidence for the relevance of manipulation of these parameters to biological and clinical effects, combined with conflicting results from previous studies has led to uncertainties regarding the scientific rationale for the clinical application of laser technologies in physiotherapy.

Objective: The purpose of this review was to provide evidence of the scientific bases by examining the effects in humans and animals of two clinical applications of low-level laser technologies.

Methods: A systematic review was completed of original research papers investigating the effects in humans and common laboratory animals of laser acupuncture for pain relief and laser-stimulated wound healing. Relevant papers were primarily sourced from PubMed using EndNote X1, and from secondary searches. Search terms were ‘laser acupuncture’, ‘laser therapy’, ‘wound healing’, ‘pain relief’, ‘inflammation’, ‘blood flow’, ‘endorphin production’, ‘enkephalin production’, ‘tissue regeneration’, ‘growth factor’ and ‘cytokine’.

Results: A total of 31 relevant papers were included in the review which comprised 15 human studies and 16 animal studies (mostly in the rat). Taken overall, results consistently demonstrated the potential of laser irradiation to reduce pain and inflammation, improve blood flow, and stimulate wound repair.

Conclusions: The consensus from the included studies on laser acupuncture and laser-stimulated wound healing is positive in terms of supporting the rationale for application of laser to decrease pain and inflammation, improve blood flow, and increase tissue regeneration.

Keywords: Low level laser, Pain relief, Wound healing, Scientific bases

Introduction

Low-level laser therapy (LLLT) is used clinically for a wide range of conditions. Applications include pain therapy, wound repair, increasing skin flap viability, leg ulcers in diabetic patients, peripheral nerve injury, supplicative diseases and scars of skin, exercise-induced skeletal muscle fatigue, and skeletal muscle recovery. These applications are informed by the literature pertaining to experimental animal and human studies that have indicated several main biological effects of laser light.

The main effects of laser light which provide a scientific rationale for its clinical use in laser therapy are:

(i) Pain relief. Laser therapy has been used to provide pain relief from the symptoms of chronic arthritis, tendonitis, carpal tunnel syndrome, fibromyalgia, knee injuries, shoulder pain, and symptoms relating to nerve injuries (sharp pain, paraesthesia). One of the mechanisms for pain relief is the reduction of inflammation and swelling. A form of laser therapy, known as laser acupuncture, is also used for the treatment of musculoskeletal pain. The application of laser light to specific acupuncture points stimulates the release of endorphins and enkephalins which are natural pain-relieving chemicals.¹²

(ii) Reduction of inflammation. Laser therapy can modulate acute inflammation by causing a reduction in the levels of pro-inflammatory cytokines such as interleukin-1 alpha (IL-1 alpha), interleukin-1 beta (IL-1 beta), tumour necrosis factor-alpha (TNF-alpha), and also an increase in the levels of anti-inflammatory growth factors and cytokines such as basic fibroblast growth factor, platelet-derived growth factor, transforming growth factor-beta
(TGF-beta). Both red and infrared laser light have been shown to be effective in this regard.3–6 In addition, laser irradiation causes dilatation of blood vessels, which also leads to a reduction in swelling caused by inflammation.

(iii) Increase in blood flow to tissues. Laser light increases the proliferation of endothelial cells, and the formation of new blood capillaries within damaged tissues.7 This is particularly important in enhancing wound healing, and also in improving the blood supply to skin flaps used for covering areas of extensive injury in reconstructive surgery.8 Uptregulation of inducible nitric oxide synthase, leading to increased production of nitric oxide, has been shown to be brought about by pulsed far infra-red laser irradiation.9 The vascular actions of nitric oxide (known formerly as endothelial-derived relaxing factor) include vasodilatation, anti-thrombotic effect, anti-inflammatory effect, and inhibition of smooth muscle hyperplasia.

(iv) Stimulation of wound healing. Irradiation with red or infrared laser light has been shown to stimulate wound healing.10 The mechanisms involve stimulating the expression and release of certain growth factors and cytokines from the cells that have invaded the wound (including fibroblasts, macrophages, lymphocytes, endothelial progenitor cells).11 These biological mediators bring about a proliferation of specific cell types within the wound or at the wound margins, and orchestrate the various stages of wound healing, resulting in accelerated reepithelialization, and filling of the wound defect by granulation tissue and collagen. Laser light also increases the vascularity of the regenerating tissue, resulting in more blood being brought to the injury site and an increased rate of healing.

(v) Stimulation of tissue regeneration. It is well established from in vitro and in vivo studies that laser light can increase the proliferation of specific cell types and stimulate the production of growth factors and cytokines involved in healing and tissue regeneration. Recent studies have indicated that laser irradiation can be used to treat spinal cord injuries.12

(vi) Reduced scarring and control of suppurative diseases of skin. Laser light stimulation of the healing process, improving blood flow to the injured area and more efficiently carrying away waste products, is associated with a reduction in scar tissue formation. Laser light has been used for treating and preventing the reoccurrence of hidradenitis suppurativa lesions.13 Also in a study of patients with dissecting cellulitis, a disorder analogous to hidradenitis suppurativa, far infra-red laser treatment resulted in a reduced reliance on systemic treatments, and a controlled or terminated disease process.14

To provide further insight and support for the main biological effects of laser light, a systematic literature search was conducted for laser acupuncture and laser-stimulated wound healing, which represent two of the most important clinical applications of low power laser technology in physiotherapy. Laser acupuncture (which may also include laser-needle acupuncture) is defined as the stimulation of acupuncture points with low-intensity, non-thermal laser irradiation; when performed without the use of acupuncture needles, it is non-invasive. Acupuncture therapy is difficult in children and in animals, and these groups can benefit from the use of techniques for stimulating acupoints which cause little or no trauma. The search fields of the literature survey were for articles reporting the effects of low-level laser therapy and laser acupuncture in regard to pain, nerve injury, muscle injury, inflammation, blood flow, endorphin and enkephalin production, growth factor and cytokine expression. This overview included reporting on:

(i) the types of study design used, e.g. placebo controlled, randomized, single- or double-blind crossover

(ii) the experimental protocols and laser treatments used, and the appropriateness of these to clinical applications in humans

(iii) the relevance of irradiation parameters to any observed effects.

Materials and Methods

Original research papers investigating the effects of laser irradiation in laser acupuncture and laser-stimulated wound healing were retrieved and used for this review. Relevant papers were sought and obtained from library sources and the online database PubMed using EndNote X1 (Thomson Corporation). Search terms were ‘laser acupuncture,’ ‘laser therapy,’ ‘wound healing,’ ‘pain relief,’ ‘inflammation,’ ‘blood flow,’ ‘nerve injury,’ ‘muscle injury,’ ‘tissue regeneration,’ ‘growth factor release,’ ‘cytokine release,’ ‘endorphin,’ ‘enkephalin’. Additional secondary sources of information included reference lists from retrieved papers, and pertinent papers identified by hand searches of relevant journals not found from the database.

We included studies that met the following criteria:

(i) studies were performed in humans or rat, mouse, rabbit (rat, mouse and rabbit are the best characterized, and most commonly used, small laboratory animal models for testing the effectiveness of therapeutic interventions)

(ii) laser (or other monochromatic light source) was investigated as the primary intervention (independent variable)

(iii) the type of laser and precise wavelength were defined or implied;

(iv) the site of application of laser light was defined

(v) at least one outcome or index of laser acupuncture or laser-stimulated wound healing was identified as the dependent variable

(vi) the outcome or index identified was pain, inflammation, blood flow, nerve injury, muscle injury, tissue regeneration, growth factor expression, cytokine expression, endorphin production, or enkephalin production.
Studies excluded from this study were:
(i) studies performed in animals other than rat, mouse, or rabbit
(ii) in vitro studies involving cells
(iii) studies reported in languages for which no English language translation was available
(iv) reviews, meta-analyses, guidelines, surveys, and single case reports
(v) studies which did not use lasers
(vi) studies which did not use laser acupuncture, or laser stimulated wound-healing
(vii) studies involving multisource/multiwavelength arrays, photodynamic therapy, laser surgery for creating wounds, laser welding of wounds, laser photocoagulation, laser ablation, laser resurfacing of skin, laser treatment of skin grafts over wounds, laser tissue welding, laser-assisted tissue soldering, confocal laser microscopy, laser Doppler flowmetry/perfusion when not part of studies involving laser acupuncture or laser-stimulated wound healing
(viii) studies for which only an abstract was available.

EndNote searches were carried out by one of the authors (PP) at the end of January 2010, and articles for inclusion and exclusion were identified and confirmed.

For included articles, the following data were extracted and tabulated by one of the authors (PP):
(i) research method (control group, randomization, and blinded outcome assessment)
(ii) sample type (human subjects or animal species/strain, number, division into groups, group sizes)
(iii) description of laser-irradiation procedure (site/sites of laser light application)
(iv) laser treatment parameters
(v) experimental outcomes
(vi) authors’ conclusion (results of laser irradiation).

Studies were then critically reviewed in terms of study design, methodology (PP), and subsequently checked independently (GDB); comments on the study characteristics were summarized (within Tables 1–12 and in the rows headed: Notes on study design and findings). The number of articles under each of the fields pain, inflammation, blood flow, endorphin and enkephalin production, growth factor and cytokine release showing a positive response to laser light was determined together with the number indicating nil or a negative response. The aim of this process was to achieve a rational conclusion as to the main actions of laser irradiation.

Results
Results from the literature search are summarized in Figs. 1 and 2 (for laser acupuncture and laser-stimulated wound healing, respectively); in total, 31 publications were included in this review (n=14 for laser acupuncture; n=17 for laser-stimulated wound healing), and are summarized in Tables 1–12.

Laser acupuncture
Ten human studies and four animal studies (n=3, rat; n=1, rabbit) were identified.

Human studies (Tables 1–4)

Pain relief. Of the 10 human studies, four had reported on subjects’ pain score; a significant reduction in pain was recorded in three of these studies (i.e. 75% of studies showed a positive response). Pain relief was obtained in patients with carpal tunnel syndrome who were laser treated with 632.8 nm at 225 and 32 J/cm² (superficial acupuncture points) together with 904 nm at 1.8–0.04 J/cm² (deeper acupuncture points),16 and 670 nm at 32.1 and 4.6 J/cm² (superficial acupuncture points);17 also in patients with radicular and pseudoradicular pain syndromes laser treated with 632.8 nm, 30 seconds each point.18 The other study involved patients with whiplash injury receiving laser treatment 632.8 nm, 15 seconds per point 0.075 J/cm² and this group showed a slight but not significant advantage in the duration of cervical collar use and the duration of drug use (muscle relaxant and analgesic) in the acute phase, and in relation to incidence of headaches, dizziness and tinnitus in the chronic phase.15

Inflammation. Only one study was identified and comprised 38 female patients with chronic pelvic inflammation. After laser treatment at 632.8 nm, 300 seconds per session, there was improvement of clinical symptoms and gynecologic signs in 33 patients (which is 87% of the patients treated).19

Blood flow. Four studies were found using laser-needle acupuncture in healthy volunteers. In one study, skin blood flow was significantly increased in the group treated at 685 nm +880–950 nm, 18.4 kJ/cm² while in the placebo group a decrease occurred.20 Two of the other studies examined blood flow in the anterior cerebral artery (ACA), posterior cerebral artery (PCA) and middle cerebral artery (MCA) when various acupoints were stimulated.21,22 Stimulation of vision-related acupoints at 685 nm, 36.8 kJ/cm² showed a trend towards an increase in mean blood flow velocity in PCA while that in MCA was not affected.21 There were significant changes of brain activity in occipital and frontal lobes.21 Stimulation at 680 nm of acupoints connected to the olfactory system according to Traditional Chinese Medicine led to a significant increase in blood flow velocity in ACA, while at the same time blood flow in PCA remained nearly unchanged.22 Furthermore, stimulation of acupoints considered to be connected to optic system showed a significant increase in blood flow velocity in PCA, whereas blood flow in ACA only changed marginally.22 Stimulation of a series of seven acupoints, which included two vision-related ones at 685.4 nm, 16.1 kJ/cm² caused an increase in blood flow velocity in the ophthalmic artery, but no significant changes occurred in blood flow velocity in MCA.23

Endorphin production. Only one study was identified, comprising 15 female and 38 male patients with
<table>
<thead>
<tr>
<th>Study</th>
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<th>Time (seconds)</th>
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<th>Wound outcomes measured</th>
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<tr>
<td>Aigner et al.¹⁵</td>
<td>Patients with whiplash injury (42 female and 8 male). Age range 17–59 years (mean approximately 30 years). Inclusion criteria were whiplash injury within 4 days before first assessment, no recent traumatic bone injuries in cervical spine region, age 18–65 years. Exclusion criteria were massive neurological symptoms, recent bone lesions, trauma more than 4 days previously, patients with a minor injury who were largely subjectively asymptomatic at first follow-up examination and whose cervical mobility was free in all planes. Patients were randomized to two treatment groups of comparable age and sex distribution. Patients in Group 1 (n=25) were treated with a cervical collar and laser acupuncture and those in Group 2 (n=25) were treated with a cervical collar and placebo laser acupuncture. All patients received a muscle relaxant (chlormezanone) combined with anaesthetic (paracetamol). Patients were blinded to the treatment given. Laser treatment was not performed at initial assessment but at first follow-up visit. Acupuncture points B10, BN40, G20, G34, TW5, SI6, LG14 and ear points 29, 37, 41, 55 were each irradiated. Patients in both groups received treatment 3 times/week until asymptomatic. Range of movement of entire cervical spine was determined in three planes and patients questioned specifically about their subjective symptoms (neck pain, headaches, dizziness/vertigo, paraesthesia and tinnitus).</td>
<td>15</td>
<td>632.8 He–Ne laser CW</td>
<td>5</td>
<td>0.075</td>
<td>15 per point</td>
<td>Range of movement of cervical spine in three planes, and a questionnaire sent to each patient 8–12 months after their injury asking about current symptoms, course of previous symptoms after end of treatment, drug consumption, use of a collar and duration of the condition</td>
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Notes on study design and findings
Group 1 obtained a slight but not statistically significant advantage only in relation to duration of cervical collar use and duration of drug treatment in acute phase, and in relation to incidence of headaches, dizziness and tinnitus in chronic phase.
### Table 1 Continued

<table>
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<tr>
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<tr>
<td>Naezer et al.</td>
<td>Patients (2 female and 9 male) with carpal tunnel syndrome (CTS). Age range 40–68 years (mean 54 years). Patients were stratified into two groups (borderline/mild CTS, moderate CTS). Eleven hands were treated.</td>
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<td>Primary measure was pain score from McGill Pain Questionnaire (MPQ). Secondary outcome measures included median nerve sensory peak latency, motor latency, Phalen sign, and Tinel sign</td>
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Step 1. Red-beam laser placed perpendicularly directly on skin at centre of distal wrist crease of affected hand, acupuncture point PC 7 (pericardium meridian), the point closest to median nerve at wrist crease.

Step 2. Circular diode (4-cm diameter) for microamps TENS device applied to skin and centred over acupuncture point PC 7, and grounding pad applied to skin and centred over acupuncture point TW 4 (Triple Warmer) located on dorsum of wrist. Power was gradually increased until a tingling sensation was felt at either electrode site, then it was immediately turned down until there was no sensation at all. During real treatment, after subthreshold intensity established and set, a pulsed frequency of 292 Hz was used for 2 minutes followed by a pulsed frequency of 0.3 Hz for 18 minutes.

Step 3. While TENS device was in place at the wrist, the red beam laser was applied to other acupuncture points on the affected hand for 3 minutes. This included 6 points on fingers and 5–8 points on hand and wrist. Also infrared laser applied to minimum of five deeper acupuncture points on upper extremity, upper trapezius, and cervical paraspinal areas. Each acupuncture point was treated at each of three pulse settings (3500, 584, 73 pulse/second).

A randomized, double-blinded, placebo-controlled, crossover trial. Significant decreases in MPQ score, median nerve sensory latency, and Phalen and Tinel signs occurred after real treatment but not after sham treatment series. Patients could perform their previous work and were stable for 1–3 years.
Table 1 Continued

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<tr>
<td>Branco et al.</td>
<td>There were 31 carpal tunnel syndrome (CTS) patients (22 female and 9 male). Age range 24–84 years (mean 55 years). A total of 36 hands were treated. Fourteen hands had failed 1–2 surgical release procedures. Patients completed pre- and post-treatment Melzack Pain Questionnaire. Primary treatments: Step 1. laser applied to wrist. Tip of laser placed on to skin at median nerve area at centre of distal wrist crease (acupuncture point, Pericardium 7) for 21 minutes (1260 seconds). Step 2. microamps TENS applied to wrist. The TENS device had two electrodes – a primary electrode and a grounding pad. The primary electrode was a metallic, circular-shaped electrode with 4 small (2-mm diameter) LED (monochromatic, but not coherent, red light). The primary electrode was placed on to same area treated in Step 1 (Pericardium 7). Grounding pad electrode was placed on to back of wrist (acupuncture point, Triple Warmer 4). Power was gradually increased until a tingling sensation was felt at either electrode site, then it was immediately turned down until there was no sensation at all. The setting was usually around 200 to 500 µA. It was important that the patient felt nothing during treatment. TENS device used 15 000-Hz carrier wave with a biphasic current. A modulated frequency of 202 Hz was used for first 2 minutes, followed by a frequency of 9.25 Hz or 0.3 Hz for next 18 minutes. Step 3. Laser applied to fingers and hand. While TENS device was in place at the wrist, the red beam laser was applied to other acupuncture points on the affected hand for 3 minutes. Secondary treatments: infrared low-level laser (904 nm, pulsed, 10 W) and/or needle acupuncture on deeper acupuncture points according to the acupuncture meridians involved and distribution of possible radiating pain; Chinese herbal medicine formulas and supplements, on a case-by-case basis. Patients treated every other day for 4–5 weeks (12–15 treatments). Each treatment lasted 45 minutes. Pre- and post-treatment Melzack pain scores.</td>
<td>5 mm diameter aperture=area 0.196</td>
<td>Laser pointer was placed directly over each acupuncture point on the skin</td>
<td>670 diode laser pointer</td>
<td>5</td>
<td>1260</td>
<td>6.3</td>
<td>32.1</td>
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</table>
A random sample of 21 in-patients (12 female and 9 male) of a neurological clinic suffering from radicular and pseudoradicular pain syndromes was selected. Age range 34–75 years (mean 52 years). Pre-trial pain duration range 1–20 weeks (mean 5.6 weeks). All patients had been treated with analgesics and physiotherapy prior to admission to clinic. Inclusion criteria were continuous segmentally radiating lumbar or cervical pain and absence of a considerable neurological deficit. Relevant psychiatric disorders were absent in all patients included in the trial. The study was done 4–8 days after admission upon completion of all diagnostic procedures and as soon as pain intensity had subsided to a level that made a period of 18–36 hours without any pain treatment other than laser irradiation acceptable for the patients. The trial was conducted after a period of 12 hours without any pain treatment. By random assignment, laser irradiation on acupuncture points or a sham laser treatment (placebo) was performed. Following an interval of 24–48 hours the treatments were repeated, with patients who had been treated with laser, receiving a placebo treatment and vice versa (crossover). Patients were blinded as to which treatment was being given. Acupuncture points were selected for different pain syndromes and consisted of four acupuncture points on the body and one corresponding point on the ear. Patients were instructed to assess overall pain intensity on a 10-point visual verbal analogue scale before each of the two treatments and 15 minutes, 1, 6, and 24 hours thereafter. Randomized single blind cross over study. Following laser treatment on acupuncture points a statistically significant reduction of overall pain intensity was observed; only 1 patient reported no pain relief. After placebo treatment, mean levels of pain intensity were not significantly changed. The differences between laser treatment and placebo were statistically significant at 15 min, 1 and 6 hours after the respective treatments.

Pain scores on a 10-point visual verbal analogue scale before each of the two treatments and 15 minutes, 1, 6, and 24 hours thereafter.
alcohol addiction. Laser treatment with an argon laser 514 nm for stimulation of auricular acupoints and a He–Ne 632.8 nm laser for stimulation of regions in the neck vessel projection caused an improvement in symptoms of depression as assessed from questionnaire scores, and there was a significant increase in plasma beta-endorphin levels both in female and male groups.

Animal studies (Tables 5–7)

Pain and inflammation. Two studies showed that laser treatment with 670 nm, 0.021 J/cm² at points selected for acupuncture in rats receiving intraplantar injection of heat-killed Mycobacterium tuberculosis in complete Freund’s adjuvant (CFA) prevented edema formation, which never reached the levels seen in untreated animals. Inflammation hyperalgesia of CFA-injected rats, and neuropathic pain in rats with sectioned or chronic constriction injury of sciatic nerve, were also reduced with laser treatment. In another study, tail flick latency in response to a heat stimulus was significantly longer after laser and laserneedle treatment at 2940 nm to a selected acupoint. The laser treated group showed two periods of anti-nociceptive action, one immediate and another 45 minutes after treatment.

Blood flow. A study in the rabbit in which transparent round chambers were inserted in the ear lobes and treated with acupuncture, short wavelength near-infrared lamp irradiation 600–1600 nm, or near-infrared laser irradiation 830 nm, showed that arteriolar diameter significantly increased in all of the treatment groups when compared to pre-treatment values and blood flow rate showed similar trends to arteriolar diameter, and treatment effect persisted for 40–50 minutes after the end of acupuncture stimulation and lamp or laser irradiation. Treatment effect persisted for 40–50 minutes after the end of acupuncture stimulation and lamp or laser irradiation.

Enkephalin production. One study in the rat using acute pain models (carrageenan inflammation) and chronic pain models [CFA inflammation and arthritis, chronic constriction injury of sciatic nerve (CCI)] demonstrated that enkephalin mRNA level was strongly upregulated in CFA and CCI animals. Laser treatment 670 nm, 0.021 J/cm² at selected acupoints further increased enkephalin mRNA expression in single neurons in CFA and CCI animals.

Laser-stimulated wound healing

Five human and 12 animal studies (n=11, rat; n=1, mouse) were identified. A significant pain relief was recorded in all of the four studies that reported on pain levels in patients as a result of laser treatment of wounds (i.e. a positive response in 100% of the patients). Human studies (Tables 8 and 9)

Pain and inflammation. Two studies showed that laser treatment with 670 nm, 0.021 J/cm² at points selected for acupuncture in rats receiving intraplantar injection of heat-killed Mycobacterium tuberculosis in complete Freund’s adjuvant (CFA) prevented edema formation, which never reached the levels seen in untreated animals. Inflammation hyperalgesia of CFA-injected rats, and neuropathic pain in rats with sectioned or chronic constriction injury of sciatic nerve were also reduced with laser treatment.

**Table 2 Laser acupuncture: human studies**

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<tr>
<td>Wu et al.¹⁹</td>
<td>Thirty-eight female patients with chronic pelvic inflammation. Age range 27–61 years. Acupoints Qihai (Ren 6), Zhongji (Ren 3), Shenhu (UB 23), Xuehai (Sp 10) and Sanyinjiao (Sp 6) were irradiated. The cases included ovaritis, ovarian cysts, salpingitis and peritendinitis.</td>
<td>Light spot diameter 1–2 mm; area 0.008–0.031; distance of radiation approximately 0.5 cm</td>
<td>632.8 He–Ne laser</td>
<td>4</td>
<td>300</td>
<td>Acupoints irradiated 4 times each session, 4 sessions per week. 10 sessions constituted one therapeutic course. The second course was started after a 7–10 day rest. Average number of irradiation sessions was 30.</td>
<td>Effectiveness of laser treatment with regard to improvement or disappearance of clinical symptoms and gynecologic signs</td>
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<tr>
<td>Peplow et al. Application of low level laser technologies for pain relief and wound healing 2010 VOL. 15 NO. 4</td>
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Notes on study design and findings

After laser treatment, 9 cases were cured, 14 markedly improved, 10 improved, and 5 ineffective. The cured and markedly improved rate was 61% and total effective rate was 87%. The number of sessions correlated with efficacy of treatment. The authors advise that patients must persist in an extended course of treatment with this method, since the biological effects of He–Ne laser irradiation are cumulative.
### Table 3 Laser acupuncture: human studies

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<td></td>
<td>Blood flow</td>
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<td>Optical fibre core diameter 0.5 mm – area of 0.00196</td>
<td>685 and 880–950 bichromatic laser diodes</td>
<td>30–40 per laser needle</td>
<td>600</td>
<td>2300 at each laser needle; for eight needles total energy density at acupuncture points 18 400 (based on average power density of 3.8 W/cm² at one acupoint, see Litscher and Shikora[25])</td>
<td>Microvascular blood flow using laser Doppler spectroscopy (provides simultaneous assessment of skin blood flow and blood flow in deeper tissue up to 6 mm). Four signal segments of 2 minutes in length before, during, and after laser needle stimulation, respectively, were selected for analysis. Alterations in tissue oxygenation in flexor carpi ulnaris muscle monitored using near-infrared spectroscopy. Four signal segments of 2 minutes in length before, during, and after laser needle irradiation, were extracted for analysis</td>
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<tr>
<td>Banzer et al.[21]</td>
<td>Thirty-three healthy non-smoking males, mean age 27 years, without vascular disease, hypertension, and use of vasoactive medications. Subjects were randomized into an experimental group (n = 18) and placebo group (n = 15). Laser needle system used for irradiation of acupuncture point Pericardium 6-Nei Guan (Pe 6). Laser needles not inserted into skin but fixed on to skin at the acupuncture point. The device consisted of eight laser needles arranged at end of optical fibres. Application of laser or placebo was computer-controlled to assure double-blinded allocation. Continuous recordings of skin blood flow and muscle oxygenation performed after acclimatization period of 10 minutes. Lasers needles put in contact to skin at acupoint Pe 6 which is located 2 cm proximal to middle point of carpal fold between tendons of flexor carpi radialis and palmaris longus. Blood flow measurements performed on right forearm, 5 cm proximal to middle point of carpal fold between tendons of flexor carpi radialis and palmaris longus. Laser Doppler spectroscopy cables secured to reduce impact of movement artefacts. The near-infrared probe placed over flexor carpi ulnaris, and blackout cloth placed over the probe to ensure maximum light filtering. Subjects lying in a relaxed, supine position during the tests, and eyes covered by an opaque mask.</td>
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</table>

Notes on study design and findings

A randomized, double-blinded, placebo-controlled trial. A statistically significant interaction was found between time and group indicating that peripheral blood flow was influenced by laser needle application. Average skin blood flow increased by 7.3% in experimental group, whereas in placebo group a decrease of 16.1% was shown (analysis segment 3 compared to baseline). After laser irradiation (analysis segment 4), skin blood flow increased by 8.1% in experimental group and decreased by 21.8% in placebo group. In the deeper tissue, after laser irradiation, perfusion increased by 2.7% in experimental group whereas perfusion was reduced by 13.6% in placebo group (analysis segment 4). In contrast, tissue oxygenation was not affected by laser irradiation.
## Study Subjects in clinical trials and study design

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects in clinical trials and study design</th>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
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<th>Power density (mW/cm²)</th>
<th>Time (seconds)</th>
<th>Energy (J) per treatment or day</th>
<th>Energy density (J/cm²) per treatment or day and number of treatments or days of irradiation</th>
<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litscher et al.²¹</td>
<td>Eighteen healthy volunteers (11 female and 7 male, mean age 25 years) investigated using functional multidirectional transcranial Doppler sonography (ITCD) and one volunteer (female, 27 years) using functional magnetic resonance imaging (fMRI). None of subjects were under influence of centrally active medication. All were free of neurological or psychological disorders including absence of visual deficits. Eight acupuncture sites were chosen and irradiated simultaneously. Laser needles were fixed to skin using plaster strips but were not inserted into skin. ITCD examination of posterior cerebral artery (PCA) and middle cerebral artery (MCA) performed simultaneously and continuously to determine alterations of cerebral blood flow velocities. Blood pressure was measured non-invasively before and after stimulation. fMRI images sensitive to blood oxygen dependent contrast were acquired with a T2 weighted gradient echo with single shot echo planar readout. Eight vision-related distal acupoints on both sides and eight placebo points were tested using two schemes (ITCD measurements), each in one session in a randomized controlled double blind cross-over study design. For ITCD investigation, testing started randomly with either acupoint or placebo stimulation. The same acupoints were used for fMRI investigation. In several test measurements needle stimulation of these acupoints led to alterations of blood flow velocity in PCA. During testing, subjects were in a relaxed and comfortable position on a bed. Interval between ITCD experiments was 20–30 minutes and subjects instructed to keep their eyes closed during whole of ITCD experiment. Similarly, during fMRI investigations subject could not see whether laser was off or on. fMRI study used a block design with alternating 1 minute resting condition and 1 minute activation condition. A total of six resting and six activation intervals were registered, taking 12 minutes. Laser applied to acupoints Hegu (LI 4) (on dorsum of hand, on middle of second metacarpal on radial side); Zusanli (S36) (one finger-breadth from anterior crest of tibia); Kunlun (B60) (in the depression between tip of lateral malleolus and tendo calcaneus); Zhiyin (B67) (located on lateral side of small toe about 0.1 cun lateral to corner of nail).</td>
<td>Optical fibre core diameter 0.5 mm² area of 0.00196</td>
<td>685</td>
<td>30–40 per laser needle</td>
<td>1200 (ITCD measurements)</td>
<td>4600 at each acupoint and a total sum of 36 800 for all acupoints (based on average power density of 3.8 W/cm² at one acupoint see Litscher and Shikora²³)</td>
<td>Mean blood flow velocity in PCA and MCA before, during, and after stimulation on vision-related acupoints and placebo points</td>
<td></td>
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</tbody>
</table>
Laser needle stimulation of vision-related acupoints showed a trend towards an increase of mean blood flow velocity in PCA measured by fTCD (but not statistically significant). Mean blood flow velocity in MCA was not affected. Stimulation of placebo points did not increase blood flow velocity in PCA or MCA. Mean arterial blood pressure before and after laser needle acupuncture was almost identical. Significant changes of brain activity were found in occipital and frontal lobes. Simulation of vision-implicated acupoints (Kunlun, Bladder 60 and Zhiyin, Bladder 67) activated the visual cortex, in accordance with other fMRI acupuncture studies.\(^{43,44}\)

Litscher\(^{22}\) Twenty-two healthy volunteers (mean age 24 years). Continuous measurement of microcirculation and temperature at the right hand were initiated after a resting period of 10 minutes. When ‘steady-state’ condition was reached, acupuncture point Hegu (LI4) on right hand was stimulated with a laser needle. The lasers used was fixed at the acupuncture point with special adhesive tape. A semiconductor 680 nm, approximately 60 mW was used as light source. The laser Doppler flowmetry probe was applied 1 cm from laser needle. In a further study series, flow profiles from left anterior cerebral artery (ACA) and right posterior cerebral artery (PCA) before, during and after laser needle acupuncture were registered continuously in the same 22 adult volunteers.

Energy dose emitted from a laser needle in 20 minutes at a distance of 1 cm warmed local skin and subcutaneous tissues about 0.7°C. Laser needle stimulation of points Yingxiang, Hegu and Pianli, which are connected to olfactory system according to traditional Chinese medicine, led to a significant increase in blood flow velocity in ACA. At the same time, blood flow in PCA remained nearly unchanged. ACA supplies major regions of frontal and median area of brain, including olfactory cortex. Laser needle stimulation of points Guangming, Taichong and Zhiyin, supposed to be connected to optic system, showed a significant increase in blood flow velocity in PCA, whereas flow in ACA only changed irrelevantly.
### Study Subjects in clinical trials and study design

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<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litscher and Shikora(^2)</td>
<td>Twenty-seven healthy volunteers (14 female and 13 male, mean age 25 years). None of subjects were under influence of centrally active medication. All were free of neurological or psychological disorders including absence of visual deficits. Seven vision-related acupoints were tested in two sessions (laserneedle acupuncture and needle acupuncture). The acupuncture scheme consisted of two traditional Chinese acupoints: UB2 Zanzhu (in depression of medial end of eyebrow); needling method: puncture transversely 0.5–0.8 cm²) and EX.3 Yangbai (at midpoint of eyebrow; needling method: puncture transversely 0.3–0.5 cm²). In addition two ear acupoints (eye and liver; needling method: puncture perpendicular 0.3 cm²) and two vision-related acupoints from Korean hand acupuncture (E2; needling method: puncture transversely 0.1–0.2 cm²) and one from Chinese hand acupuncture (Yan Dian, on ulnar side of thumb distal to first metacarpal; needling method: puncture perpendicular 0.2 cm²). The combination of these three different acupuncture systems showed an enhanced effect on the parameters measured. The laser radiation of eight 55 mW laser diodes was coupled to eight optical fibres and laserneedles arranged at distal ends of optical fibres. Laser needles were fixed to skin using plaster strips but were not inserted into skin. The acupoint scheme was the same as for needle acupuncture. During testing, subjects were in a relaxed and comfortable position on a bed. After a 10-minute resting period the laserneedles or acupuncture needles were applied. The choice for initial stimulation was randomized. Blood flow in ophthalmic artery (OA) and middle cerebral artery (MCA) were measured by multidirectional transorbital and transtemporal Doppler sonography. Blood pressure was measured non-invasively before, during and after stimulation.</td>
<td>Optical fibre core diameter 0.5 mm² area of 0.00186</td>
<td>685</td>
<td>30–60 per laser needle</td>
<td>3800 at each acupoint determined by integration of intensity curve (intensity versus optical fibre diameter curve)</td>
<td>600</td>
<td>2300 at each acupoint; for 7 acupoints total energy density 16 100</td>
<td>Mean blood flow velocity in OA and MCA were measured simultaneously and continuously. Each subject was studied with laserneedle acupuncture and needle acupuncture. The choice of measuring procedure was randomized and interval between the experiments was 20–30 minutes.</td>
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</table>

There were significant increases in mean blood flow velocity in OA during laserneedle acupuncture and needle acupuncture. No significant changes occurred in blood flow velocity in MCA.
Table 4  Laser acupuncture: human studies

<table>
<thead>
<tr>
<th>Study Subjects in clinical trials and study design</th>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
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<tr>
<td>Endorphin production</td>
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<tr>
<td>Zalewska-Kaszubska et al.¹</td>
<td>Fifty-three patients (15 female and 38 male) with alcohol addiction. Age range 31-63 years (mean 47 years). At pre-treatment and end of second session, patients completed a questionnaire which reflects cognitive and affective symptoms of depression. Pregnant and breast-feeding females were excluded; also patients with cancer diagnosis or serious somatic problems. In the course of the study, patients who did not abstain from alcohol or complete daily laser applications were excluded. Patients were treated with two types of laser stimulation. An argon laser was used for stimulation of auricular points and a He–Ne laser for stimulation of regions in the neck vessel projection. Laser acupuncture stimulation with argon laser was applied to auricle where acupoints are located, 10 times, every second day. Successive stimulation with He–Ne laser was administered for 20 consecutive, daily sessions (except Sundays). After each session, there was a one-week interval without laser exposure. Patients received laser acupuncture treatment with argon laser into auricular concha, bilaterally to 5 points: 82, 83, 87, 51, 55 by tactile method. In addition, patients were subject to He–Ne irradiation in a spot on neck vessel projection. Blood samples were taken before laser treatment, and next day after each treatment series.</td>
<td>0.05</td>
<td>514 Ar laser</td>
<td>100</td>
<td>10, each point</td>
<td></td>
<td></td>
<td>Effectiveness of laser treatment with regard to improvement in cognitive and affective symptoms of depression from completed questionnaires. Plasma beta-endorphins by radioimmunoassay</td>
</tr>
</tbody>
</table>

Notes on study design and findings

Improvement in questionnaire scores and increase in beta-endorphin level were observed (plasma concentrations doubled both in female and male groups).
Table 5 Laser acupuncture: animal studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Animals in experimental studies and study design</th>
<th>Irradiated area or spot size (cm²)</th>
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<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pain and inflammation</strong></td>
<td>Lorenzini et al. (24) rat, Sprague-Dawley, male 2 month-old, 48 in total. Both continuous wave (CW) laser and pulsed wave (PW) laser tested in acute inflammatory pain models (CFA paw inflammation, myofascial pain). Only pulsed wave laser tested on neuropathic pain model (axotomy of sciatic nerve) and visceral pain (cystitis). A modified acupoint detector designed for humans was used to identify the acupoints in rats. Acupoints ST36 Zu San Li (Stomach meridian) and TH5 Waiguan (Sanjiao meridian) were used. CFA paw inflammation. Rats were divided into three groups: untreated ($n=5$); CW laser treated ($n=5$); PW laser treated ($n=5$). Inflammation induced by unilateral intraplantar injection of 100 µl heat-killed Mycobacterium tuberculosis (30 µg) suspended in complete Freund's adjuvant (CFA) into right hind paw. Immediately after paw volume measurement, to assess nociceptive responses to thermal stimuli, paw withdrawal latency was measured at same time-points. Myofascial pain and grip strength. Rats divided into three groups as above ($n=5$ per group). Carrageenan was injected into triceps bilaterally (4 mg suspended in 150 µl PBS per triceps). Laser application was performed 3 hours after carrageenan injection and grip force of animals tested 10 minutes after laser application. Test was repeated 6 hours after the injection. Axotomy and spontaneous pain. Two groups were used: untreated ($n=4$); PW laser treated ($n=5$). Neuropathy was produced on right hind paw by complete section of sciatic nerve. Cystitis induction and visceral pain. Two groups were used: untreated ($n=4$); PW laser treated ($n=5$). Cyclophosphamide 75 mg/kg was administered i.p. every 3 days for 4 times in adult female Sprague-Dawley rats. Control rats ($n=5$) received volume-matched injections of saline.</td>
<td>&lt;5 Laser applied at ST36 (antero-inferior depression of head of fibula) and TH5 [2 'cun' above mid-point of dorsal transverse crease of wrist, between radius and ulna. (cun = length of phalanx 2 of rat's digit)] 670 laser diode, &lt;0.03 pulse 0.1 ms with duty cycle of 1% (pulse period 10 ms, frequency 100 Hz)</td>
<td>35</td>
<td>20</td>
<td>0.021</td>
<td>For acute inflammatory pain and muscle pain, laser applied once for 20 seconds at acupuncture points ST36 and TH5. For visceral and neuropathic pain, laser treatment applied at same acupuncture points every 3 days.</td>
<td>CFA paw inflammation. Paw volume for ipsilateral and contralateral hind paws measured using a water displacement plethysmometer. Paw volume measurements were plotted at each time point 4, 8, 12, 24, 48, 72 hours after CFA injection. Paw withdrawal latency measured with rats placed in a plastic chamber with plexiglass floor and a radiant heat source under the floor beneath hind paw to be tested. Myofascial pain and grip strength. Animals were tested at 0, 3, 6 hours after carrageenan injection. Forelimb grip force was measured using a computerized grip force meter when each animal applies force to a wire grid connected to a gauge located at front of measuring apparatus. Axotomy. For evaluation of neuropathic pain, rats were placed in plastic cylinder and position of hind limbs in relation to the floor observed. Cystitis induction. Behaviour was recorded 3 times every 15 minutes for 3 hours Assessors were blinded to laser treatment.</td>
<td></td>
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</tbody>
</table>
Intraplantar injection of CFA induced a rapid and progressive hind paw edema that reached its peak 24 hours after injection after which it started to decline. A single CW or PW laser application to acupuncture points, performed 2 hours after injection, prevented edema formation, which never reached that observed in untreated rats. Laser treatment with PW significantly reduced inflammation hyperalgesia in response to thermal stimuli. Injection of carrageenan into both triceps produced a significant reduction in grip force. Laser treatment was performed once at the inflammation peak (3 hours after i.m. injection of carrageenan) and grip force measured 10 minutes after laser application. PW, but not CW laser application, resulted in better muscular performance of rats. 25 days after surgery, rats in the sciatic nerve ligation group displayed neuropathic pain behaviour. Rats treated with very low level laser displayed a slight but significant reduction in overall neuropathic pain behaviour. For cyclophosphamide-induced bladder inflammation, the time course profiles of PW-treated rats overlapped those of untreated animals.

Data regarding number of rats is not consistent with that reported in abstract. Before treatment, all rats showed similar tail-flick latency of 4.9 ± 0.2 seconds. Tail-flick latency was longer after treatment in all groups, but mean latency was significantly longer in laser and needle groups. Increase in response latency was about 2.2 times higher in laser group and 2.9 times higher in needle group than in restraint group. At 55 minutes (45 minutes after treatment) tail-flick latency was significantly longer in laser group than in needle group or restraint group. Laser group had therefore two periods of anti-nociceptive action, one immediate and another 45 minutes after treatment.
Table 5 Continued

<table>
<thead>
<tr>
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<th>Animals in experimental studies and study design</th>
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</thead>
<tbody>
<tr>
<td>Giuliani et al.²⁵</td>
<td>rat, Sprague-Dawley, male 250-275 g</td>
<td>Very lower power laser tested in acute pain models (carrageenan inflammation) and chronic pain models (CFA inflammation and arthritis, chronic constriction injury of sciatic nerve). For acute experiments, laser applied at two selected points which in humans correspond to anteroinferior depression of head of fibula, and to above midpoint of transverse crease of wrist, bilaterally between radius and ulna, or directly on dorsal surface of hind paws of gently restrained animals. For chronic experiments, laser treatment was applied at same points every 3 days.</td>
<td>&lt;5</td>
<td>670 Biolite laser</td>
<td>0.03 obtained through 1% duty cycle modulation of 3 mW peak power diode, frequency 100 Hz</td>
<td>35 at each point</td>
<td>0.021</td>
<td>Carrageenan paw inflammation. Paw volume for ipsilateral and contralateral hind paws measured using a water displacement plethysmometer. Paw volume measure just prior to carrageenan injection was used as control volume. CFA inflammation and arthritis. A total score for edema and reddening i.e. sum of scores for different areas was assigned during each observation. Spontaneous pain in arthritic rats was evaluated by placing rats in plastic cylinder and position of hind limbs in relation to the floor observed. Neuropathic pain. Behavioural testing carried out. Thermal hyperalgesia test. Rats placed in a plastic chamber with plexiglass floor and a radiant heat source under the floor beneath hind paw to be tested. Intensity of light source was calibrated to induce withdrawal within 8-10 seconds in normal animals. Withdrawal latency of both hind paws measured. Mechanical hyperalgesia test. A graded mechanical force was applied on to convex surface of paw, Rats withdrew hind paw or vocalized when applied force reached nociceptive threshold.</td>
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</table>

Very low power laser treatment at points usually selected for acupuncture was effective in reducing edema and hyperalgesia in acute and chronic inflammation. Neuropathic pain and thermal hyperalgesia were reduced in rats with chronic constriction injury of sciatic nerve.
### Table 6 Laser acupuncture: animal studies

<table>
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<tr>
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</table>
| Blood flow Komori et al.  
rabbit, Japanese domestic white rabbits 2.5-3.0 kg, 40 in total. Transparent round chambers (REC) were inserted in the ear lobes. REC was composed of a disc with a central round table and three peripheral pillars, cover plate and holder ring. Disc was designed to leave a cavity 50 μm thickness, 6.4 mm diameter between central round table and cover plate. New microvessels arose from the blood vessels of dermis and covered entire cavity within 6 weeks. RECs were observed microscopically. Rabbits randomly assigned to four groups: acupuncture, near-infrared lamp irradiation, near-infrared low-power laser irradiation, no irradiation, control (n=10 in each group). In acupuncture group, five needles were placed around crus of helix at centre of outer ear, which is controlled by vagus. Needles left in place for 20 minutes without electrical stimulation. The stimulated region was located around 4 cm from centre of REC. In the lamp, laser, and control groups, the same site as that of acupuncture needles in acupuncture group was irradiated or applied the contact probe for 10 minutes. | 0.385 | 600–1600 optical filter for lamp | 1540 | 4000 | 1 second followed by 4 seconds of treatment cessation. | During baseline period after surgical preparation, arterioles with diameters 20–100 μm were selected. Blood vessel diameter, blood flow velocity, and blood flow rate for up to 60 minutes after acupuncture or irradiation treatment were compared with baseline values |
|             | Notes on study design and findings                                                                                                                                         |                                    |                 |             |                        |                |                                |                                                                                                              |
|             | Arteriolar diameter significantly increased to 131% in acupuncture group, 129% in lamp group, and 128% in laser group when compared with pre-treatment value (100%). Maximum values were reached 20 minutes after end of acupuncture stimulation, and 10 minutes after the end of lamp and laser irradiation. The three groups showed significant increases in arteriolar diameter when compared with control group. Blood flow velocity and blood flow rate showed similar trends to arteriolar diameter. Treatment effect persisted for 40–50 minutes after end of stimulation and irradiation. |                                    |                 |             |                        |                |                                |                                                                                                              |
Table 7 Laser acupuncture: animal studies

<table>
<thead>
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<tr>
<td>Enkephalin production</td>
<td>Sprague-Dawley, male 250–275 g Very lower power laser tested in acute pain models (carrageenan inflammation, n=6) and chronic pain models (CFA inflammation and arthritis, n=6; chronic constriction injury of sciatic nerve, CCI, n=6). For acute experiments, laser applied at two selected points which in humans correspond to anteroinferior depression of head of fibula, and to above midpoint of transverse crease of wrist, bilaterally between radius and ulna, or directly on dorsal surface of hind paws of gently restrained animals. For chronic experiments, laser treatment was applied at same points every 3 days. In situ hybridization. Animals in each group were euthanized, lumbar spinal cord and lumbar dorsal root ganglions removed, immersed in ice-cold saline, and 14 μm sections cut in a cryostat at −20°C. An oligonucleotide probe complementary to preproenkephalin 322–360 was labelled at 3'-end with α-[35S]-dATP. Sections were incubated with labelled probe for 15–20 hours at 50°C and exposed to betamax films for 20 days at −20°C. Films were developed. Sections were dipped in nuclear track emulsion for 3 weeks before being developed and fixed.</td>
<td>&lt;5</td>
<td>670</td>
<td>Biolite laser</td>
<td>0.03 obtained through 1% duty cycle modulation of 3 mW peak power diode, frequency 100 Hz</td>
<td>35 at each point</td>
<td>0.021</td>
<td>Quantitative analysis was performed on emulsion-dipped sections. mRNA expression was measured in external layers of dorsal horn of spinal cord, bilaterally, in all CFA- and CCI-treated animals.</td>
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</table>

Notes on study design and findings
Enkephalin mRNA level was strongly upregulated in CFA animals and laser treatment further increased the mRNA level in single neurons. Enkephalin mRNA was also upregulated in both sides of CCI animals compared to naive animals, but the level on the lesione side was significantly higher than on the non-lesioned side. Enkephalin mRNA expression was increased in laser-treated CFA and CCI animals.
Table 8 Laser and wound healing: human studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects in clinical trials and study design</th>
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<td>Pain and inflammation</td>
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<tr>
<td>Sharon-Buller et al.</td>
<td>One female patient 50 years and one male patient 65 years with ulcerative lesion of lower labial frenulum and tongue, respectively, as a side effect of radiation therapy. A thin film of Elmex gel was placed on the lesion (to reduce beam absorption by soft tissue) and lesion was laser irradiated with a circular motion</td>
<td>27</td>
<td>10 600</td>
<td>CO₂ laser</td>
<td>CW</td>
<td>1000–1500</td>
<td>5</td>
<td>Reporting of pain by patients following treatment</td>
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<td></td>
<td>Notes on study design and findings</td>
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<td>Following laser irradiation of lesion, patients reported immediate pain relief or no further pain</td>
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<tr>
<td>Moreira et al.</td>
<td>Two patients with spastic cerebral palsy, one female 6 years and one male 2 years. Patients with internal mucosa and lower lip traumatism caused by oral reflex automatism with spastic tonic bite and lower lip interposition. Laser therapy applied to all injured areas with a 24-hour interval between first and second administration, and a 7-day interval between the two subsequent ones.</td>
<td>28</td>
<td>685</td>
<td>InGaAlP laser</td>
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<td>At first reevaluation, 24-hour later, there was a striking reduction in inflammation, a decrease in vascular congestion, and a reduction of ulcerated area with spasticity and pain reduction. At 14-day reevaluation, significant clinical differences in advanced healing process were observed.</td>
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<tr>
<td>Schaffer et al.</td>
<td>Seven patients (six female age range 42–66 years suffering from painful mastitis after breast ionizing irradiation, and one male 56 years with radiogenic ulcer of neck). In all six females, laser irradiation performed for the complete breast affected. In male, radiogenic ulcer irradiated by laser light including a margin of 5 cm in length and width of non-damaged tissue. Laser treatment was performed five times per week, resulting in average of 25 laser sessions for each patient. MRI scans of each patient performed 1 day before beginning of laser treatment and 1 day after completion of all laser sessions.</td>
<td>29</td>
<td>780</td>
<td>laser</td>
<td></td>
<td>100</td>
<td>5 per session</td>
<td>Patients reporting of pain and MRI scans</td>
<td></td>
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<tr>
<td></td>
<td>Already at 2 weeks after beginning of laser treatments, all six females showed significant pain relief. After laser therapy had been concluded (average 5 weeks, 25 sessions), the inflamed tissue showed complete remission of mastitis symptoms: decrease in discomfort, decrease in skin edema and swelling. Similar results were found for male with radiogenic ulcer of neck.</td>
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<td>Study</td>
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</tr>
<tr>
<td>Bensadoun et al.⁵⁰</td>
<td>Thirty patients (4 female and 26 male) with carcinoma of oropharynx, laryngopharynx and oral cavity being treated by external radiotherapy without prior surgery or concomitant chemotherapy. Age range 36–78 years (mean 60 years). All patients were requested to stop smoking tobacco and drinking alcohol, to prevent their potential worsening effect on oral mucositis. Patients assigned to either laser treatment or sham treatment by computer randomization (n=15 patients for each group). No associated anti-inflammatory or other mucositis treatment was authorized. Prescription of analgesics was allowed but not during the 2 days before evaluation each week. Patients received laser applications daily for 5 consecutive days each week during the 7 weeks of radiotherapy, before the radiation sessions. The malignant tumour had to be located outside test zones selected for randomized laser application. The 9 laser treatment areas included posterior third of internal surfaces of cheeks, soft palate and anterior tonsillar pillars. Laser operator was the only person to know whether or not patient was being sham-treated. In the laser-treated group, 14 patients had laser treatment for skin of neck included in fields of radiation. In the sham group, 7 patients had laser treatment for skin of neck included. Patients did not know whether they were being sham-treated or receiving laser applications. Whole irradiation field, oral cavity and visible oropharynx were inspected weekly during the 7 weeks by a physician blinded to type of treatment. Laser treatment over the 7 weeks of radiation treatment significantly reduced mean intensity scores for oral mucositis in laser treatment fields. Preventative use of laser application significantly reduced oral pain for treatment area as assessed by patients. There was a reduction in the incidence of severe pain (expressed as a number of weeks for the whole population of patients) from 25 weeks for sham-treated group to 2 weeks for laser-treated group.</td>
<td>Each treatment area=1</td>
<td>632.8 He–Ne laser using a 1.2-mm optical fibre</td>
<td>60 (29 patients) or 25 (one patient)</td>
<td>33 or 80</td>
<td>2 per point, 9 points</td>
<td>Criteria used for evaluation were standard WHO staging for mucositis and modified visual analogue scale for pain (patient self-evaluation)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
studies). These studies involved patients with ulcerative lesion of the lower labial frenulum and tongue laser treated at 10 600 nm,27 patients with spastic cerebral palsy and with internal mucosa and lip trauma treated at 685 nm, 190 J/cm²,28 female patients with painful mastitis, and a male patient with an ulcer of the neck treated at 780 nm, 5 J/cm² each session,29 patients with carcinoma of oropharynx, laryngopharynx, and oral cavity who developed oral mucositis treated at 632.8 nm, 18 J/cm².30 A reduction in inflammation and edema was reported in two of the studies (i.e. a positive response in 50% of the studies).28,29

Blood flow. One study had been performed with patients having diabetic microangiopathy and a reduced skin temperature over the forefoot region.31 A rise in skin temperature occurred after a single transcutaneous laser irradiation at 632.8 nm, 30 J/cm² indicating an improved skin blood circulation of the forefoot region. Sham treatment caused a small decrease in skin temperature.31

Animal studies (Tables 10–12)

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects in clinical trials and study design</th>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
<th>Energy density (J/cm²)</th>
<th>Power (mW)</th>
<th>Power density (mW/cm²)</th>
<th>Time (seconds)</th>
<th>Energy (J) per treatment or day and number of treatments or days of irradiation</th>
<th>Wound outcomes measured</th>
<th>Notes on study design and findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow Schindl et al.</td>
<td>Thirty patients (14 female and 16 male, mean age 62 years) showing a reduced temperature profile over forefoot region (mean temperature, 29°C) and levels of glycosylated hemoglobin. Exclusion criteria were clinical or blood-chemical signs of infection and medication with drugs that might influence platelet aggregation, vasodilatation, or both. Subjects were randomized by blocks of two into two groups (n = 15): Group 1 received a single session of simultaneous laser irradiation over both forefoot regions, while both forefeet in Group 2 were sham irradiated.</td>
<td>3.0</td>
<td>632.8</td>
<td>30</td>
<td>3000</td>
<td>30</td>
<td>Skin blood circulation of forefoot region as indicated by temperature recordings was detected at 0, 20, 50 minutes after start and 15 minutes after end of irradiation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inflammation. Nine of the 11 rat studies had reported inflammation, and three had been performed in STZ-diabetic rats. One of the studies with STZ-diabetic rats involved making two parallel incisions on the dorsum, one on the left side and one on the right side, using a steel scalpel, and sutured.34 The wound on the left side was laser irradiated at 808 nm, 10 J/cm² and the degree of inflammation on day 10 was found to be lower than for the right side which was not irradiated.34 In another study with STZ-diabetic rats, three incisions were made on the dorsum, one on the left side using a diode laser and other incisions on right side using a steel scalpel and a diode laser, and sutured.32 The wound on the left side was laser irradiated at 808 nm, 10 J/cm². On day 10 there was a lower inflammation for the irradiated diode wound compared to non-irradiated scalpel and non-irradiated diode wounds.32 In the third study with STZ-diabetic rats, a third-degree burn was induced using a specially designed instrument which was heated until red hot and applied to the skin for 20 seconds.35 Laser stimulation with 660 or 780 nm, 20 J/cm² was performed immediately post-burn and repeated until the day before sacrifice. At day 7, the acute inflammatory reaction of laser-irradiated wounds was less than for non-irradiated wounds.35 Thus, there was a decrease in inflammation of incisional wounds and burns for all three studies with STZ-diabetic rats.

Two of the rat studies involved making incisions in normal (non-diabetic) rats only. In one of these, incisions were made on the left and right sides of the dorsum using a diode laser.33 The wound on the left
### Table 10 Laser and wound healing: animal studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Animals in experimental studies and study design</th>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
<th>Power (mW)</th>
<th>Power density (mW/cm²)</th>
<th>Time (seconds)</th>
<th>Energy (J) per treatment or day and number of treatments or days of irradiation</th>
<th>Energy density (J/cm²) per treatment or day and number of treatments or days of irradiation</th>
<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation</strong></td>
<td><strong>Akyol and Gungormus</strong>&lt;sup&gt;32&lt;/sup&gt; rat, Wistar, 250-300 g, 18 STZ-diabetic. Three incisions (approximately 15 mm length) were made on dorsum of each rat. One incision was made on left side of dorsum using a diode laser, and other incisions made using a steel scalpel and diode laser on right side of each rat. Tissues were incised without any elevation of a full thickness flap. Incisions were then sutured with 3-0 polyglycolic acid. Incisions made using steel scalpel served as control group. Wound on left side of each diabetic rat received laser stimulation. Rats were divided into three groups: Group 1, scalpel (n=18); Group 2, diode (n=18); Group 3, diode + laser stimulation (n=18). Erbium&amp;diode laser were used to induce diode laser incision and LLLT. Laser treatment was started immediately after surgery and repeated on 2nd, 4th, 6th and 8th days after surgery (five sessions).</td>
<td>1 laser applied approximately 1 cm from surface of wound</td>
<td>808 GaAlAs laser CW</td>
<td>100</td>
<td>20</td>
<td>10 (total dose)</td>
<td>10 (total dose)</td>
<td>Rats euthanized on days 10 and 20 to compare degree of inflammation and reepithelization in each group</td>
<td></td>
</tr>
<tr>
<td><strong>Gungormus and Akyol</strong>&lt;sup&gt;33&lt;/sup&gt; rat, Wistar, 250-300 g, total of 18. Two incisions (approximately 15 mm length) were made on left and right sides of dorsum of each rat using a diode laser. Wound on left side of each rat received laser stimulation (diode + LLLT group). Incisions on right side used as control group (diode group). Erbium&amp;diode laser used to induce laser incision and LLLT. Laser treatments were started immediately after surgery and repeated on 2nd, 4th, 6th and 8th days after surgery (five sessions).</td>
<td>1 laser applied approximately 1 cm from surface of wound</td>
<td>808 GaAlAs laser CW</td>
<td>100</td>
<td>20</td>
<td>10 (total dose)</td>
<td>10 (total dose)</td>
<td>Rats euthanized on days 10 and 20 to compare degree of inflammation and reepithelization in each group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes on study design and findings

Sex of rats was not reported. Total number of rats does not agree with number given in Table of article. On day 10 there was a significantly lower inflammation for diode + laser stimulation rats compared to scalpel rats and also to diode rats. On day 20 there was no significant difference between groups in inflammation and reepithelization.

Sex of rats was not reported. On day 10 there was a significant difference in inflammation between the groups: a predominance of chronic inflammation in diode laser group whereas diffuse acute inflammation in diode laser + LLLT group. On day 10 there was no significant difference in reepithelization between diode laser and diode laser + LLLT group. On day 20 there was no significant difference between the two groups for inflammation. On day 20 there was a significantly greater degree of reepithelization in the diode laser + LLLT group than in diode group.
### Study Animals in experimental studies and study design

<table>
<thead>
<tr>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
<th>Power (mW)</th>
<th>Power density (mW/cm²)</th>
<th>Time (seconds)</th>
<th>Energy (J) per treatment or day</th>
<th>Energy density (J/cm²) per treatment or day and number of treatments or days of irradiation</th>
<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gungormus and Akyol²⁴ rat</td>
<td>1150</td>
<td>808 GaAlAs laser CW</td>
<td>100</td>
<td>20</td>
<td>10 (total dose)</td>
<td>Diabetic and non-diabetic rats euthanized on days 10 and 20 to compare degree of inflammation and reepithelization in each group</td>
<td></td>
</tr>
<tr>
<td>Meireles et al.²⁵ rat</td>
<td>2 mm diameter</td>
<td>660 laser</td>
<td>35</td>
<td>5 per point, total 20</td>
<td>7, 14, and 21 days post-burn</td>
<td>Rats euthanized on 3, 5, 7, 14, and 21 days post-burn. Wound specimens fixed, sectioned, stained with H&amp;E and examined by histology</td>
<td></td>
</tr>
</tbody>
</table>

On day 10 there was a significantly lower degree of inflammation in nondiabetic scapel group than in diabetic scapel group. The degree of inflammation for diabetic scapel with laser stimulation was significantly lower than in diabetic scapel group. Also degree of reepithelization for diabetic scapel with laser stimulation was significantly greater than in diabetic scapel group. On day 20 there was no statistically significant differences between all groups for inflammation and reepithelization.

At day 7 in control diabetic rats, while the acute inflammatory reaction was mostly intense, the chronic inflammatory reaction was less. In diabetic rats treated with 660 nm laser, acute inflammatory reaction was moderate in all subjects, and chronic inflammatory reaction was either slight or moderate. In 780 nm laser-treated diabetic rats the acute inflammatory reaction was slight. Diabetic rats treated with laser energy 660 nm had positive effects on amount and quality of granulation tissue, fibroblast proliferation, collagen deposition and orientation, epithelization and local microcirculation.
<table>
<thead>
<tr>
<th>Study</th>
<th>Animals in experimental studies and study design</th>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
<th>Power (mW)</th>
<th>Power density (mW/cm²)</th>
<th>Time (seconds)</th>
<th>Energy (J) per treatment or day and number of treatments or days of irradiation</th>
<th>Energy density (J/cm²) per treatment or day and number of treatments or days of irradiation</th>
<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yasukawa et al.</td>
<td>Sprague-Dawley, male 238–330 g, 8 weeks, 55 in total. 10 mm operative wounds made using scalpel on dorsal thoracic region, dorsal lumbar region and dorsal buttock region. Each wound was closed with simple interrupted 4-0 nylon sutures (3 sutures/wound). Rats were divided into 11 groups (n=5): one group as a non-irradiation control, and 10 irradiation groups which received either 8.5 or 17.0 mW of 15 seconds laser irradiation per operative wound a day with one of five different irradiation frequencies i.e. daily (from 1st to 6th day following surgery), every other day (1st, 3rd, 5th day), on only 1st day, on only 3rd day, on only 5th day; 1st day was day following surgery. Animals sacrificed on 7th day. Specimens of dorsal skin with operative wounds harvested from all rats.</td>
<td>Tip of laser probe 10 mm from operative wound</td>
<td>632.8 He–Ne</td>
<td>8.5</td>
<td>139</td>
<td>15</td>
<td>2.09</td>
<td>4.21</td>
<td>Tensile tests and histological examination of skin specimens collected on 7th day after surgery. Sections stained with H&amp;E and observed under a light microscope.</td>
</tr>
<tr>
<td>Viegas et al.</td>
<td>Wistar, male 200–250 g, 64 in total. A standardized circular wound 8-mm diameter was created on dorsum of each rat using a surgical punch. Rats were divided into four groups: Group A, control; Group B, received i.m. injection of meloxicam (1 mg/0.1 ml) immediately after surgery and at 48 hours; Group C, irradiated with laser 685 nm; Group D, irradiated with laser 830 nm. Groups C and D received laser treatment immediately after surgery and at 48 hours post-injury.</td>
<td>0.02827 Laser energy applied trans-cutaneously in 4 equidistant points around wound margin</td>
<td>685 laser CW</td>
<td>35</td>
<td>180</td>
<td>1 per session</td>
<td>Rats euthanized at 12, 36, 72 hours and 7 days. Wound specimens fixed, sectioned, stained with H&amp;E and examined by histology. Expression of IL-1 beta in specimens evaluated by PCR.</td>
<td>Only Group B showed decreases in intensity of polymorphonuclear infiltrates and edema. Rats in Group C showed the best healing pattern. Quantification of IL-1 beta mRNA did not show any reduction in inflammatory process in laser irradiated groups compared to other groups.</td>
<td>Only Group B showed decreases in intensity of polymorphonuclear infiltrates and edema. Rats in Group C showed the best healing pattern. Quantification of IL-1 beta mRNA did not show any reduction in inflammatory process in laser irradiated groups compared to other groups.</td>
</tr>
<tr>
<td>de Araujo et al.</td>
<td>Swiss, male approximately 30 g, 30 in total. Two standardized 6-mm diameter circular full-thickness wounds were made on the median region of dorsum using skin biopsy punch. Wounds were separated by 1 cm from each other. Experimental lesion was laser irradiated. Lesions that were not irradiated served as control. Lesions were randomly divided into laser or control group. Laser group lesions were irradiated at 1, 5, 8, 12 and 15 days post-wounding (p.w.). Two circular skin biopsies with 8-mm diameter containing total dermis were collected.</td>
<td>Laser beam diameter approximately 2 mm expanded to 6 mm</td>
<td>632.8 He–Ne laser</td>
<td>10</td>
<td>180</td>
<td>1 per irradiation</td>
<td>Mice were sacrificed on 8, 15 and 22 days p.w. Before sacrifice, animals were injected with ^3H-proline. Wound specimens were used for TEM, light microscopic studies including autoradiography and immunohistochemistry.</td>
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</tbody>
</table>
Laser irradiated wounds showed a faster reepithelization than control wounds. Laser treatment reduced the local inflammation and appeared to influence the organization of collagen fibrils in the repairing areas. Pinheiro et al. [39] rat, Wistar, male age approximately 4 months, 30 in total. One standard excisional wound was created on dorsum of each rat with a scalpel. Rats were divided into five groups (n=6): Group 1, untreated control; Group 2, laser therapy (685 nm, 20 J/cm²); Group 3, laser therapy (685 nm, 40 J/cm²); Group 4, polarized light (400–2000 nm, 20 J/cm²); Group 5, polarized light (400–2000 nm, 40 J/cm²). Rats in Groups 2, 3, 4, and 5 were transcutaneously irradiated or illuminated immediately and at every 48 hours during 7 consecutive days. At the end of the experimental period, rats were sacrificed and specimens taken for light microscopy.

Control specimens showed presence of ulceration, hyperemia, discrete edema, intense and diffuse inflammation, irregular collagen deposition, and myofibroblasts arranged parallel to wound margins. Wounds treated by laser therapy at 20 J/cm² showed mild hyperemia, inflammation varied from moderate to intense, number of fibroblasts was large, and distribution of collagen fibres was more regular.

Increasing the laser dose to 40 J/cm² evidenced exuberant neovascularization, severe hyperemia, moderate to severe inflammation, large collagen deposition, and fewer myofibroblasts. Mendez et al. [40] rat, Wistar, male 200–250 g, 60 in total. Rats divided into seven groups: Group 1, control; Group 2, laser 685 nm 20 J/cm²; Group 3, laser 830 nm 20 J/cm²; Group 4, laser 685 nm and 830 nm 20 J/cm²; Group 5, laser 685 nm 50 J/cm²; Group 6, laser 830 nm 50 J/cm²; Group 7, laser 685 nm and 830 nm 50 J/cm². A single standardized wound 8-mm² circular defect 1 mm deep was created on dorsum of each rat with a punch. Immediately after surgery, all groups except control were irradiated and additional laser sessions were carried out at 48-hour intervals during 7 consecutive days. Irradiation performed transcutaneously on 4 points 2 mm of border of wound, fractionating dose of each session when two wavelengths used. Total dose varied according to time of sacrifice. At end of experimental periods of 3, 5, and 7 days, two control and three experimental animals were sacrificed. Wound specimens were collected.

Histology of Group 4, Group 6 and Group 7 indicated that inflammation was in different stages at end of experimental period. There was regression in inflammation when compared to controls.
<table>
<thead>
<tr>
<th>Study</th>
<th>Animals in experimental studies and study design</th>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
<th>Power (mW)</th>
<th>Power density (mW/cm²)</th>
<th>Time (seconds)</th>
<th>Energy (J) per treatment or day</th>
<th>Energy density (J/cm²) per treatment or day and number of treatments or days of irradiation</th>
<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whittaker et al.⁴¹</td>
<td>Sprague-Dawley, female mean 390 g. Chest opened through fourth intercostal space to expose basal region of heart. Left coronary artery was occluded by tying 5-0 polypropylene suture around the artery. Rats (n=25) that survived to 24 hours after occlusion were randomly assigned to either 5 or 10 mW laser treatment or sham treatment. Rats were subjected to 3 minutes exposures twice daily for 4 days, beginning on second day after infarction. Laser was placed in contact with skin above fifth intercostal space and pointed towards the heart. At 7 days after infarction, hearts were removed, aorta cannulated, and hearts connected to column of formalin set to height equivalent to a pressure of 15 mm. In this way, hearts were fixed in an approximation of the end-diastolic configuration.</td>
<td>780 GaAlAs laser</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td>0.64</td>
<td>1.27</td>
<td>A tissue slice from centre of infarct in each heart was processed for histology and sections stained with H&amp;E and Picrosirius red. Extent of infarct healing assessed by measuring degree of inflammatory cell infiltration into infarct and collagen content.</td>
</tr>
</tbody>
</table>

The thicker laser-treated infarcts often contained large regions of necrotic muscle devoid of inflammatory cells. The proportion of infarct devoid of inflammatory cells was greater in hearts exposed to 5 mW treatment dose than in shams. Collagen content of infarcts did not differ between groups in any region of infarct. Inhibition of inflammation by 5 mW laser treatment dose and failure to increase collagen indicated that healing was not enhanced.
Table 11 Laser and wound healing: animal studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Animals in experimental studies and study design</th>
<th>Irradiated area or spot size (cm²)</th>
<th>Wavelength (nm)</th>
<th>Power (mW)</th>
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<th>Energy density (J/cm²) per treatment or day and number of treatments or days of irradiation</th>
<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow</td>
<td>Nunez et al.¹² rat, Wistar, male approximately 300 g, 15 in total. Rats were divided into three groups. Flow registers were performed at two selected sites on the skin, over the vertebral column in anterior-posterior direction. The first site, denoted as lesion site (LS), was located at middle of the back at 3 cm from base of tail and received an injury. The second site, denoted as control site (CS), was located at 1 cm from base of tail. Distance of laser flow meter probe from skin was adjusted using 1 mm spacer. For both sites (LS and CS) a 6 mm diameter area was selected in which three 30 second-distinct measurements were carried out to calculate a mean blood flow at each site. In the LS, 6 mm diameter lesions were produced using a cylindrical brass rod cooled to 77 K. Contact was made in two sequences of 15 seconds each with an interval of 5 minutes. Laser of flow meter produced a small spot over measured area, resulting in an irradiance of 2.0 W/cm²; averaged irradiance over entire lesion area was 3.5 mW/cm². Five rats comprising Flowmeter Control Group had their blood flow monitored on 1st day before lesion, immediately after lesion and then on 14th and 21st days. Five rats denoted as Laser Group received laser treatment at days 1, 2 and 3. Skin blood flow registers were performed before lesions, immediately after lesions prior to irradiation, and 7 and 20 minutes after laser irradiation. Five rats of Laser Control Group received same procedure as Laser Group with exception of He–Ne laser treatment.</td>
<td>Spot diameter 6 mm = area 0.2828</td>
<td>632.8 He–Ne laser polarized CW</td>
<td>10</td>
<td>6</td>
<td>180</td>
<td>1</td>
<td>Blood flow measurements at LS and CS</td>
<td></td>
</tr>
</tbody>
</table>

Notes on study design and findings

Laser Doppler flowmetry technique did not cause significant alterations in blood microcirculation during skin repair process. No significant sustained effect on microcirculation was found with 6 mW, 1 J/cm² He–Ne laser dose.

The authors concluded that further studies are needed to test influence of other sets of laser parameters on blood microcirculation.
<table>
<thead>
<tr>
<th>Study</th>
<th>Animals in experimental studies and study design</th>
<th>Irradiated area or spot size (cm²)</th>
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<th>Wound outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth factor and cytokine expression</td>
<td>Rocha Junior et al.¹⁵, Wistar, male 160–220 g, 6–8 weeks, 30 in total. Rats randomly divided into two groups: Group 1 (controls, n=15) and Group 2 (laser treatment, n=15). A circular fragment of skin was removed from dorsum using a 10-mm punch. In Group 2, experimental wounds were laser irradiated, with the treatment performed three times for each rat, with first session immediately post-surgery, second one 48 hours later, and third one 7 days post-surgery. In control group, no laser treatment was given. At 10 days post-surgery animals were sacrificed and samples from skin lesions collected.</td>
<td>870</td>
<td>Ga-As laser</td>
<td>15</td>
<td>15</td>
<td>3.8</td>
<td>Presence of TGF-β₂ in skin samples investigated in sections of formalin-fixed tissue by incubation with rabbit anti-TGF-β₂ antibody. Also in situ detection of apoptosis using Apoptag Plus in-situ detection kit</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes on study design and findings
TGF-β₂ was expressed within connective tissue by inflammatory cells, fibroblasts, and endothelial cells. There were more TGF-β₂-positive cells, recognized morphologically as fibroblasts, endothelial cells, and inflammatory cells, in connective tissue in samples from laser-treated group compared to control group.
side was laser-irradiated 808 nm, 10 J/cm² and on day 10 showed diffuse acute inflammation, whereas a predominance of chronic inflammation was present in the non-irradiated wound on the right side. In the other study, a wound was made with a scalpel in one of three different locations on the dorsum, sutured, and laser irradiated 632.8 nm, 2.09 or 4.21 J/cm². Of the various irradiation frequencies tested, irradiation with 4.21 J/cm² every other day was found to provide the most significant benefits in terms of prevention of excessive inflammation and increased formation of collagen fibres and repair of tissues. In both studies of incisional wounds in normal animals there was an alteration in the degree of inflammation.

Three of the rat studies, and the one performed in the mouse, created excisional wounds on the dorsum in normal animals (usually a single circular wound made using a skin punch). In one of the rat studies, wounds were irradiated at 685 or 830 nm, or using both wavelengths, and different total doses. Using the combined wavelengths at 20 J/cm² per session, and also for 830 nm, 50 J/cm² per session, and 685 nm, 50 J/cm² per session, there was a regression of inflammation when compared to non-irradiated wounds.
wounds. In the other two rat studies, which also used irradiation at 685 and 830 nm, no reduction in the inflammatory process was observed in the laser-treated wounds. In the mouse study, laser treatment 632.8 nm, 1 J/cm² per irradiation at 1, 5, 8, 12 and 15 days post-wounding reduced the local inflammation and influenced the organization of collagen fibres in the repair areas. Hence, a decrease in inflammation of excisional wounds in normal animals was found in 50% of the studies.

One of the rat studies investigated the effects of laser irradiation 780 nm at 0.64 and 1.27 J/cm², applied transcutaneously in the fourth intercostal space, on heart infarct healing. The thicker laser-treated infarcts often contained large regions of necrotic muscle devoid of inflammatory cells. The proportion of infarct devoid of inflammatory cells was greater in hearts exposed to skin irradiation 0.64 J/cm².

Blood flow. A study in the rat measured blood flow at two sites, lesion and control, on the dorsum situated at 3 and 1 cm from the base of the tail. The lesion was produced by placing a metal rod cooled to 77 K in contact with the skin in two sequences of 15 seconds each. Lesions were laser-irradiated at 632.8 nm, 1 J/cm² on days 1, 2 and 3. No significant sustained effect on the microcirculation was found.

Growth factor and cytokine expression. A study in the rat reported more TGF-β2-positive cells in the connective tissue of circular excisional skin wounds after laser irradiation at 870 nm, 3.8 J/cm² than in non-irradiated wounds. These cells were recognizable morphologically as fibroblasts, endothelial cells, and inflammatory cells.

Discussion
This review aimed to provide an overview of the evidence for the scientific bases of the main effects of low-level laser light that underpin two of its most important clinical applications. These include relief of pain, decreased inflammation, increased blood flow to tissues, enhanced wound healing and tissue regeneration. Many of these effects are inter-related: for example, a decrease in inflammation, and an increase in blood flow to tissues, would lead to a reduction in pain; also, an earlier resolution of the inflammatory response together with increased blood flow and tissue regeneration would accelerate wound healing. A total of 31 eligible studies in humans and common laboratory animals were included in this review based upon EndNote PubMed searches for laser acupuncture and laser-stimulated wound healing, which were two chosen clinical applications of laser light. Most of the studies had used red or infrared laser light, and were evaluated in terms of whether they provided support or otherwise for the scientific bases mentioned above. The consensus reached from the included studies was positive in terms of supporting the rationale for laser intervention.

This review highlighted a number of important issues relating to the nature of study designs and protocols used, the types of conditions that were treated, and the appropriateness of these to physical therapy applications in humans, and the relevance of irradiation parameters to any observed effects.

Research design and protocols used
In many studies, patients were blinded to the treatment given, and frequently a randomized, single- or double-blinded, placebo-controlled, crossover design was used. However, in some instances, laser light and electrical treatments were combined (e.g. patients with carpal tunnel syndrome received laser acupuncture and TENS) and it was not possible to determine the relative contribution of either treatment to the observed effects. Furthermore, some patients received medications and supplements which might have influenced reported outcomes.

As might be expected, both subjective and objective measurements were used in the human studies (e.g. pain scores on a 10-point visual verbal analogue scale, as well as more objective measures such as range of movement, median nerve sensory peak latency, motor latency, laser Doppler flowmetry), whereas all measurements in the animal studies were objective.

There was considerable variation in the number of patients and gender representation in the human studies. In one carpal tunnel syndrome study there were 11 patients (two female, nine male) while in another study there were 31 patients (22 female, 9 male). It is important that there are adequate numbers of females and males especially in regard to carpal tunnel syndrome, for which there is a much higher incidence in females. Major differences in the numbers of male and female patients are likely to limit the ability to explore whether gender has any significant effect on observed outcomes of treatment. Two of the wound healing studies reported on only two patients: in one study, a female and a male, with ulcerative lesion of lower labial frenulum and tongue, respectively, caused by radiation therapy; in the other, a female and a male with internal mucosa and lower lip traumatism. In other studies, the numbers of females and males were not reported. There were also marked variations in mean age for the human studies, which ranged from 2 to 60 years.

It was noticed that different techniques had been used in human and animal studies for measuring outcomes of laser treatment. For example, in female patients with chronic pelvic inflammation and treated...
with laser acupuncture, the degree of inflammation was determined by tenderness, presence of a mass on palpation, or by ultrasound examination.\textsuperscript{19} In the wound healing studies with patients having internal mucosa and lower lip traumatisms, and female patients with mastitis, the effect of laser irradiation on inflammation of the lip mucosa was assessed clinically,\textsuperscript{28} and for the breast by MRI scans.\textsuperscript{29} In the laser acupuncture animal studies, inflammation was induced by intraplantar injection of heat-killed \textit{Mycobacterium tuberculosis} in complete Freund’s adjuvant or intraperitoneal administration of cyclophosphamide to induce cystitis, and inflammation was assessed from paw volume and histological analysis of urinary bladder.\textsuperscript{24} Histological analysis was used for assessing inflammation of laser irradiated wounds in rats.\textsuperscript{40} Also in the animal studies on laser-irradiated wound healing, different experimental models had been used. In some, incisional wounds were made on the dorsum of rats, sutured, and then irradiated. However, in studies involving irradiation of excisional wounds in the rat and mouse (which are loose-skinned animals), the wounds would have healed largely by contraction, as part of which wound margins are drawn inwards. This differs from the healing of excisional wounds in humans (fixed-skinned), in which re-epithelization and granulation tissue formation are the main processes of healing. To mimic the healing of human wounds, the excisional wounds in rat and mouse need to be splinted by use of metal or silicone rings attached to the skin using adhesives and/or sutures,\textsuperscript{45–48} or adhesive dressings attached to the skin with adhesive agents.\textsuperscript{49,50} In our previous review on laser photobiomodulation of wound healing in the rat and mouse (using different search terms and based on a large number of articles, and including outcomes such as area, histological and immunohistochemical analysis of wounds),\textsuperscript{51} very few studies had covered the wounds with an adhesive dressing; in those that did, the dressings remained in place only for a few days.

\textbf{Types of conditions treated and appropriateness to physical therapy applications}

A wide range of conditions had been treated in human patients including whiplash injury, carpal tunnel syndrome, radicular and pseudoradicular pain syndromes, chronic pelvic inflammation, depression, ulcerative lesions of lower labial frenulum and tongue, internal mucosal and lower lip ulceration, mastitis, carcinoma of pharynx and oral cavity, and diabetic microangiopathy.\textsuperscript{15–20,27–31} In addition, normal healthy volunteers were used for studying the effects of laser treatment on skin blood flow and muscle oxygenation, blood flow in anterior cerebral artery, posterior cerebral artery, middle cerebral artery, ophthalmic artery and cerebral oxygenation.\textsuperscript{20–23} While many of these conditions are relevant to clinical practice, studies involving laser-dependent reductions in pain and inflammation associated with ionizing radiation treatment for cancer must be interpreted with caution and it is questionable whether the findings can be directly extrapolated to skeletal muscle and tendon injury. Also resolution of oral mucositis cannot be equated with the healing of chronic skin wounds. However, in spite of these reservations, treatment of whiplash injuries, carpal tunnel syndrome, other pain syndromes, depression, diabetic microangiopathy by laser treatment provides a non-invasive procedure that has been shown to be successful in bringing about improvement and in some cases a resolution of symptoms over a considerable period of time. For patients with carpal tunnel syndrome, this avoids a need for surgery and loss of time spent recovering post-operatively. Studies in healthy volunteers showing that blood flow can be increased in the anterior cerebral artery and posterior cerebral artery by laser intervention holds promise for treating patients with early warning signs of an ischemic stroke. Increased blood flow in the ophthalmic artery by laser acupuncture could benefit patients including smokers, patients with diabetes, or of advanced age to retard or decrease glaucomatous damage to the eyes.\textsuperscript{52} In addition, that skin blood flow and muscle oxygenation can be increased by laser irradiation provides a modality for treating slow-to-heal skin ulcers, such as in diabetic patients, and muscle ischemia leading to muscle fatigue.

\textbf{Relevance of laser parameters to observed effects}

In the studies reviewed, laser irradiation at wavelengths ranging from 514 (Argon laser) to 10 600 nm (CO\textsubscript{2} laser) had been used and gave positive outcomes. There was a very wide range of energy doses employed: for the human studies, the dose varied from 0.04 to 36 800 J/cm\textsuperscript{2}; for the animal studies it varied from 0.021 to 50 J/cm\textsuperscript{2}, with the majority of the wound healing trials performed with 2 to 10 J/cm\textsuperscript{2}, which is in keeping with that considered to be most suitable for stimulating wound healing.\textsuperscript{51} One of the human studies using an extremely low dose of 0.075 J/cm\textsuperscript{2} was for patients with whiplash injury, and reported no significant benefit of treatment; a higher dose would have been beneficial. The other study using a low dose was for carpal tunnel syndrome patients, where an infrared laser was applied to five deeper acupuncture points with doses ranging from 1.8 J/cm\textsuperscript{2} at the highest frequency to 0.04 J/cm\textsuperscript{2} at the lowest frequency.

\textbf{Conclusion}

Based upon the current review, clinical applications of laser irradiation in human and animal subjects in
the areas indicated are well founded in the scientific evidence relating to a reduction of pain and inflammation, and an increase in blood flow and tissue repair. A re-examination and re-defining of laser parameters (wavelength, power, power density, spot size, time of irradiation, energy, energy density, number of irradiations, interval between irradiations) based on new well-designed trials in human subjects and improved animal models such as splinted wounds of genetic diabetic mice should potentially lead to further improvements in treatment outcomes. In the future, there is likely to be increased clinical use of laser therapy for the treatment of inflammation and pain following injury, in stimulating wound healing by direct laser irradiation over the skin, skeletal muscle and/or tendon, and for reducing scarining.

References

18 Knasner BD, Hamzavi FH, Murakawa GJ, Hamzawi H. Low level laser technologies for pain relief and wound healing Physical Therapy Reviews 2010 VOL. 15 NO. 4


