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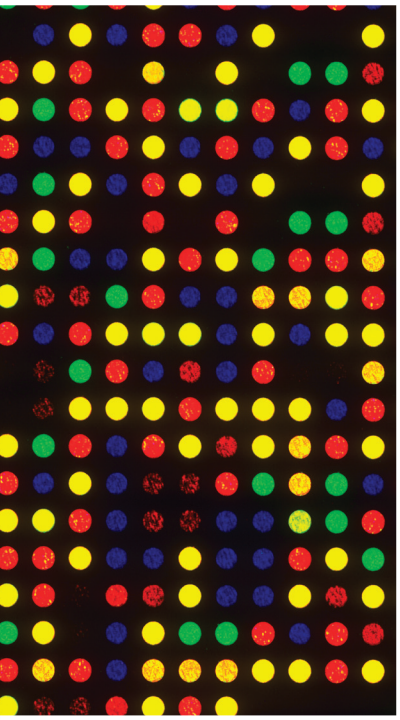
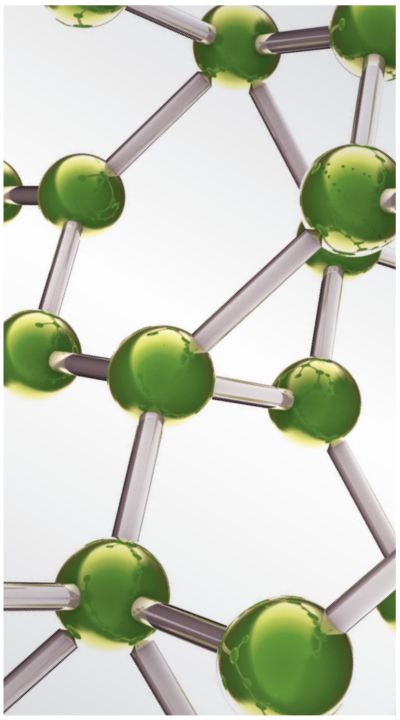
High-Tech Acupuncture and Integrative Laser Medicine 2013

Guest Editors: Gerhard Litscher, Xin-Yan Gao, Lu Wang, and Bing Zhu



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Editorial Board

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Editorial

High-Tech Acupuncture and Integrative Laser Medicine 2013

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This is an annual special issue. The current issue is the 2013 issue which includes eleven interesting papers.

Acupuncture has been used for medical treatment for thousands of years. A large number of empirical data is available, but the technical quantification of effects was not possible up to now. Using electroacupuncture, needle, or laser stimulation and modern biomedical techniques, it was possible for the first time to quantify changes in biological activities caused by acupuncture.

As mentioned above, this special issue contains eleven publications, of which six are related to laser acupuncture, three to electroacupuncture, two to needle acupuncture, and one to photoluminescent bioceramic material. Apart from body acupuncture, special emphasis is also given to auricular acupuncture. One review article, 22 pages in length, deals with the topic auricular acupuncture with laser. The investigations cover animal experimental studies and studies in healthy volunteers, preterm neonates, and patients, as well as basic and clinical research on evidence-based high-tech acupuncture, integrative laser irradiation, and translational medicine.

It has to be mentioned that this annual special issue 2013 contains, among others, the following topics:

(i) modernization of acupuncture (evidence-based medicine, integrative laser medicine),

(ii) high-tech acupuncture,

(iii) development of innovative acupuncture stimulation methods,

(iv) scientific evaluation of complementary medical methods (acupuncture, electroacupuncture, and low level laser therapy),

(v) clinical effects of acupuncture,

(vi) intravenous laser blood irradiation,

(vii) laser needle acupuncture,

(viii) red and infrared laser stimulation,

(ix) auricular acupuncture with laser,

(x) biomedical assessment of acupuncture,

Modernization of acupuncture is performed by high-tech acupuncture and integrative laser stimulation. Eastern and Western medicine are getting closer to each other using modern biomedical engineering technology, as described in this annual special issue.

Conflict of Interests

The authors state that there is no conflict of interests regarding the publication of this editorial.

Acknowledgments

As in the first annual special issue, the Lead Guest Editor again wishes to thank the other three Guest Editors, Professor Bing Zhu, (Director of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, Beijing, China), Associate Professor Xin-Yan Gao (Head of

the Department of Physiology, Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, Beijing, China), and Professor Lu Wang (Stronach Research Unit for Complementary and Integrative Laser Medicine and TCM Research Center Graz, Medical University of Graz, Graz, Austria), for the excellent cooperation. In this context, I would also like to thank all authors for their excellent contributions and patience during the review process. The work of all reviewers on the papers within this special issue is highly appreciated.

We want to thank Ms. Ingrid Gaischek (Stronach Research Unit for Complementary and Integrative Laser Medicine, Research Unit of Biomedical Engineering in Anesthesia and Intensive Care Medicine, and TCM Research Center Graz, Medical University of Graz, Graz, Austria) for her valuable support in every respect.

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Lu Wang
Bing Zhu

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Research Article

Clinical Effect of Acupuncture on Endemic Skeletal Fluorosis: A Randomized Controlled Trial

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Academic Editor: Lu Wang

Objective. To evaluate the effect of acupuncture on endemic skeletal fluorosis (ESF) through the randomized controlled trial. *Methods*. Ninety-nine cases were divided into the treatment group (68 cases) and the control group (31 cases) randomly. Normal acupuncture combined with electroacupuncture was used in treatment group, while Caltrate with vitamin D tablets were applied in control group. After 2 courses, the VAS, urinary fluoride, serum calcium, and serum phosphate were evaluated before and after treatment. *Results*. Both of these two methods could relieve pain effectively and the effect of acupuncture was better ($P < 0.05$). In treatment group, the content of urinary fluoride after treatment was higher than before ($P < 0.05$), while the content of serum calcium and phosphate was lower ($P < 0.05$). *Conclusion*. The effect of acupuncture on relieving pain and promoting discharge of urinary fluoride is better than that of western medicine. Acupuncture can reduce the content of serum calcium and phosphate.

1. Introduction

Endemic fluorosis, known as endemic skeletal fluorosis (ESF), is a kind of chronic poisoning disease caused by excessive intake of fluorine for a long time during people's lives. It usually includes dental and skeletal fluorosis. The disease, endemic skeletal fluorosis in drink-water type, is caused by high content of fluorine in drinking water in prevalent areas. At present, the pandemic disease has affected 35 countries and regions.

The pathogenesis of skeletal fluorosis is unclear, so the method of descending fluorine though water improvement is the only effective one [1, 2], but it needs a long time for the elimination of fluorine in body and the improvement of clinical symptoms. Drug therapy usually includes chemical elements such as calcium, magnesium, and aluminum, vitamins, and amino acid [3]. Though these drugs can produce certain effect, their unstable effect and side effects still should be considered.

This study applies acupuncture therapy to treat skeletal fluorosis, which promotes qi circulation, regulates meridians, dredges collaterals to relieve pain, and improves the joint motion. The mechanism of acupuncture analgesia has been studied profoundly and gets a positive effect. The dysfunction of joints actually belongs to a fibrous ankylosis, a reversible change, which results from low tensity of muscle, tendon, and ligament, around the affected joint after pain relief [4]. The calcium can coordinate with fluorion in the intestinal tract, and the coordination compound-calcium fluoride is excreted to lower the absorption of fluorion; on the other hand, the diet structure with profuse calcium is suitable for patients with skeletal fluorosis [5].

The study is designed as a randomized controlled trial and obeys the scientific research principle of random, blindness, control, and so forth, also follows the TCM treating principle of promoting blood circulation to remove blood stasis, dispelling wind to eliminate dampness, and dredging collaterals to resolve pain, and aims to evaluate the acupuncture treatment on skeletal fluorosis for 99 patients by means of clinical outcomes as pain alleviation, urinary fluoride, serum phosphate, and serum calcium.

2. Materials and Methods

2.1. General Data. From May 2009 to June 2010, the study enrolled 99 patients suffering from skeletal fluorosis

diagnosed by WS 192–2008 health industry standard of China. All of them lived in Caosi village, Qing country, Cangzhou, Hebei province (the fluorine content in water from 2.67 to 7 mg/L).

2.2. Inclusive and Exclusive Criteria. Inclusive criteria are as follows:

(1) age from 30 to ~75 years old (including 30 and 75 years old);

(2) the syndrome which is blood stasis and phlegm and dampness blockage (the detailed syndrome differentiation can be referred in the addendum);

(3) life history with long-time consuming water of high-content fluorine;

(4) in accordance with WS 192-2008 health industry standard of China;

(5) patients who signed the informed consent volunteer.

Exclusive criteria are as follows:

(1) the etiology that was due to burning the coal with high fluoride or drink the brick-tea water with high fluoride content;

(2) the disease that is at the severe stage, and also patients suffers from bedridden status, paralysis, or cannot live without others;

(3) complications of severe diseases from primary cardiocerebral vascular, hepatic, nephritic, hemopoietic, and nervous systems, drug addicts, or psychopaths;

(4) patients suffering from hepatitis B, tuberculosis, and malignancy;

(5) women with pregnancy or lactation;

(6) trauma of bone or joints within 2 months, without fully recovery;

(7) continuous intake of medicine for tonifying kidney or calcium supplement 3 months before treatment;

(8) intake of purified or cleaned water with normal content of fluoride;

(9) severe adverse reaction to acupuncture, Chinese herbal medicine, and western medicine of the study (eg., fainting during acupuncture, drug allergy).

2.3. Method. The randomized controlled method was conducted, and the stratification method was also employed with severity of disease as the layering factor. The ratio of patients assigned to the treatment and control group was 2 : 1, and the sequence number was generated and kept in the randomized center. Finally, the treatment group consisted of 27 male patients and 42 female patients (3 patients dropped off), with age 54.76 ± 10.22 years old; the control group consisted of 12 male patients and 19 female patients (5 patients dropped off), with average age of 60.03 ± 9.44 years old.

The treatment group (acupuncture group) based on the TCM treating principle of promoting blood circulation to remove blood stasis, dispelling wind and eliminating dampness, as well as dredging collaterals to resolve pain, employed the acupuncture treatment with electroacupuncture. The basic points group was combined with local points group.

The Basic Points. The basic points are Dazhui (Du 17), Geshu (BL 17), Quchi (LI 11), Hegu (LI 4), Xuehai (SP 12), Yinlingquan (SP 10), Sanyinjiao (SP 6), and Fenglong (ST 40).

Local Points. Local point is added in the case of pain in the local part (shoulder: Jianyu (LI 15); elbow: Chize (LU 5), wrist: Yangchi (SJ 4); neck: Tianzhu (BL 10); waist: Yaoyangguan (DU 3); hip: Huantiao (GB 30) or Juliao (GB 29); knee: Dubi (ST 35); ankle: Jiexi (ST 41)). And a local Ashi point was also combined.

Points Applied with Electroacupuncture. The basic points are Quchi (LI 11) and Hegu (LI 4) in the same side, Quchi (LI 11) and Hegu (LI 4) in the same side, and local points in both sides. The dilatational wave was applied.

The Frequency of Treatment. Once every other day, 30 min for each time, and points without electroacupuncture were applied with lifting and thrusting and rotating manipulation by hand for 1 min every 15 min, with reducing method. The treatment was applied 3 times a week, 1 course lasted for 1 month, and 2 courses in total.

The control group (Caltrate with vitamin D tablets group) based on rectifying the disorder of calcium-phosphorus metabolism and enhancing the fluoride dispelling applied Caltrate with Vitamin D tablets (Tianjin Wyeth Pharmaceutical, 600 mg/tablet, 60 tablets/bottle) to patients. The oral intake method was 600 mg one time, twice a day, one in morning, and the other in the evening. One course lasted for 1 month and 2 courses in total. The study was approved by the Ethics Committee of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, and written informed consent was obtained from all the patients.

2.4. Clinical Outcomes

2.4.1. VAS Scores of Joint Pain. Visual Analogue Scale (VAS) measured the pain intensity with a 10 cm scale ruler. One end was marked with no pain, and the other end with worst pain. A moving ruler slid from 0 to ~10 in the front, and 0~10 scale numbers just corresponded in the back. Patients were asked to slide the ruler to the position that symbolized the pain intensity for themselves, and the observer got the corresponding scale number in the behind to be the pain score. In front of the ruler, there was no mark, the left end was no pain, and the right end was the worst pain (Figure 1).

2.4.2. Urinary Fluoride. Urinary fluoride was measured by electrode method using the Urinary Fluoride Tester (HACH-F523) in the laboratory department of Caosi Center Hospital

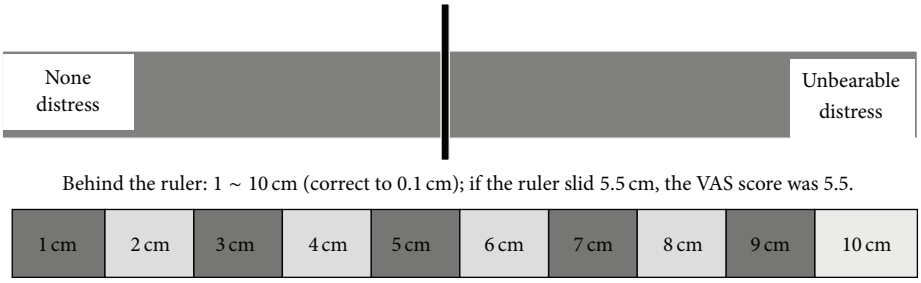

Figure 1: The VAS ruler. VAS scores were measured before and after treatment in both groups.

of Hebei Province. The analysis and calculation was based on the standard curve method (WS/T 89-1996 health industry standard of China). Urinary fluoride was measured before and after treatment in both groups.

2.4.3. Serum Phosphorus and Serum Calcium

Serum Calcium. Methyl thyme phenol blue colorimetric method (MTB): the adult: 2.03~2.54 mmol/L; children: 2.25~2.67 mmol/L.

Serum Phosphorus. Molybdenum acid salt method: the adult: 0.97~1.45 mmol/L, children: 1.45~2.1 mmol/L.

Both of them were measured in the laboratory department of Caosi Center Hospital of Hebei Province.

2.4.4. Statistic Analysis. SPSS 17.0 was adopted to analyze data. According to the clinical data and certain statistical principle, the baseline, therapeutic effect, and safety were analyzed and expressed by mean ± standard deviation for measurement data. Measurement data was dealt by normality test, and if it was in accordance with normal distribution, t test would be employed then; if the measurement data was not in accordance with normal distribution, parametric test would be employed after data altered to normal change. If the data was not in accordance with normal distribution, rank sum test was adopted. The rank sum test was also adopted for the ranked data. $P < 0.05$ (bilateral sides) meant comparison between groups had statistical significance.

3. Results and Discussion

3.1. Results

3.1.1. General Data. There was no statistical significance between the water fluoride in residential area, time of residence, and labor intensity of two groups ($P > 0.05$) (Tables 1(a) to 1(c)).

3.1.2. VAS. The VAS after treatment in two groups both decreased than those before treatment (both $P < 0.01$) (Figure 1). The effect on decreasing VAS in treatment group

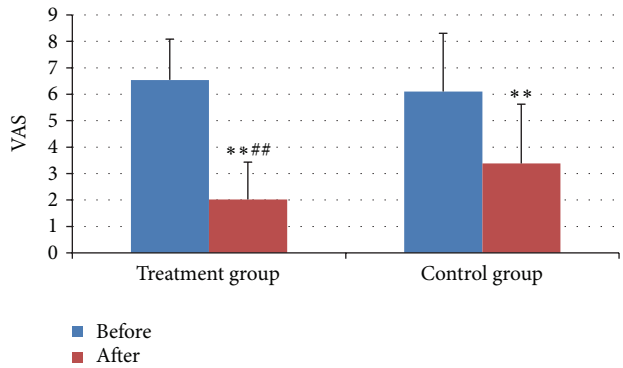

Figure 2: VAS before and after treatment in two groups. $^{**}P < 0.01$, compared with that before treatment; $^{\#\#}P < 0.01$, compared with control group.

Table 1: (a) Water fluoride in residential area between two groups. (b) Time of residence between two groups. (c) Labor intensity between two groups.

(a)

Group	Cases	Water fluoride ($\overline{X}$ ± SD)	Z	P
Treatment group	68	4.48 ± 1.44	−1.002	−0.316
Control group	31	4.88 ± 1.73		

(b)

Group	Cases	Time of residence ($\overline{X}$ ± SD)	Z	P
Treatment group	68	44.21 ± 16.72	−0.151	0.88
Control group	31	44.65 ± 17.44		

(c)

Group	Cases	Labor intensity ($\overline{X}$ ± SD)	Z	P
Treatment group	68	1.75 ± 0.72	−1.22	0.219
Control group	31	1.58 ± 0.77		

was better than that in control group, and the difference was statistically significant ($P < 0.01$) (Figure 2).

3.1.3. Urinary Fluoride. The urinary fluoride after treatment was higher than that before in treatment group (Figure 3).

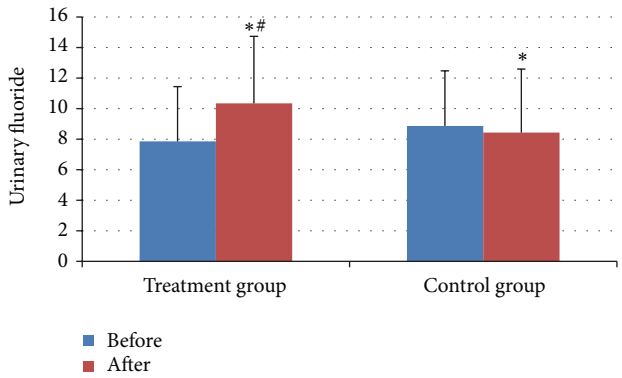

Figure 3: Urinary fluoride before and after treatment in two groups. $^{*}P < 0.05$, compared with that before treatment; $^{\#}P < 0.05$, compared with control group.

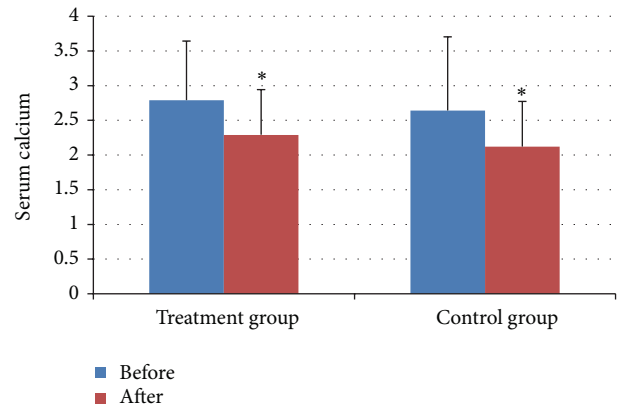

Figure 4: Serum Calcium before and after treatment in two groups. $^{*}P < 0.05$, compared with that before treatment.

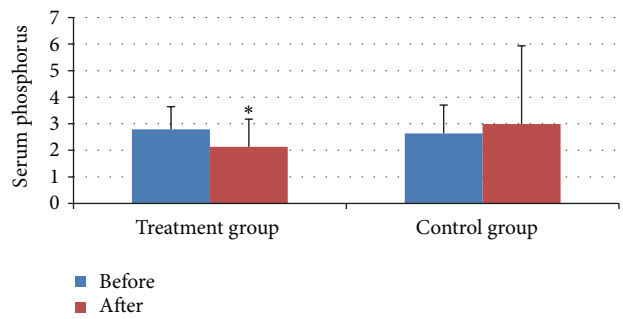

Figure 5: Serum Phosphorus before and after treatment in two groups. $^{*}P < 0.05$, compared with that before treatment.

The urinary fluoride after treatment in treatment group was higher than that in control group. And both of the differences were statistically significant (both $P < 0.05$) (Figure 3).

3.1.4. Serum Calcium and Phosphorus. The serum calcium after treatment was lower than that before in both groups (Figure 4). The serum phosphorus after treatment was lower than that before in treatment group (Figure 5). And all of the differences were statistically significant (all $P < 0.05$).

3.2. Discussion. Though there are lots of western therapy and medicine for treating skeletal fluorosis, the effect of chemical elements preparation (calcium, magnesium, aluminum, etc.), vitamin, and amino acid [3], antioxidant, and anti-inflammatory analgesic dugs is limited and their effect on reliving pain, improving motion of joint, and discharging of urinary fluoride is not so good caused by unclear pathogenesis. Anti-inflammatory analgesic drugs are not suitable to use for a long time. Operation only can be used in its indications, and most patients in remote mountainous areas cannot pay for it. Improvement of water decreasing fluorine is a responsible approach to treat drinking water type of skeletal fluorosis, while the necessary government and economic support is huge, so it is difficult to carry out in short order for some less developed areas. The more important is that the metabolic process of fluorous is long (months to several decades), so most patients' clinical symptoms are still obvious in a long time except for very few ones, and these symptoms affect their quality of life seriously. According to the theory that kidney controls bone and marrow and presides urination and defecation and patients' clinical symptoms, traditional Chinese medicine has achieved obvious effect on skeletal fluorosis aiming at regulating kidney to reinforcing bone and promoting the metabolism of fluorosis.

Skeletal fluorosis is a disease manifested as injuries in skeletons and soft tissue caused by excessive intake of fluoride for a long time and high content of fluorine in environment [6]. Main symptoms include painful and rigid joints, joint deformity, and amyotrophy. According to the theory of TCM, excessive fluoride, a kind of pathogenic factor, stays in body and causes the formation of blood stasis and phlegm. These pathogenic products block meridians and influence joints' normal movement, and pain appears when qi and blood circulation is influenced. There have been some reports on skeletal fluorosis and local kaschin-beck disease and endemic diseases treated by acupuncture in the domestic literatures. These diseases belong to Bi syndrome in TCM theory, and the treatment experience of acupuncture on Bi syndrome is rich and its clinical effect is reliable. Acupuncture is a green therapy with little side effect, and it cannot increase or reduce some materials or elements related to skeletal fluorosis directly. The use of acupuncture on skeletal fluorosis and the observation on its effect is very important for us to treat some endemic disease such as skeletal fluorosis and local kashin-beck disease.

At present, the mechanism study on acupuncture's analgesia effect on skeletal fluorosis has been very deep, and main research directions are as follows. The first is the effect of acupuncture on peripheral nerves. Experiments indicate that acupuncture or electroacupuncture on nerves conducting pain can block the conduction of pain fibers in these nerves. The second is the effect of acupuncture on nervous centralis. Acupuncture can stop new and old spinothalamic tract to conduct pain into central nervous system and then transmit relieved pain and acupuncture stimulation into different levels of central nervous system though new and

old spinothalamic tract. Pain changes through the integration of neurohumor and pain modulatory system, and then the sensation and reaction caused by pain is stopped. This is the process of analgesia effect [7]. The last acupuncture can increase neurotransmitter with analgesia effect (such as ACH, 5-HT, and opioid substances in brain) or strengthen their effect and reduce neurotransmitter with antianalgesia effect (such as NA, DA) [8].

Acupuncture's effect on improving range of motion of joints is related to its analgesia effect. Studies on joint injury of skeletal fluorosis indicate that the joint dysfunction of skeletal fluorosis is caused by fibrous ankylosis actually, and this change is reversible. When pain relieves or disappears obviously, the joint dysfunction relieves or disappears too. This is the result that the tension of muscle, tendon, ligament, and so forth around joints gets lower after pain relieving [4].

Besides relieving pain of joints, the treatment group can also increase urinary fluoride, while the effect in control group is not significant statistically. This indicates that acupunctures' effect on skeletal fluorosis may be related to fluorine's excretion through urine. The level of serum calcium and phosphorus in treatment group also reduced obviously, while only the level of serum calcium in control group reduced. Calcium and phosphorus, normal elements in bone, form calcium-phosphorus metabolic pathway with effects of PTH, CT, vitamin D, and so forth, and calcium-phosphorus metabolism is closed related to osteolysis and osteogenesis [1]. Based on RCT designing methods, this clinical study indicated that acupuncture can influence serum calcium, serum phosphorus and urinary fluoride, but its mechanism is unclear. So, we may study acupuncture's effect on skeletal fluorosis through its regulating effect on calcium-phosphorus metabolism and bone metabolism in the next step.

Followup was not applied in this study due to the time limitation, but followups in 6 months, 1 year, and 3 years are suggested strongly because of the slow developing course and recurrent episodes of this disease.

Considering of the therapeutic effects and side effect, we suggest acupuncture as the first choise for the endemic skeletal fluorosis, especially for relieving the pain caused by it.

4. Conclusion

Both acupuncture and western medicine can relieve the pain of skeletal fluorosis patients, and the effect of acupuncture is better than that of western medicine.

The urinary fluoride of skeletal fluorosis patients increases after acupuncture treatment, while that does not change after taking western medicine.

Both acupuncture and western medicine can decrease the serum calcium of skeletal fluorosis patients, and acupuncture can decrease the serum phosphorus also.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] S. Chen, B. Li, S. Lin et al., "Change of urinary fluoride and bone metabolism indicators in the endemic fluorosis areas of southern China after supplying low fluoride public water," *BMC Public Health*, vol. 13, 156 pages, 2013.

[2] V. Ganvir and K. Das, "Removal of fluoride from drinking water using aluminum hydroxide coated rice husk ash," *Journal of Hazardous Materials*, vol. 185, no. 2-3, pp. 1287–1294, 2011.

[3] I. Błaszczyk, E. Birkner, I. Gutowska, E. Romuk, and D. Chlubek, "Influence of methionine and vitamin E on fluoride concentration in bones and teeth of rats exposed to sodium fluoride in drinking water," *Biological Trace Element Research*, vol. 146, no. 3, pp. 335–339, 2012.

[4] C.-Q. Huang, Z.-B. Feng, and H.-W. Xu, "The study of clinical of IIstage on treatment endemic skeletal fluorosis by using Fenbid," *Chinese Journal of Control of Endemic Diseases*, vol. 17, no. 2, pp. 77–81, 2002.

[5] S. K. Jha, V. K. Mishra, D. K. Sharma, and T. Damodaran, "Fluoride in the environment and its metabolism in humans," *Reviews of Environmental Contamination and Toxicology*, vol. 211, pp. 121–142, 2011.

[6] J. H. Lv, "Clinical experiences of integration of TCM and western medicine for treating skeletal fluorosis," *Journal of Tianjin Medical University*, vol. 1, no. 2, pp. 74–76, 1995.

[7] X. F. Mi and W. F. Wang, "Mechanism of acupuncture analgesia and discussion on point selecting principle," *Shanxi Journal of Traditional Chinese Medicine*, vol. 21, no. 4, pp. 42–43, 2005.

[8] J. S. Yang, *Science of Acupuncture and Moxibustion*, People's medical publishing house, Beijing, China, 1997.

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Research Article

The Infrared Radiation Temperature Characteristic of Acupoints of Mammary Gland Hyperplasia Patients

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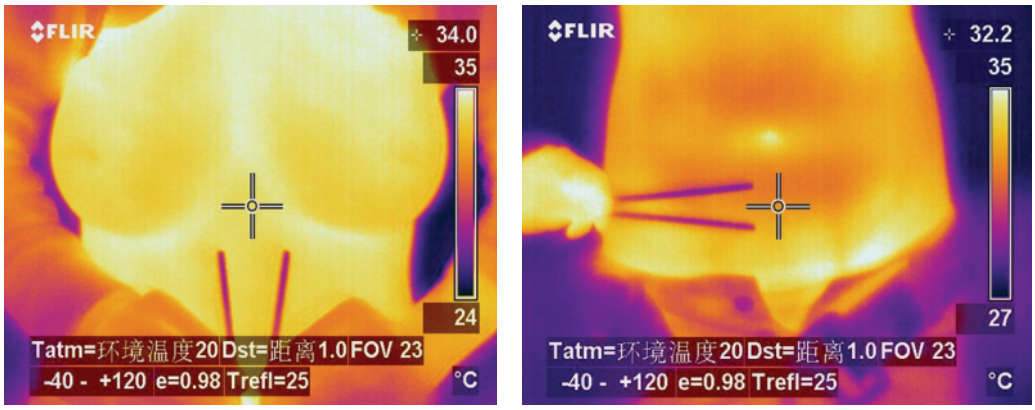
Objective. To ascertain pathological information on hyperplasia of mammary glands (HMG) of patients via the infrared radiation temperature of acupoints. *Method*. Patients with HMG and healthy controls were tested using an infrared thermal imager. *Results*. In controls, no significant difference in temperature was observed between points with the same name ($P > 0.05$). The temperature of all tested points was found to be higher in the group with HMG than in that of the healthy controls, except for the left and right Zusanli (ST36). The temperature of the right Rugen (ST18), Guanyuan (CV4), Qihai (CV6), and Hegu (LI4) reached a statistically significant heightened level ($P = 0.046 \sim P < 0.001$). The temperature of the Zusanli (ST36) and Hegu (LI4) present on the right side was significantly higher than that of the left ($P = 0.001$ and $P = 0.004$, resp.), while the temperature of the left Youmen (KI21) was significantly higher than that of the right ($P = 0.008$). *Conclusion*. The temperature of the bilateral acupoints in healthy controls was symmetrical, and the raised temperatures observed of the Rugen (ST18), Guanyuan (CV4), Qihai (CV6), and Hegu (LI4) acupoints of HMG patients and the imbalance of the temperature of the bilateral acupoints Zusanli (ST36), Youmen (KI21), and Hegu (LI4) carried special pathological information about HMG disease.

1. Introduction

Acupuncture points are sites where qi and the blood of zang-fu organs and meridians are transported. The changes in qi and blood can be reflected in these acupoints. Thus, acupuncture points not only can receive stimulation by acupuncture and moxibustion but also may reflect disorders in patients. Therefore, when there are disorders in zang-fu organs, there could be tenderness, pain, soreness, numbness, or thermosensitivity in the related acupoints. The changes can also be reflected as changes in the biophysical character of the acupoints, such as changes in the volt-ampere characteristics of the points [1, 2] or an infrared radiation intensity change [3]. The human body is a natural source of infrared radiation; it continuously emits infrared radiation into the surrounding environment. When people suffer from ailments or any physiological changes, their systemic or local thermal equilibrium is affected or destroyed. As a result of this, the temperature of their tissues is observed to increase or decrease. Thus, the infrared radiation temperature of acupoints can also carry specific pathological information of diseases related to them. Hyperplasia of the mammary gland (HMG), also known as breast dysplasia, is classified under the "Rupi" category in TCM, which accounts for 75% of all breast disease; ~40% of childbearing women have this disease, which makes it the most common breast disease [4]. With increasing environmental pollution, social pressure, and life-pace acceleration, the incidence of HMG is rising, and the age onset has decreased. Studies have shown that malignant transformation of normal breast tissue undergoes a multistage and gradual process as follows: hyperplasia → dysplasia → carcinoma in situ → invasive cancer, and is considered reversible before developing into invasive cancer [5]. In our research, we study HMG patients and record the infrared radiation temperature of the chosen acupoints to explore its specific pathological information.



(a) (b)

Figure 1: Infrared radiation temperature of acupoints. Bamboo stick in picture (a) points to Youmen (KI21) on both sides. In picture (b), it points to Guanyuan (CV4) and Qihai (CV6). The centre of the cross indicates the focus when the picture was taken. Data written on the top right of the picture is the temperature of the point.

2. Patients and Methods

2.1. Patients. This study included 101 female HMG patients from the breast clinic of Longhua Hospital, which is affiliated to the Shanghai University of Traditional Chinese Medicine. All of them volunteered to participate in the program and signed informed consent forms. Out of these patients, 73 had bilateral HMG, 13 had only right-lateralized HMG, and 15 were only left-lateralized. The youngest patient was 21 years old and the oldest was 51; the mean age of the sample was 33.14 ± 8.001 years. Thirty-five healthy controls who participated in this study were all interns and staff members of the hospital working at different posts. Seven were medical care workers and the remaining 28 were from house-keeping, finance, and the catering departments. In the control group, the youngest participant was 18 years old and the oldest was 49. The mean age of the control group was 32.23 ± 10.085 years. There was no significant difference in the age distribution between the two groups ($P = 0.631$). Detection time was from September 2008 to November 2008 and from August 2009 to December 2009.

2.2. Inclusion and Exclusion. The diagnostic and syndrome differentiation standards adopted in 2002 at the 8th meeting of the Professional Committee for Breast Diseases of China TCM Surgery Society [6] were used in this trial. Patients who met the inclusion criteria were between 18 and 55 years of age. These patients voluntarily participated and showed good compliance when enrolled. Those patients with the following conditions were excluded: mastitis, a simple benign tumour of the mammary gland, a malignant tumour of the mammary gland, or complicated syndromes that were difficult to differentially diagnose. Patients were also excluded if they were pregnant, lactating, had irregular menstruation, functional uterine bleeding, mental disease, were taking endocrine hormones within the previous 6 months, had gynaecomastia, showed mammary development before menarche, or had serious heart, liver, kidney, or hematopoietic system disease.

The control group consisted of women who had no mammary disease or serious heart, liver, kidney, or cerebral disease, were 18–55 years old, had normal menstruation, voluntarily participated, and showed good compliance when enrolled.

2.3. Testing Equipment and Environment. For infrared testing, a 5th generation high-performance uncooled focal plane economic infrared thermograph ThermaCAMTMP30 (FLIR, Sweden) was used. It uses the focal plane array (FPA) principle, an uncooled microbolometer, and has ultra-high thermal sensitivity (0.08°C). The temperature measuring range is 0–500°C; the IFOV, 1.3 mrad; and the wavelength range, 7.5–13 μm. This study was conducted at room temperature of 22 ± 3°C and at an air humidity of 55 ± 10%. There was no obvious air flow, strong noise, or electromagnetic sources around.

2.4. Testing Acupoints and Methods. According to the national standard GB12346-90 Acupoints Location released by the State Bureau of Technical Supervision in 1990 [7], we chose 7 relevant acupoints in HMG patients for infrared thermograph: Rugen (ST18), Qimen (LR14), Youmen (KI21), Guanyuan (CV 4), Qihai (CV 6), Zusanli (ST 36), and Hegu (LI4). In addition, bilateral acupoints sharing the same name were used. Therefore, a total of 12 points were tested.

Patients entered the lab, loosened their underwear, and sat down for 20 min to adapt to the environment. During this period, they are asked questions to obtain the relevant required information. After questioning, the patients exposed their testing sites, and the analyser sat 1 meter away from the testing site in a fixed position prepared to film the procedure. The infrared camera was placed right above the testing site to obtain infrared images. Figure 1 is an infrared image of acupoints on the upper and lower abdomen.

2.5. Statistical Method. The thermaCAM reporter 2000 professional analysis system, matching the infrared thermography, was used to analyse the infrared thermograph picture

Table 1: Infrared radiation temperature comparison of the tested points of patients and healthy controls ($\overline{x} \pm s$, °C).

Acupoints	Patients	Healthy controls	t	P
Left Rugen (ST18)	33.888 ± 1.0 (n = 101)	33.503 ± 1.3 (n = 35)	1.559	0.126
Right Rugen (ST18)	33.878 ± 0.94 (n = 101)	33.340 ± 1.5 (n = 35)	2.053	**0.046**
Left Qimen (LR14)	33.943 ± 0.9 (n = 101)	33.811 ± 1.2 (n = 35)	0.662	0.509
Right Qimen (LR14)	33.871 ± 0.9 (n = 101)	33.629 ± 1.2 (n = 35)	1.266	0.208
Left Youmen (KI21)	33.677 ± 1.1 (n = 100)	33.246 ± 1.5 (n = 35)	1.615	0.113
Right Youmen (KI21)	33.600 ± 1.0 (n = 100)	33.286 ± 1.3 (n = 35)	1.422	0.157
Guanyuan (CV4)	33.756 ± 1.1 (n = 101)	32.746 ± 1.5 (n = 35)	3.717	**0.001**
Qihai (CV6)	33.428 ± 1.1 (n = 101)	32.543 ± 1.3 (n = 35)	3.907	**0.000**
Left Zusanli (ST36)	32.584 ± 0.9 (n = 101)	32.677 ± 1.0 (n = 35)	−0.499	0.618
Right Zusanli (ST36)	32.696 ± 1.0 (n = 101)	32.760 ± 1.0 (n = 35)	−0.334	0.739
Left Hegu (LI4)	32.781 ± 1.2 (n = 97)	32.160 ± 1.3 (n = 35)	2.539	**0.012**
Right Hegu (LI4)	32.904 ± 1.2 (n = 97)	32.263 ± 1.3 (n = 35)	2.595	**0.011**

Table 2: Infrared radiation temperature comparison of bilateral acupoints with the same name of healthy controls ($\overline{x} \pm s$, °C).

Acupoints	Left side	Right side	t	P
Rugen (ST18) (n = 35)	33.503 ± 1.3	33.340 ± 1.5	1.568	0.126
Qimen (LR14) (n = 35)	33.811 ± 1.2	33.629 ± 1.2	1.648	0.109
Youmen (KI21) (n = 35)	33.246 ± 1.5	33.286 ± 1.3	−0.824	0.416
Zusanli (ST36) (n = 35)	32.677 ± 1.0	32.760 ± 1.0	−1.370	0.180
Hegu (LI4) (n = 35)	32.160 ± 1.3	32.263 ± 1.3	−1.135	0.264

and obtain the skin temperature at the detected acupoints; Microsoft Excel was used to construct a database and the SPSS 11.5 (SPSS Inc., Chicago) software package was used to analyse the statistical data. We used mean and standard deviation to analyse the measurement data, group t-tests for comparison between patients and healthy controls, and paired t-tests for comparison between bilateral acupoints with the same name ($\alpha = 0.05$).

2.6. Results of the Comparison of Infrared Radiation Temperature between the Healthy Controls and HMG Patients. Except for bilateral Zusanli (ST36), the infrared radiation temperature of 10 out of 12 testing points was higher than that of the healthy controls. Among these 10 testing points, the temperature of the right Rugen (ST18), Guanyuan (CV4), Qihai (CV6), and bilateral Hegu (LI4) was significantly higher than that of the healthy controls ($P = 0.046 \sim P < 0.001$) (Table 1).

2.7. The Infrared Radiation Temperature Comparison of Bilateral Acupoints That Share the Same Name in Healthy Controls. Out of 5 testing acupoints in healthy controls, the infrared radiation temperatures of Rugen (ST18) and Qimen (LR14) on the left side were higher than those on the right side, while the temperature of the other 3 acupoints on the right side was higher than those on the left side. However, the difference was not statistically significant ($P > 0.05$) (Table 2).

2.8. The Infrared Radiation Temperature Comparison of Bilateral Acupoints That Share the Same Name in HMG Patients. Out of 5 testing acupoints in patients, the infrared radiation temperature of Rugen (ST18), Qimen (LR14), and Youmen (KI21) on the left side was higher than that on the right side, while the temperature of the other 2 acupoints was higher on the right side than that on the left. The temperature of Youmen (KI21) on the left side was significantly higher than that on the right side ($P = 0.008$), while the temperature of Hegu (LI4) and Zusanli (ST36) on the right side was significantly higher than that on the left side ($P = 0.001$, $P = 0.004$) (Table 3).

3. Discussion

It's recorded in the *Zhen Jiu Jia Yi Jing* that: "Therapy of breast pain and swelling of the chest lies in the Rugen point." *Pu Ji Fang* mentioned that "Therapy of breast pain lies in Rugen point." Nowadays, many textbooks [8–11] take Rugen (ST18) as an important acupoint to treat Rupi, and clinical reports [12, 13] have proven that Rugen (ST18) is particularly effective in HMG treatment. Qimen (LR14) is the front-mu point of the liver meridian. Our previous research on the infrared radiation spectrum found that, to some extent, Qimen (LR14) reflects the pathological nature of the liver qi stagnation of HMG patients [14]. Youmen (KI21) is the crossing point of the kidney meridian and the Chong meridian that is in close proximity to the breast. Guanyuan (CV4) and Qihai (CV6) are important acupoints in the Ren meridian. Guanyuan (CV4), Qihai (CV6), and Youmen (KI21) can reflect the disturbances of HMG patients [15] in the Chong and Ren meridians. Zusanli (ST36) is an important acupoint in the stomach meridian of Foot Yangming. This meridian travels down the inside of the breast. There have been reports showing that Zusanli (ST36) is an important acupoint in HMG therapy [16]. Therefore, in this research, we selected

Table 3: Infrared radiation temperature comparison of bilateral acupoints with the same name of patients ($\overline{x} \pm s$, °C).

Acupoints	Left side	Right side	t	P
Rugen (ST18) (n = 101)	33.888 ± 1.0	33.878 ± 1.9	0.217	0.829
Qimen (LR14) (n = 101)	33.943 ± 1.0	33.871 ± 0.9	1.592	0.114
Youmen (KI21) (n = 100)	33.677 ± 1.1	33.600 ± 1.0	2.709	**0.008**
Zusanli (ST36) (n = 101)	32.584 ± 1.0	32.696 ± 1.0	−3.513	**0.001**
Hegu (LI4) (n = 97)	32.781 ± 1.2	32.904 ± 1.2	−2.928	**0.004**

Rugen (ST18), Qimen (LR14), Youmen (KI21), Guanyuan (CV4), Qihai (CV6), Zusanli (ST36), and Hegu (LI4) as testing points. Except for bilateral Zusanli (ST36), the detected infrared radiation temperature of the other 10 points was higher than that of healthy controls. The temperature of the right Rugen (ST18), Guanyuan (CV4), Qihai (CV6), and bilateral Hegu (LI4) reached statistical significant levels ($P < 0.05$). Researchers [17, 18] reported that thermography of hyperplasia mammary gland is manifested as a cluster and sheet-shaped high-temperature hot zone. Its peripheral area is shown as a low heat area, and it has clear but irregular boundaries and an ectatic vascular shadow. Its distribution is continuous and irregular. The raised temperature of the areola is the most obvious, making it the high-temperature area. This is basically consistent with the temperature trend of acupoints on the trunk as reflected in this test result. It points out that the overall pathological condition of HMG patients exceeds that of controls. Infrared radiation temperature of the right Rugen (ST18) was significantly higher than that of healthy controls, which reflected the raised temperature phenomenon of the local breast tissue. The temperature of Guanyuan (CV4) and Qihai (CV6) on the Ren meridian was significantly higher than that of healthy controls, which may be a reflection of the disorders of the Chong and Ren meridians. Hegu (LI4) is the source point of the large intestine meridian, and the temperature of this point is significantly higher than that of healthy controls. We believe that there are two possibilities: first, the findings of the clinical observations suggest that many HMG patients often have gastrointestinal disorder symptoms such as constipation, which could be reflected on Hegu (LI4) of the large intestine meridian; second, pain is a primary clinical symptom of HMG patients, and the temperature-rising phenomenon is likely to be an important reaction to breast pain.

There have been ancient records for Hegu (LI4) on the treatment of painful diseases. *Xi Hong Fu* says: "Unbearable pain of the hands and shoulders should be treated with Hegu and Taichong." *Sheng Yu Ge* says: "Soreness and pain of the hands, which makes it hard to hold things, should be treated with Hegu, Quchi, and Jianyu." There are also reports on Hegu (LI4) for pain treatment in a modern clinic [19–22]. Researchers [23] proved that electric acupuncture on Hegu (LI4) can elicit compound action, respectively, on trigeminal nerve branches, and the pain threshold of the head, chest, and abdominal skin rapidly becomes higher. This phenomenon disappears when we completely block the points. So, it is suggested that Hegu (LI4) is an important acupoint for treating pain and an important reflection point of breast pain. According to the human body symmetry, in order to measure the temperature difference of human bilaterally corresponding parts with thermal images, determination of the occurrence of disease is a common diagnostic method of thermal imaging. Feldman filmed thermal images of the cervical vertebrae and upper limbs of healthy people in 1994 [24]. He believed that normal temperature differences of bilateral sides of the upper body would be no more than 0.62°C; Uematsu et al. [25] thought that the temperature difference should be between 0.2 and 0.5°C and the maximum difference is 1°C [26]. Yan's study showed that the surface temperature of a normal human body and that of a rabbit are basically symmetrical, while the spleen-deficiency diarrheogenic rabbit model showed significant asymmetry in temperature change of the left and right side of the body [27, 28]. He present a study that showed that there was no statistically significant difference in the infrared radiation temperature of bilateral points sharing the same name and that of healthy controls ($P > 0.05$). This was consistent with the symmetry law of bilateral, normal, skin temperature, while the infrared radiation temperature difference of bilateral Youmen (KI21), Zusanli (ST36), and Hegu (LI4) of HMG patients was statistically significant ($P < 0.05$). This showed the temperature imbalance phenomenon of bilateral acupoints of HMG patients and indicated the pathological nature of liver qi stagnation, phlegm and blood stasis, and Chong and Ren meridian disturbance of HMG patients.

4. Conclusion

The infrared radiation temperature of the bilateral acupoints in healthy controls was found to be symmetrical, and the raised temperatures observed of the Rugen (ST18), Guanyuan (CV4), Qihai (CV6), and Hegu (LI4) acupoints of mammary gland hyperplasia patients and the imbalance of the infrared radiation temperature of the bilateral acupoints Zusanli (ST36), Youmen (KI21), and Hegu (LI4) carried special pathological information about mammary gland hyperplasia disease. These results can be used as an auxiliary diagnosis for mammary gland hyperplasia. This experiment provides new research ideas and methods for TCM diagnosis using acupoints, the research of acupoint specificity, acupoint selection at the clinic, and the objective research on the evaluation of curative effectiveness.

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References

[1] X.-Y. Shen, G.-H. Ding, J.-Z. Wei et al., "Observation of the volt-ampere characteristics before and after menstruation," *Study Journal of Traditonal Chinese Medicine*, vol. 24, no. 9, pp. 1589–1591, 2006.

[2] L.-N. Wang, J.-Z. Wei, H.-J. Mao et al., "Effect of devascularization on volt-ampere characte-ristic of acupoints," *Chinese Acupuncture and Moxibustion*, vol. 32, no. 12, pp. 1095–1097, 2012.

[3] X. Shen, G. Ding, H. Deng et al., "Analysis on pathological information of infrared radiation spectrums at acupuncture point neiguan (PC6) for patients with coronary heart disease," *Journal of Infrared and Millimeter Waves*, vol. 25, no. 6, pp. 443–446, 2006.

[4] Y. Lin and H.-J. Tang, *Breast Disease of Modern TCM*, vol. 5, People's Medical Publishing House, Beijing, China, 2003.

[5] S. R. Lakhani, "The transition from hyperplasia to invasive carcinoma of the breast," *Journal of Pathology*, vol. 187, no. 3, p. 272, 1999.

[6] Y. Lin and H.-J. Tang, *Breast Disease of Modern TCM*, vol. 5, People's Medical Publishing House, Beijing, China, 2003.

[7] State Bureau of Technical Supervision, *State Standard of the People's Republic of China GB-12346-90*, China Standard Press, Beijing, China, 1990.

[8] Q.-C. Wang, *Subject of Acupuncture and Moxibustion Theraphy*, China Press of Traditional Chinese Medicine, Beijing, 2003.

[9] F.-R. Liang, *Science of Acupuncture and Moxibustion*, China Press of traditional Chinese medicine, Beijing, China, 2005.

[10] H.-Z. Xu, *Science of Acupuncture and Moxibustion*, People's Medical Publishing House, Beijing, China, 2002.

[11] D.-F. Gong and Y.-H. Li, *Subject of Acupuncture and Moxibustion Theraphy*, Science and Technology Literature Press, Beijing, China, 2006.

[12] Y.-M. Guo and C.-J. Guo, "Clinical observation on acupuncture for treatment of 1076 patients with hyperplasia of mammary glands," *Journal of Shanxi College of Traditonal Chinese Medicine*, vol. 24, no. 2, p. 30, 2001.

[13] W.-H. Zhang, Y.-M. Guo, X. R. Guo et al., "Acupuncture the 1678 cases with Mammary gland hyperplasia in Wuyi((ST 15), Rugen(ST18) et al points," *Shanxi Journal of Traditional Chinese Medicine*, vol. 28, no. 6, pp. 714–715, 2007.

[14] J. Ying, X.-Y. Shen, G.-H. Ding et al., "Compare the infrared radiation spectrum of opint LR14 with non-acupoint of patients with hyperplasia of mammary gland," *Liaoning Journal of Traditonal Chinese Medicine*, vol. 36, no. 8, pp. 1145–1147, 2008.

[15] J.-Z. Li, J. Fu, and Y.-H. Fan, "Only choose acupuncture, "Qihai" treatment of the gas for the disease," *Shanxi Journal of Traditional Chinese Medicine*, vol. 27, no. 6, pp. 711–713, 2006.

[16] S.-X. Wang, W.-H. Zhang, Y. Li et al., "Study on hyperplasia of mammary acupoint selection of acupuncture treatment," *Journal of Shanxi College of Traditional Chinese Medicine*, vol. 32, no. 4, pp. 55–56, 2009.

[17] J.-F. Yang, J.-Z. Xu, Q.-X. Zhao et al., "Research on Application of medical thermography in diagnosis of breast disease," *Laser and Infrared*, vol. 30, no. 1, pp. 42–44, 2000.

[18] Y.-K. Ao, W.-B. Zou, X.-Q. Yu et al., "Application of medical infrared thermography in the diagnosis of breast diseases (report of 500 cases)," *Practical Clinical Journal of Integrated Traditional Chinese and Western Medicine*, vol. 7, no. 6, pp. 78–79, 2007.

[19] B. Wang, C.-Y. Zhang, J.-Q. Zhang et al., "Clinical study on treatment of odontalgia by acupunctrue of point Hegu(LI4)," *Shanghai Journal of Acupuncture and Moxibustion*, vol. 25, no. 8, pp. 6–9, 2006.

[20] L. He and X. Lin, "Effects of Hegu (LI 4) and Taichong (LR 3) on vascular dilation and constriction in migraine patients," *Journal of Acupuncture and Tuina Science*, vol. 7, no. 5, pp. 305–307, 2009.

[21] C.-H. Li, Y.-Z. Wang, and X.-X. Guo, "Acupuncture at Siguan points for treatment of primary dysmenorrhea," *Chinese Acupuncture & Moxibustion*, vol. 28, no. 3, pp. 187–190, 2008.

[22] J. Qiu and X. Chen, "Application of acupuncture analgesia in natural childbirth," *Tianjin Medical Journal*, vol. 34, no. 4, pp. 279–280, 2006.

[23] S.-L. Chen, Z.-G. Jin, X.-H. Jing et al., "Morphological study on the afferent pathways of "Hegu" point and mouth-face region," *Acupuncture Research*, vol. 29, no. 3, pp. 217–221, 2004.

[24] F. Feldman and E. L. Nickoloff, "Normal thermographic standards for the cervical spine and upper extremities," *Skeletal Radiology*, vol. 12, no. 4, pp. 235–249, 1984.

[25] S. Uematsu, D. H. Edwin, W. R. Jankel, J. Kozikowski, and M. Trattner, "Quantification of thermal asymmetry. Part 1: normal values and reproducibility," *Journal of Neurosurgery*, vol. 69, no. 4, pp. 552–555, 1988.

[26] H.-H. Niu, P.-W. Lui, J. S. Hu et al., "Thermal symmetry of skin temperature: normative data of normal subjects in Taiwan," *Chinese Medical Journal*, vol. 64, no. 8, pp. 459–468, 2001.

[27] Z.-Q. Yan, Y.-Y. Chi, J. Cheng et al., "Topographic mapping of the physical Informations on the body surfaces of human and animal models," *Acta Biophysica Sinica*, vol. 8, no. 2, pp. 356–364, 1992.

[28] Z.-Q. Yan, Y.-Z. Wang, L. Zhang et al., "Observation on temperature imbalance of meridian and ear points for patients with coronary heart disease," *Journal of Traditional Chinese Medicine*, no. 1, pp. 51–53, 1985.

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Research Article

Effects of Low Level Laser Therapy on Ovalbumin-Induced Mouse Model of Allergic Rhinitis

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Introduction. This study was designed to investigate the effects of low level laser therapy (LLLT) on experimental allergic rhinitis (AR) models induced by ovalbumin. *Materials and Methods*. AR was induced by 1% ovalbumin in mice. Twenty-four mice were divided into 4 groups: normal, control, low, and high dose irradiation. Low and high dose LLLT were irradiated once a day for 7 days. Total IgE, cytokines concentrations (IL-4 and IFN-γ), and thymus and activation regulated chemokine (TARC) were measured. Histological changes in the nasal mucosal tissue by laser irradiation were examined. *Results*. LLLT significantly inhibited total IgE, IL-4, and TARC expression in ovalbumin-induced mice at low dose irradiation. The protein expression level of IL-4 in spleen was inhibited in low dose irradiation significantly. IL-4 expression in EL-4 cells was inhibited in a dose dependent manner. Histological damages of the epithelium in the nasal septum were improved by laser irradiation with marked improvement at low dose irradiation. *Conclusion*. These results suggest that LLLT might serve as a new therapeutic tool in the treatment of AR with more effectiveness at low dose irradiation. To determine the optimal dose of laser irradiation and action mechanisms of laser therapy, further studies will be needed.

1. Introduction

Allergic rhinitis (AR) is a condition characterized by one or more of the following nasal symptoms: congestion, rhinorrhea (anterior and posterior), sneezing, and itching [1], which is estimated to affect up to 40% of the population worldwide [2]. At present, some pharmacological therapies like antihistamines, corticosteroids, decongestants, and mast cell stabilizers are available for the AR patients [3]; however, AR symptoms are not always satisfactorily controlled by medication, and some patients fail to respond to conventional treatments [4].

Acupuncture is one of the most popular complementary and alternative medical treatments and classified according to stimulating materials or methods such as metal needle, laser stimulation, or herbal extracts (pharmacopuncture) [5]. In case of AR, acupuncture treatment has been reported to show beneficial effects through clinical trials. Moreover, EX-HN9 (内迎香) is used as one of the main acupuncture points for its treatment [6]. However, there are some controversies about the effectiveness of acupuncture treatment on AR and some limitations for applying acupuncture in the clinical field (e.g., pain or discomfort due to needle pain). Therefore, another modality of acupuncture to improve the effectiveness on AR and compliance with treatment is necessary to be investigated.

Low level laser therapy (LLLT) has been clinically used to aid wound healing [7], relieve pain in musculoskeletal diseases [8, 9], and stimulate acupuncture points [10], and its anti-inflammatory and immunosuppressive effects have been examined in the laboratory [11–13]. However, there are few studies that have examined the effects of LLLT on AR.

In the present study, to investigate the effects of laser acupuncture on AR, the ovalbumin (OVA) induced allergic

rhinitis model was used. Particularly, we irradiated the low level laser into the intranasal cavity of the mouse for recreating the laser acupuncture on EX-HN9 according to the dose of irradiation. The serological parameters of allergic inflammation including IgE and cytokines (IL-4 and IFN-γ) and thymus and activation regulated chemokine (TARC) were examined with the *in vitro* detection of IL-4 expression in Th2 cell as well. Histological examination was also carried out to measure the changes on inflammatory cell infiltration of intranasal epithelium by LLLT.

2. Materials and Methods

2.1. Laser. The laser device made by Gwangju Institute of Science and Technology (Gwangju, Korea) had the following characteristics: low-power laser of 658 nm infrared wavelength, aluminum gallium indium phosphide (InGaAlP) semiconductor, fiber diameter of material (Core/Cladding) as 50/125 μm, and 30 mW of output. Under anesthesia and with maximal exposure of mouse nostril to laser beam, the LLLT to mice was conducted for irradiating overall mucosal surface of nasal cavity including EX-HN9. The laser was irradiated 10 mm apart from each nostril of mouse with vertical setting of the laser device. The parameters of laser irradiation in the present study were shown in Table 1.

Table 1: Laser irradiation parameters involved in this study.

Parameters	Values
Power output	30 mW
Wavelength	658 nm
Mode of action	Continuous
Fiber diameter (Core/Cladding)	50/125 um
Cell	
Power density	3.157×10^{-3} W/cm^2
Spot size	9.5 cm^2
Irradiation time	32 s (100 mJ/cm^2), 160 s (500 mJ/cm^2), 320 s (1000 mJ/cm^2), 640 s (2000 mJ/cm^2)
Mouse	
Power Density	15×10^{-2} W/cm^2
Spot size	0.2 cm^2
Irradiation time	320 s (1000 mJ/cm^2), 640 s (2000 mJ/cm^2)

2.2. Th2 Cell Culture and Cell Viability. Murine thymic lymphoma cells (EL-4) were purchased from the KoreaCell Line Bank, Seoul, Korea. EL-4 cell lines were cultivated in RPMI-1640 medium with extra L-glutamine (4 mM), sodium pyruvate (1.0 mM), 4.5 g L^{-1} glucose, penicillin (100 U/mL), streptomycin (100 U/mL), and 10% v/v fetal bovine serum. For differentiation of EL-4 cells, phorbol-12-myristate-13-acetate (PMA) and 4-tert-Octylphenol (OP) (Sigma-Aldrich, MO, USA) were treated at a density of 5×10^5 cells/well. Control cells were incubated without MPA or OP. Cells were collected by gently scraping (cell scraper) in culture medium and centrifuged for 8 min at 300 g, and the supernatant was removed. Cell pellets were washed twice in ice cold PBS and directly analyzed or stored at −80°C for further analysis for RT-PCR. Optimal PMA and OP concentration was selected by determining the percentage of differentiated cells and their viability. Laser irradiation was delivered to the culture plate from above via an optical fiber. The laser beam was clipped to cover the entire area of the plate using cap. The laser densities were 100 mJ (30 mW, 32 s), 500 mJ (30 mW, 160 s), 1,000 mJ (30 mW, 320 s), and 2,000 mJ (30 mW, 640 s).

2.3. Animal and Grouping. All experiments were carried out in accordance with the guidelines of Kyung Hee University for animal care. Male Balb/c mice, aged 6 weeks and weighing 18–20 g, were randomly divided into 4 groups: normal (OVA untreated and no irradiation, $n = 6$), control (treated only OVA, $n = 6$), low dose irradiation (1,000 mJ irradiation, $n = 6$), and high dose irradiation (2,000 mJ irradiation, $n = 6$).

2.4. ELISA Assay. Serum of OVA mouse was separated from the whole blood collected by the cardiac puncture and stored in the deep-freezer for the serum IgE assay. After the final laser irradiation, spleen tissues were kept in coldlysis buffer (Cell signaling, Carlsbad, CA, USA) for IL-4, INF-γ, and TARCELISA assay. IgE, IL-4, INF-γ, and TARCELISA kits (R&D Systems, Inc., Minneapolis, MN, USA) were used and the cytokine concentrations were assessed according to the manufacturer's cytokine ELISA protocol.

2.5. OVA Sensitization and Challenging. Through allergen exposure using the chronic exposure protocol, mice were sensitized and challenged, shown in Figure 1. Briefly, 6 week-old mice were systemically sensitized to OVA (10 μg/0.1 mL chicken egg albumin, ovalbumin, grade V, 98% pure, Sigma, St. Louis, MO, USA) by intraperitoneal (i.p.) injection on days 7, 14, and 21. Exposures to aerosolized OVA (10 mL of 10 mg OVA/mL 1% saline solution) were commenced on day 28 after the first i.p. (Figure 1). In this AR model, 1,000 mJ (30 mW, 320 s) and 2,000 mJ (30 mW, 640 s) laser were irradiated once a day for 7 days.

2.6. Western Blot. All spleen samples were lysed in ice-cold lysis buffer containing Tris-HCl (pH 7.4) (20 mM), 1% Triton X-100, 0.1% SDS, EDTA (2 mM), and phenylmethyl sulfonyl fluoride (PMSF) (1 mM). The amount of protein in each sample was determined using the Bradford assay. The samples were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein spots were electrotransferred to a polyvinylidenedifluoride (PVDF) membrane. The membrane was incubated with block buffer (PBS containing 0.05% Tween-20 and 5% w/v nonfat dry milk) for 1 h, washed with PBS containing 0.05% Tween-20 (PBST) three times, and then probed with IL-4 antibody (1 : 1,000 diluted, Cell Signaling Technology, USA) overnight at 4°C. In addition, the intensity of the blots probed with 1 : 1,000 diluted solution of antibody β-actin (Cell Signaling Technology, USA) was used as the control to ensure that a constant amount of protein was loaded into each lane of the gel. The membrane was washed for 5 min (3 times) in PBST,

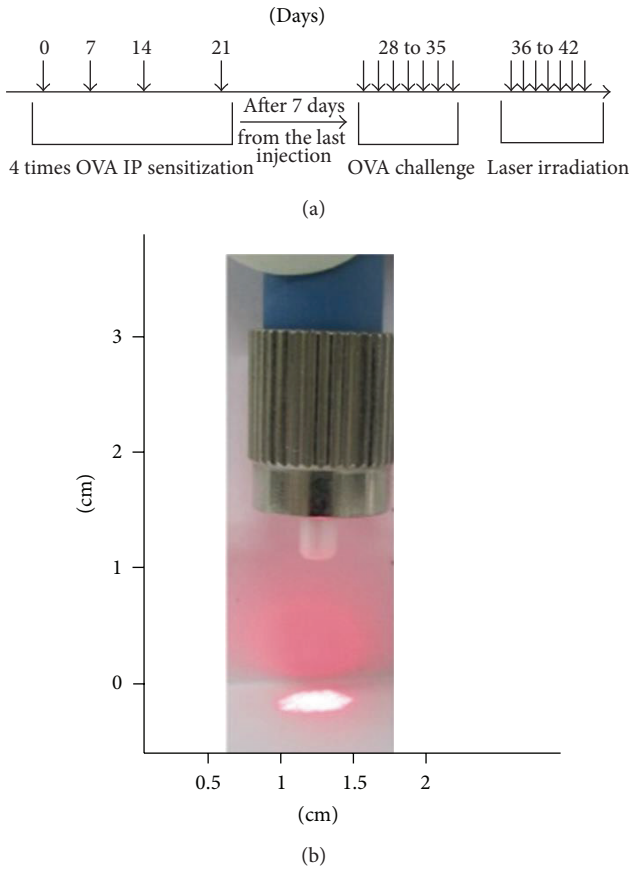

Figure 1: (a) Protocol for sensitization of ovalbumin (OVA). Sensitization was performed by intraperitoneal injection of OVA on days 0, 7, 14, and 21. After 1 week later, mice were challenged OVA aerosol daily for 1 week to induce local sensitization. Next day, laser was irradiated daily for 1 week. Normal group mice were injected and challenged with PBS instead of OVA. (b) Laser instrument used for the intranasal irradiation.

shaken in a solution of HRP-linked anti-rabbit IgG secondary antibody (1 : 2,000 diluted) for 2 h at RT, and again washed for 5 min (3 times) in PBST. The expressions of proteins were detected by enhanced chemiluminescent (ECL) reagent (Millipore, Billerica, MA, USA).

2.7. RT-PCR. First strand cDNA synthesis with 5 μg of total RNA was performed using MMLV reverse transcriptase and oligodT primer for 1 h at 42°C. Subsequently, the PCR-amplification was performed by a modified method originally described by Saiki et al. [14]. Firstly, 5 μL of cDNA was added to 2.5 μL of 10x PCR buffer, 1 μL of 25 mM $MgCl_2$, 1 μL of 2.5 mM dNTP, 0.5 μL of polymerase (1 U), 1 μL of each primer (4 pmol), and DEPC-H_2O to give a final volume of 25 μL. The sequences of IL-4 primers were as follows: 5′-TAGTTGTCATCCTGCTCTT-3′ as forward primer and 5′-CTACGAGTAATCTTGC-3′ as reverse primer. PCR-products were separated on a 1.5% agarose gel, visualized by ethidium bromide using i-MAX gel image analysis system (CoreBioSystem, Seoul, Korea), and analyzed using Alpha

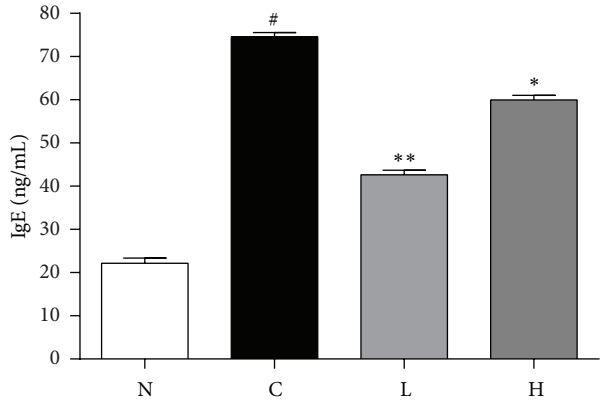

Figure 2: Measurement of serum total IgE level. Level of total IgE was expressed as optical density (OD) at 450 nm. N: normal group (no stimulation), C: control group (stimulation by ovalbumin without irradiation), L: low dose irradiation on control group (30 mW/320 s), and H: high dose irradiation on control group (30 mW/640 s). $^{\#}P < 0.05$ versus the normal group. $^{*}P < 0.05$ versus the control group. $^{**}P < 0.01$ versus the control group.

Easy FC software (AlphaInnotech Corporation, San Leandro, CA, USA). Results from at least three separate experiments were used for statistical analysis.

2.8. Hematoxylin and Eosin (HE) Staining. For histological studies, paraffin-embedded tissue sections (5 μm thick, coronal section) were stained by HE staining. The sections were deparaffinized and rehydrated in xylene, 100, 95, 80, and 70% ethanol. The sections were overstained with hematoxylin, usually 3–5 min, and rinsed off excess stain in deionized water. Then they were destained a few seconds in acidic alcohol until sections look red, usually 4-5 dips, and rinsed briefly in deionized water to remove the acid. Hematoxylin stained slides from the last tap water were rinsed and placed in 70% ethanol for 3 min. Slides were placed in eosin for 2 min and taken slides through 95 and 100% ethanol and xylene. After HE staining, slides were mounted with Canada balsam.

2.9. Statistical Analysis. All quantitative data derived from this study were analyzed statistically. The results were expressed as the mean ± SEM. Differences between groups were assessed by one-way ANOVA using the SPSS version 12.0 software package for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance at $P < 0.05$ has been given respective symbols in figures.

3. Results

3.1. EL-4 Cell Differentiation Condition and Cell Viability. Before investigating the effects of LLLT on the AR mice, cell viability was assessed upon various doses of irradiation (100, 500, 1,000, and 2,000 mJ) to determine the irradiating doses of laser. As a result, laser treatments up to 1,000 mJ did not induce any changes in terms of cell cytotoxicity. After 2,000 mJ of laser irradiation, cell viability was decreased than that of untreated cell; however, there was no significance (data

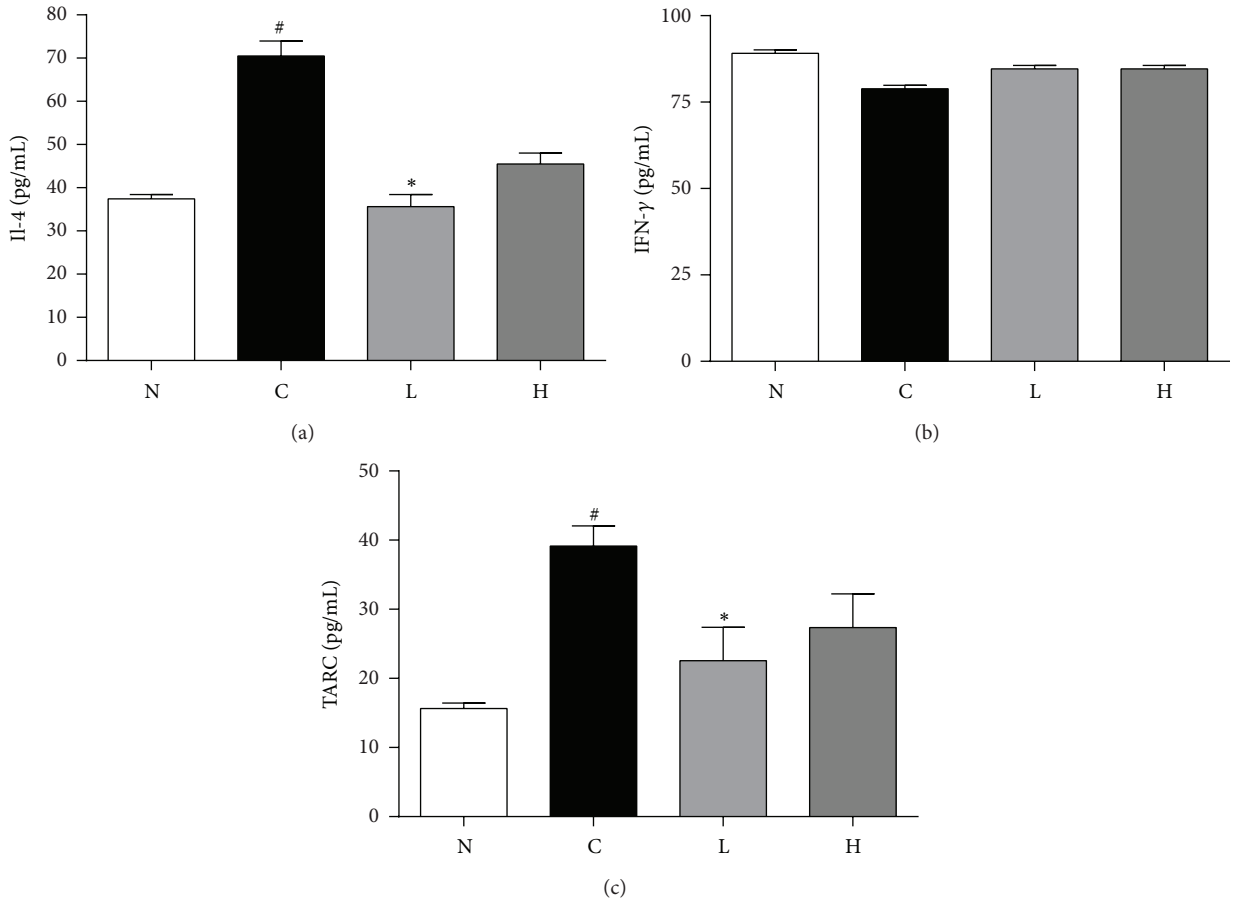

Figure 3: Measurement of serum cytokines production. Serum cytokine levels of IL-4 (a), TARC (b), and IFN-γ (c) were presented. Levels of IL-4, TARC, and IFN-γ were expressed as optical density (OD) at 450 nm. N: normal group (no stimulation), C: control group (stimulation by ovalbumin without irradiation), L: low dose irradiation on control group (30 mW/320 s), and H: high dose irradiation on control group (30 mW/640 s). $^{\#}P < 0.05$ versus the normal group. $^{*}P < 0.05$ versus the control group.

not shown). Based on this, doses for animal experiments were chosen as 1,000 mJ as low dose irradiation and 2,000 mJ as high dose irradiation, respectively. According to the scheme as shown in Figure 1, 3 groups of animals were exposed and treated as indicated.

3.2. Effects of LLLT on Total IgE Level in the OVA-Induced AR Mice Serum. To further analyze the allergic response, we measured the serum levels of total IgE. The concentration of total IgE level was significantly increased in control group (74.54 ± 1.05%) compared to that of normal group (22.17 ± 1.21%, Figure 2). The total IgE levels of low dose irradiation group (42.64 ± 1.05%) and high dose irradiation group (59.93 ± 1.10%) were significantly decreased than those control group, respectively (Figure 2).

3.3. LLLT Reduces Cytokines IL-4 and TARC Production. To further examine the effect of LLLT on AR, the production of cytokines was evaluated through the ELISA test. Serum concentrations of IL-4, TARC, and IFN-γ were determined and are shown in Figure 3. The IL-4 level in control group (70.48±3.49%) showed elevated production of IL-4 compared to that in normal group (37.40 ± 1.01%), while the low dose irradiation group (35.65 ± 2.77%) and high dose irradiation group (45.50 ± 2.52%) were significantly decreased than that of control group (Figure 3(a)). In addition, TARC production in serum showed similar results as observed in IL-4 production. TARC level in control group (39.16 ± 2.90%) was increased than that in normal group (15.63 ± 0.78%), while the low dose irradiation group (22.56 ± 4.80%) and high dose irradiation group (27.36 ± 4.87%) were significantly decreased than that of control group (Figure 3(c)). The levels of IFN-γ production in normal, control, low dose irradiation, and high dose irradiation groups were 89.12 ± 1.00%, 78.91 ± 1.00%, 84.64 ± 1.01%, and 84.61 ± 1.01%, respectively. There was a slight difference between groups; however, there was no statistical significance (Figure 3(b)).

3.4. LLLT Reduces IL-4 Expression Levels in Mice Spleen and EL-4 Cells. Western blot was performed to determine the effect of laser irradiation on protein expression in spleen of AR mice. β-Tubulin was used as internal control. As the result indicated in Figure 4(a), IL-4 protein level in control group (144.85 ± 9.82%) was increased as compared to normal group

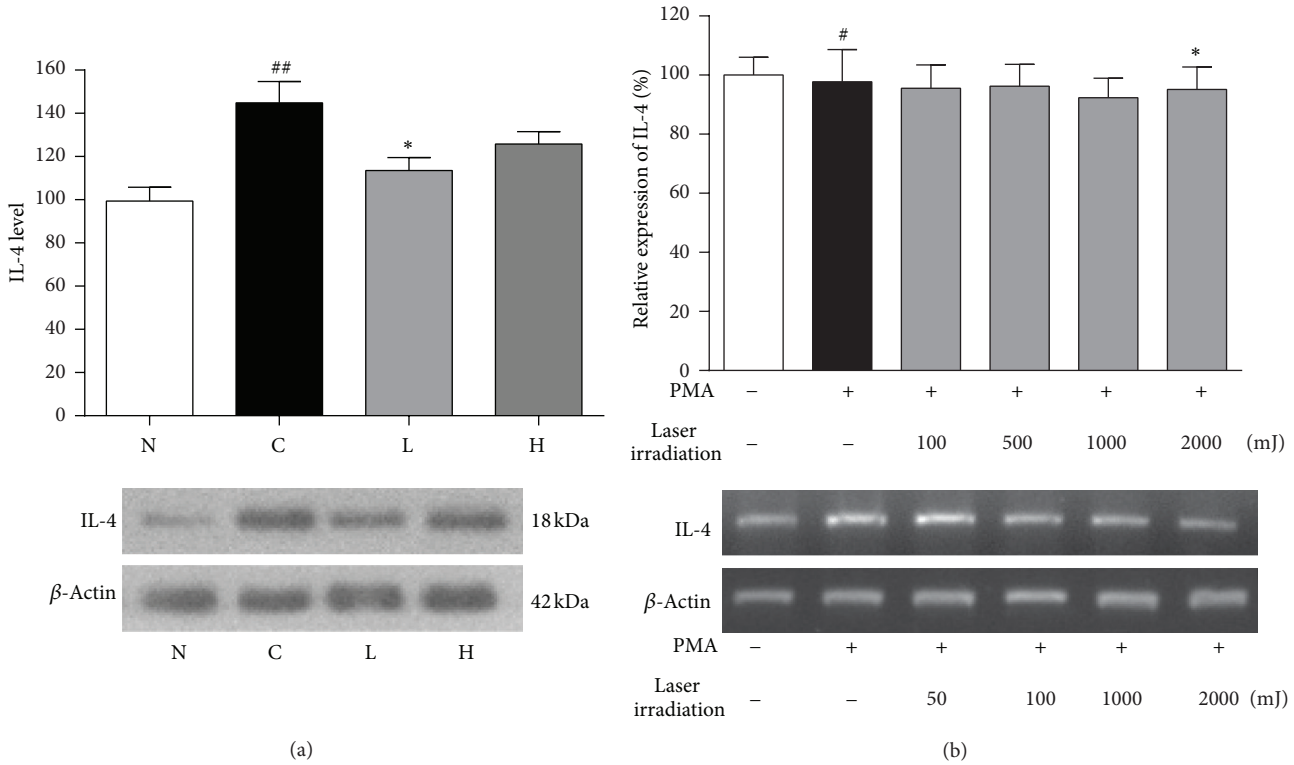

Figure 4: The IL-4 expression in mice spleen and EL-4 cells. (a) Protein expression levels of IL-4 in mouse spleen. The representative bands of IL-4 from electrophoresed gel were shown. N: normal group (no stimulation), C: control group (stimulation by ovalbumin without irradiation), L: low dose irradiation on control group (30 mW/320 s), and H: high dose irradiation on control group (30 mW/640 s). $^{\#}P < 0.05$ versus the normal group. $^{\#\#}P < 0.01$ versus the normal group. $^{*}P < 0.05$ versus the control group. (b) mRNA levels of IL-4 in EL-4 cells. The representative bands of IL-4 from electrophoresed gel after RT-PCR reaction. Normal: no stimulation; control: stimulation by PMA. $^{\#}P < 0.05$ versus the normal group. $^{*}P < 0.05$ versus the control group.

(99.41 ± 6.45%), while the IL-4 protein levels of low dose irradiation group (113.59 ± 5.95%) and high dose irradiation group (125.77 ± 5.74%) were significantly decreased than those of control group (Figure 4(a)). To confirm the effects of LLLT on IL-4 expression, we investigated the IL-4 mRNA level in the EL-4 cells. IL-4 expression of control group (112.43 ± 4.11%) was increased upon PMA treatment as compared to that of normal group (100.00 ± 5.43 %), while the laser irradiation groups (100, 500, 1,000 and 2,000 mJ) were decreased than those of control group (106.61 ± 8.46%, 92.98 ± 7.98%, 91.87 ± 8.97%, and 78.37 ± 9.53%, resp., Figure 4(b)).

3.5. Histological Changes after Laser Irradiation. HE staining was carried out to observe the histological changes of nasal septum. The respective numbers of inflammatory cells in the epithelium of nasal septum in the control group (Figure 5(b)) were significantly higher than those observed in the normal group (Figure 5(a)). Low dose irradiation group (Figure 5(c)) and high dose irradiation group (Figure 5(d)) showed reduced number of infiltrated inflammatory cells in the epithelium as well as recovered epithelium structure from OVA-induced epithelium damage. Especially, in low dose irradiation group, the effect of laser irradiation was most effective as compared to that observed in normal group (Figures 5(a) and 5(c)).

4. Discussion

Medical application of laser therapy has been classified into two categories: high level laser and low level laser. High level laser has a range between 3,000 and 10,000 mW and has been used in surgical purpose, while low level laser which has been applied as a laser acupuncture in traditional Korean medicine has below 1,000 mW of output [15]. Laser acupuncture is a painless, noninvasive treatment modality and presents no risk of infection. According to a literature review, evidence was found to support the use of laser acupuncture in the treatment of myofascial pain, postoperative nausea, and vomiting and for the relief of chronic tension headache [10]. It has been reported that low level laser stimulates certain types of cells and tissues using photoenergy, thus increases blood circulation, collagen synthesis, cell growth and bone regeneration and relieves inflammation, pain, and edema [16–22].

In this study, to investigate the effect of LLLT on animal model of AR, we determined the dose of laser irradiation at first, because targeting the acupuncture point of the nasal

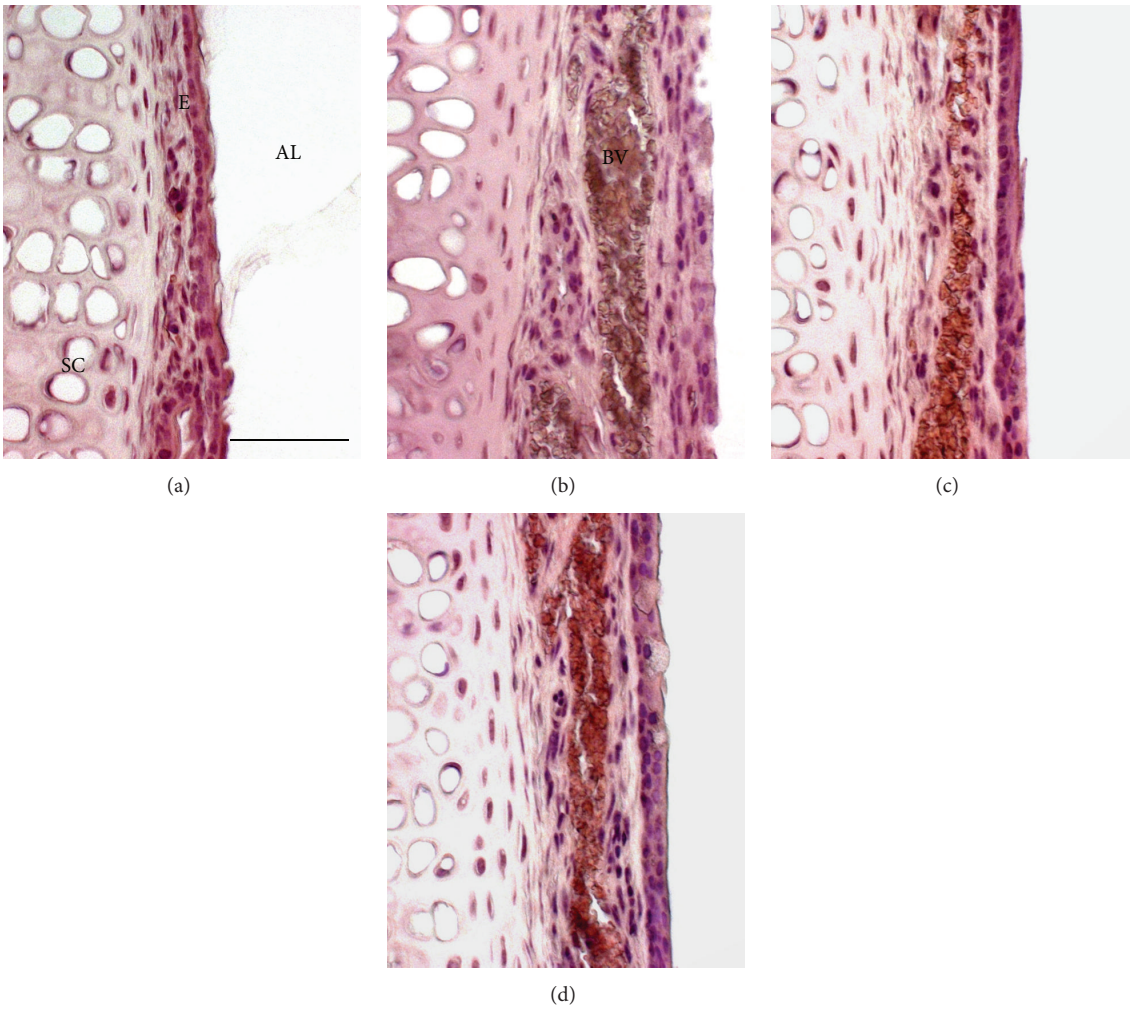

Figure 5: Hematoxylin and eosin staining of nasal septum. Mice were sensitized and challenged intranasally with no treatment (a), OVA treatment (b), 1,000 mJ laser-irradiation (c), and 2,000 mJ laser-irradiation (d). Images were obtained at an objective magnification of ×400. Scale bars = 100 μm. Airway lumen (AL), epithelium (E), blood vessels (BV), and septum cartilage (SC) were shown, respectively.

cavity as therapeutic purpose of laser treatment has not been tried yet. For this, EL-4 cells were subjected to viability assays by exposing to increasing doses of laser irradiation up to 2,000 mJ. Results showed that laser irradiation did not induce any notable cell death of EL-4 cell. Because the EL-4 is a cancerous cell, we could not exclude the possibility that the viabilities of these cells may be affected by higher rate of metabolic circumstances in cytoplasm. However, there were no morphological changes of EL-4 cells as observed in necrosis and cytotoxic environment. As a result, we applied two different doses of laser irradiation (1,000 and 2,000 mJ) to mouse AR model and examined serum level of IgE, cytokines (IL-4 and INF-γ), and TARC. In the low dose irradiation group, a significant inhibition of IgE, IL-4, and TARC was indicated. In addition, the expression of IL-4 was significantly inhibited in the low dose irradiation group, and the dose-dependent inhibition of EL-4 cells was detected. Particularly, inflammatory cell infiltration of nasal septum was decreased by laser irradiation in terms of histological examination with marked decrease in low dose irradiation group.

Type I hypersensitivity of AR is mediated by IgE immune response against foreign allergen and leads to the development of acute inflammation through IgE sensitive mast cells and other immune modulators [23]. When nasal mucosa is exposed to allergen, many immune cells such as mast cells, neutrophils, eosinophils, and lymphocytes are activated and cause infiltration of cells followed by blood vessel damage and histological change of nasal tissues [23].

IgE triggers allergic symptoms by activating the secretion of granules from mast cell and basophil through binding to its surface receptors [24]. Increasing IgE level in serum can induce acute hypersensitivity and subsequently cause symptoms for hay fever, asthma, and anaphylaxis. In our experiments, OVA-induced mouse showed increased IgE production in serum, and this was decreased by the low level laser irradiation, suggesting that LLLT has inhibitory effects on IgE production in the AR mouse model.

It has been known that IL-4 plays an important role in development of early stage of inflammation at AR, and it sustains the inflammatory process through the induction of

Th2 cell differentiation and function [25, 26]. On the other hand, IFN-γ which is secreted from Th1 cells stimulates antibody production of B cells and induces IgG mediated immune responses [27]. It has been also reported that increasing level of IFN-γ could reduce the early and the late stage of allergic reaction and relieve the symptom [28, 29]. Thus, it has an antagonic action against IL-4 function and IgE production. TARC (CCL17) is a receptor expressed in Th2 cells and binds to CCL4 with high affinity [30]. It has been known to be involved in leukocytes migration and regarded as one of key inflammatory factors with IgE in atopic immune responses [24]. To investigate the effect of LLLT on cytokines production in OVA-induced AR mice, we performed ELISA assays. Results showed that the low level laser irradiation was significantly reducing serum IL-4 and TARC levels, while INF-γ level was not affected by any doses of LLLT. In the present study, the inhibitory effect of LLLT on IL-4 production was confirmed by RT-PCR analysis and western blot using mouse spleen and EL-4 Th2 cells, respectively. These results strongly suggest that the effect of LLLT on AR mice was derived from Th2 mediated immune responses by regulating IgE and IL-4 production rather than those of Th1 related immune responses. In addition, IL-4 is involved in synthesis and secretion of IgE by B cells. Thus, inhibiting the expression of IL-4 might be related to the serum levels of IgE. However, molecular mechanisms by which LLLT reduces the IgE and IL-4 production remain to be studied.

Histological change of nasal mucous membrane is an important indicative factor in AR development. Therefore, we examined whether the effect of LLLT is accompanied by histological improvement of epithelium of nasal septum. OVA-induced AR mice showed clear damages in nasal epithelium as observed by infiltration of cells across the blood vessel, and this was relieved by LLLT not only in number of infiltrated cells but also in the overall histological damages of nasal septum. Together with the effect of LLLT on the regulation of cytokines expression, anti-inflammatory effects of LLLT have been reported. In animal model of acute lung inflammation induced by intestinal ischemia/reperfusion or LPS, LLLT has relieved airway inflammation through the induction of IL-10 and reduction of TNF and macrophage inflammatory protein-2 (MIP-2) expression [31, 32]. Considering the maximal effects at low dose irradiation in the present study, the effective irradiation dose of LLLT should be determined when it subject to the application for the therapeutic use in human.

5. Conclusion

LLLT might be an alternative therapeutic method for treating AR or its symptoms, and low dose irradiation (1,000 mJ) could be more effective than high dose irradiation (2,000 mJ). This could be explained by the function of low level laser irradiation that inhibits the progression of AR through the regulation of Th2 cell activation, differentiation, and expression of inflammatory molecules such as cytokines and IgE. To explain the clinical effectiveness, optimal doses of laser irradiation, or the detailed mechanisms, further investigations including clinical applications to human or molecular studies will be needed.

Authors' Contribution

Binhye Choi and Mun Seog Chang contributed equally to the study and are cofirst authors.

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References

[1] D. V. Wallace, M. S. Dykewicz, D. I. Bernstein et al., "The diagnosis and management of rhinitis: an updated practice parameter," *The Journal of Allergy and Clinical Immunology*, vol. 122, no. 2, pp. S1–S84, 2008.

[2] J. Bousquet, N. Khaltaev, A. A. Cruz et al., "Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA2LEN and AllerGen)," *Allergy*, vol. 63, supplement 86, pp. 8–160, 2008.

[3] M. Plaut and M. D. Valentine, "Allergic Rhinitis," *The New England Journal of Medicine*, vol. 353, no. 18, pp. 1934–1944, 2005.

[4] R. A. Nathan, "The burden of allergic rhinitis," *Allergy and Asthma Proceedings*, vol. 28, no. 1, pp. 3–9, 2007.

[5] Q. Q. Li, G. X. Shi, Q. Xu, J. Wang, C. Z. Liu, and L. P. Wang, "Acupuncture effect and central autonomic regulation," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 267959, 6 pages, 2013.

[6] J. Roberts, A. Huissoon, J. Dretzke, D. Wang, and C. Hyde, "A systematic review of the clinical effectiveness of acupuncture for allergic rhinitis," *BMC Complementary and Alternative Medicine*, vol. 8, article 13, 2008.

[7] C. S. Enwemeka, J. C. Parker, D. S. Dowdy, E. E. Harkness, L. E. Sanford, and L. D. Woodruff, "The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study," *Photomedicine and Laser Surgery*, vol. 22, no. 4, pp. 323–329, 2004.

[8] R. T. Chow, M. I. Johnson, R. A. Lopes-Martins, and J. M. Bjordal, "Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials," *The Lancet*, vol. 374, no. 9705, pp. 1897–1908, 2009.

[9] A. Petrucci, F. Sgolastra, R. Gatto, A. Mattei, and A. Monaco, "Effectiveness of low-level laser therapy in temporomandibular disorders: a systematic review and meta-analysis," *Journal of Orofacial Pain*, vol. 25, no. 4, pp. 298–307, 2011.

[10] G. D. Baxter, C. Bleakley, and S. McDonough, "Clinical effectiveness of laser acupuncture: a systematic review," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 1, no. 2, pp. 65–82, 2008.

[11] Y. Sakurai, M. Yamaguchi, and Y. Abiko, "Inhibitory effect of low-level laser irradiation on LPS-stimulated prostaglandin E2 production and cyclooxygenase-2 in human gingival fibroblasts," *European Journal of Oral Sciences*, vol. 108, no. 1, pp. 29–34, 2000.

[12] F. Aimbire, R. Albertini, M. T. T. Pacheco et al., "Low-level laser therapy induces dose-dependent reduction of TNFα levels in acute inflammation," *Photomedicine and Laser Surgery*, vol. 24, no. 1, pp. 33–37, 2006.

[13] R. G. De Oliveira, F. M. Aarestrup, C. Miranda, B. J. Vieira, A. P. Ferreira, and L. C. Andrade, "Low-level laser therapy reduces delayed hypersensitivity reaction to ovalbumin in balb/C mice," *Photomedicine and Laser Surgery*, vol. 28, no. 6, pp. 773–777, 2010.

[14] R. K. Saiki, T. L. Bugawan, and G. T. Horn, "Analysis of enzymatically amplified β-globin and HLA-DQα DNA with allele-specific oligonucleotide probes," *Nature*, vol. 324, no. 6093, pp. 163–166, 1986.

[15] J. B. Kim, J. Y. Jung, J. C. Ahn, C. K. Rhee, and Y. H. Oh, "Preventive and therapeutic effects of low level laser irradiation on gentamicin-induced vestibulotoxicity in rat utricles," *Korean Jornal of Otorhinolaryngology-Head and Neck Surgery*, vol. 52, pp. 19–28, 2009.

[16] M. K. Marei, S. H. Abdel-Meguid, S. A. Mokhtar, and S. A. Rizk, "Effect of low-energy laser application in the treatment of denture-induced mucosal lesions," *Journal of Prosthetic Dentistry*, vol. 77, no. 3, pp. 256–264, 1997.

[17] K. U. Kim, "Effects of low level laser irradiation with 904 nm pulsed diode laser on the extraction wound," *Journal of Korean Academy of Oral Medicine*, vol. 23, pp. 301–309, 1998.

[18] C. Clokie, K. C. Bentley, and T. W. Head, "The effects of the helium-neon laser on postsurgical discomfort: a pilot study," *Journal of the Canadian Dental Association*, vol. 57, no. 7, pp. 584–586, 1991.

[19] J. Kert, "Low level laser therapy used pre-operatively," *Laser News*, vol. 4, pp. 26–32, 1992.

[20] A. K. Røynesdal, T. Bjornland, P. Barkvoll, and H. R. Haanaes, "The effect of soft-laser application on postoperative pain and swelling. A double-blind, crossover study," *International Journal of Oral and Maxillofacial Surgery*, vol. 22, no. 4, pp. 242–245, 1993.

[21] R. M. Pick and G. L. Powell, "Laser in dentistry. Soft-tissue procedures," *Dental Clinics of North America*, vol. 37, no. 2, pp. 281–296, 1993.

[22] M. Miller and T. Truhe, "Lasers in dentistry: an overview," *The Journal of the American Dental Association*, vol. 124, no. 2, pp. 32–35, 1993.

[23] H. M. Lee, M. S. Park, I. H. Park et al., "A comparative pilot study of symptom improvement before and after phototherapy in Korean patients with perennial allergic rhinitis," *Photochemistry and Photobiology*, vol. 89, pp. 751–757, 2013.

[24] T. J. Kindt, R. A. Goldsby, and B. A. Osborne, *Kuby Immunology*, W. H. Freeman, New York, NY, USA, 6th edition, 2006.

[25] B. S. Bochner and R. P. Schleimer, "The role of adhesion molecules in human eosinophil and basophil recruitment," *The Journal of Allergy and Clinical Immunology*, vol. 94, no. 3, pp. 427–439, 1994.

[26] Y. Ohashi, Y. Nakai, H. Okamoto et al., "Serum level of interleukin-4 in patients with perennial allergic rhinitis during allergen-specific immunotherapy," *Scandinavian Journal of Immunology*, vol. 43, no. 6, pp. 680–686, 1996.

[27] R. G. Oliveira, A. P. Ferreira, A. J. Côtes, B. J. Aarestrup, L. C. Andrade, and F. M. Aarestrup, "Low-level laser reduces the production of TNF-α, IFN-γ, and IL-10 induced by OVA," *Lasers in Medical Science*, 2013.

[28] F. Rousset, J. Robert, M. Andary et al., "Shifts in interleukin-4 and interferon-γ production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis," *The Journal of Allergy and Clinical Immunology*, vol. 87, no. 1, pp. 58–69, 1991.

[29] S. R. Durham, S. Ying, V. A. Varney et al., "Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for Interferon-γ," *The Journal of Allergy and Clinical Immunology*, vol. 97, no. 6, pp. 1356–1365, 1996.

[30] H. Saeki and K. Tamaki, "Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases," *Journal of Dermatological Science*, vol. 43, no. 2, pp. 75–84, 2006.

[31] F. M. De Lima, A. B. Villaverde, R. Albertini et al., "Dual Effect of low-level laser therapy (LLLT) on the acute lung inflammation induced by intestinal ischemia and reperfusion: action on anti- and pro-inflammatory cytokines," *Lasers in Surgery and Medicine*, vol. 43, no. 5, pp. 410–420, 2011.

[32] F. Mafra de Lima, A. B. Villaverde, M. A. Salgado et al., "Low intensity laser therapy (LILT) in vivo acts on the neutrophils recruitment and chemokines/cytokines levels in a model of acute pulmonary inflammation induced by aerosol of lipopolysaccharide from Escherichia coli in rat," *Journal of Photochemistry and Photobiology B*, vol. 101, no. 3, pp. 271–278, 2010.

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Research Article

Wave-Induced Flow in Meridians Demonstrated Using Photoluminescent Bioceramic Material on Acupuncture Points

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The mechanisms of acupuncture remain poorly understood, but it is generally assumed that measuring the electrical conductivity at various meridians provides data representing various meridian energies. In the past, noninvasive methods have been used to stimulate the acupuncture points at meridians, such as heat, electricity, magnets, and lasers. Photoluminescent bioceramic (PLB) material has been proven to weaken hydrogen bonds and alter the characteristics of liquid water. In this study, we applied the noninvasive PLB technique to acupuncture point irradiation, attempting to detect its effects by using electrical conductivity measurements. We reviewed relevant literature, searching for information on meridians including their wave-induced flow characteristics.

1. Introduction

Acupuncture meridians are traditionally believed to comprise channels that connect the surface of the body with internal organs. Twelve primary meridians are believed to be located bilaterally in the body. The left and right meridians are symmetrical and influence each other through the interconnected meridian channels [1–4]. A network of meridian channels is believed to be located within soft connective tissues. Traditional Chinese medicine (TCM) has extensively

described the related normal physiological functions, pathological conditions, transmission of the senses, and possible disease mechanisms [5]. The mechanisms of acupuncture remain poorly understood and require further investigation with scientific methods to explore the nature of meridian lines and acupuncture points. It is generally assumed that measuring the electrical conductivity at various meridians provides data relevant to the so called "meridian energy" [1].

In 1950, Dr. Yoshio Nakatani measured the electrical resistance of the skin of his patients, revealing poor electrical conductivity. He discovered that some patients presented excessive skin resistance. Because this line exhibited the increased electrical conductivity and seemed to follow a traditional meridian, he named it the "Ryodoraku," meaning a strong conductive line or electrical pathway. The Ryodoraku lines were defined as they corresponded to various classical Chinese meridian pathways [6].

In this study, we used Ryodoraku equipment to measure meridian current flow by using electrodermal measurement on the Ryodoraku meridian points. Many previous studies have measured current levels by applying Ryodoraku techniques [1, 6–10]. The 24 measured Ryodoraku meridian points are located on the twelve primary meridians; thus, based on the theories of TCM, the resulting electrodermal measurements are regarded as measurements of meridian energy [1].

A previous study of meridian energy or current flow suggested that specific diseases may be related to more than a single meridian [1] (e.g., gastric diseases to the stomach). A nonspecific relationship exists between abnormal organic findings and the corresponding meridian energies. These results support the hypothesis that all meridians are interconnected [1–4].

During the traditional acupuncture, a needle penetrates the dermis of the skin and subcutaneous tissue. It then passes through interstitial connective tissues, potentially reaching deeper structures such as muscles, nerves, bones, or other vital organs. Although adverse reactions to traditional acupuncture are rare, complications such as regional subcutaneous or systemic infections, neuropathy, spinal injuries, pneumothorax, and cardiac injury are occasionally reported [11, 12]. In this study, we used a non-invasive technique of PLB to irradiate acupuncture points and applied electrodermal measurement to assess whether PLB reinforces the effects of needle acupuncture on meridians. It was previously proven that PLB weakens hydrogen bonds and alters the characteristics of liquid water [13, 14]. In this study, we applied the PLB technique to the Ryodoraku meridian point irradiation, attempting to detect its effects on the meridian current flow by the possible alteration of liquid characteristics in the meridian channels; thus, this study may demonstrate the fluid-wave characteristics of meridians [13, 14]. In the past, noninvasive methods have been used to stimulate the acupuncture points, such as heat, electricity, magnets, and lasers, but none of these can replace the traditional acupuncture needles. Using PLB acupuncture point irradiation is painless, nontraumatic, and poses no risk of infection; this study should demonstrate the advantages of the PLB method [15]. We reviewed relevant research, to support our hypothesis that PLB irradiation is suitable for application on meridians.

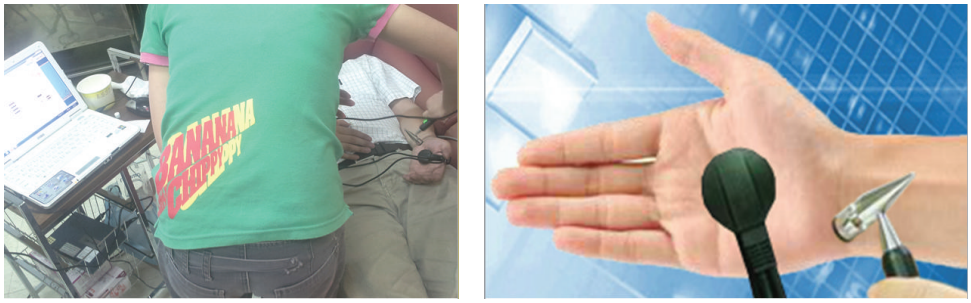
2. Materials and Methods

2.1. Participants. The participants were adults who consented to participate in this trial offered by the Departments of Traditional Medicine and Diagnostic Radiology, from March 2013 to June 2013. There are totally 147 meridian points on different candidates that were involved in this study. The study was approved by the human subjects committee at the hospital (approval number: TMU-JIRB201207024).

2.2. Measurements. We measured the meridian current by using a MEAD Me-Pro, 6th generation (Hanja International CO. Ltd., Taoyuan, Taiwan) device, which yielded electrodermal measurements of the 24 Ryodoraku meridian points (lung (LU9), pericardium (PC7), heart (HT7), small intestine (SI4),triple energizer (SJ4), large intestine (LI5), spleen (SP3), liver (LR3), kidney (KI4), bladder (BL65), gallbladder (GB40), and stomach (ST42)) and was similar to the equipment used in previous studies [1, 7–9]. The MEAD device was designed based on the Ryodoraku theory. The machine comprises two electrodes: the first, a metal cylinder held in the left hand of the patient, and the second, connected to a spring-loaded probe containing cotton moistened with physiological saline solution. A trained technician applies (Figure 1) the second electrode to the 24 acupuncture points along the 12 meridians (12 left plus 12 right). The measurements begin using a low current, which is probably gradually increased to a maximal value of 200 μA. The electrical conductivity readings for the meridian points are recorded into a computerized system. The participants with normal or abnormal meridian current findings are divided into six categories: extremely high current level, moderately high current level, normally high current level, extremely low current level, moderately low current level, and normally low current level.

This MEAD device is advantageous because the overall current levels remain in good repeatability when the device is operated by a trained technician (with at least 60 minutes of training or past examinations of at least 10 patients). According to the results of the mean overall meridian current, the technician selected the most abnormal current level on meridian channels and its specific Ryodoraku meridian points. The lowest current level meridian measurement (LCLMM) and highest current level meridian measurement (HCLMM) were both selected.

2.3. Photoluminescent Bioceramic Materials. Ceramic powder was obtained from the laboratory of radiology of Taipei Medical University (Taipei, Taiwan). The bioceramic material consisted of microsized particles produced from various elemental components [13, 14, 16–29]. Of the bioceramic material, 7% was embedded in a silicon sticker with good translucence (YY Rubber Company, Foshan, Guangdong, PRC). Photoluminescence is a special type of luminescence, referring to materials that absorb light energy and then



(a) (b)

Figure 1: Electrodermal measurements of various acupuncture points on the meridian line (a), using a metal cylinder, electrode, and spring-loaded probe (b).

(a) (b)

Figure 2: PLB irradiation on acupuncture points and remeasurement.

release that energy in the form of light; it also describes the interaction between electromagnetic radiation and matter. The PLB of this bioceramic material absorbs some specific wavelength spectrum (including near, middle, and far infrared) and was provided using visible light source irradiation, which was directed to the silicon sticker placed on the selected meridian lines at the corresponding acupuncture point (Figure 2). The light sources were visible light-emitting diodes (LEDs), which emitted wavelengths of a visible white light spectrum between 480 nm and 780 nm. We strictly controlled the level of illumination at 450 lux ± 50 lux, avoiding thermal effects on the skin of the participants.

2.4. To Analyze Possible Effects on Meridian Points on Different Candidates. The participants with meridian current findings (Figure 3) were divided into six categories: extremely high current level, moderately high current level, normally high current level, extremely low current level, moderately low current level, and normally low current level. After selecting the most abnormal current levels on meridian lines (LCLMM or HCLMM) and their corresponding acupuncture points for each patient, we performed PLB irradiation for 15 minutes (Figure 2). The meridian current was reexamined by using the MEAD Me-Pro, which yielded new electrodermal measurements of the 24 meridians. The second result of the overall mean of meridian currents was compared with the previous measurements from 15 minutes prior.

We calculated and compared before and after PLB irradiations on the mean current levels of each candidate the normalizing ability of PLB irradiations on extremely high and moderately high current level group and also on extremely low and moderately low current level group on different meridian channels.

2.5. To Observe the Possibility of One Specific Meridian Channel Indirectly to Affect Another Meridian Channel (Crossover Effect). After PLB treatment of one specific meridian channel (e.g., gall bladder, lung or triple energizer), we observe that the current level of another specific meridian channel could be influenced. We supposed the specific meridian channel is being influenced as (1) change of current level over 50%; (2) change of its current level category (six categories of extremely high current level, moderately high current level, normally high current level, extremely low current level, moderately low current level, and normally low current level). In other word, the influence is defined as positive "crossover" effect by PLB treatment.

2.6. Data Analysis. The 2006 version of AutoCAD was used for data processing, calculating the percentage change in

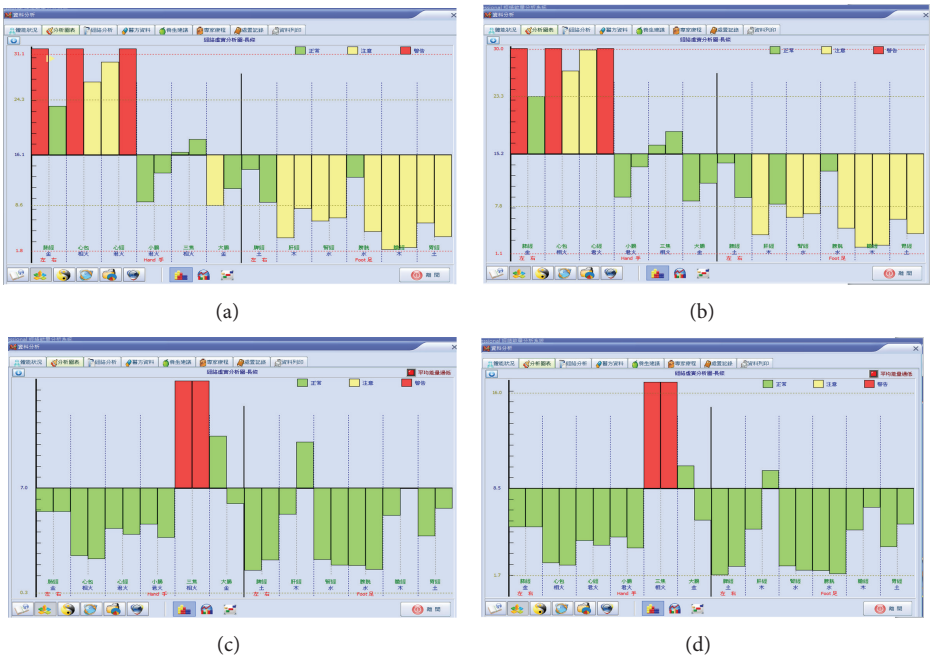


Figure 3: (a, c) Two examples of candidates on instrumental measurements; (b, d) the repeated measurements (within 15 minutes) showed good repeatability.

the scale and ratio of the current levels at various meridian channels before and after the PLB irradiation of acupuncture points. Statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, Ill, USA). We examined the individual variables by using percentages, means, and standard deviations, exploring group differences by using paired samples t-test.

2.7. Literature Research. Using Google Scholar (http://scholar.google.com.tw/), we searched for relevant literature published from 2000 to 2013, using keywords combinations such as *meridian, acupuncture, propagation, stimulation, current, water, fluid, anatomy, energy, transport, flow*, and *propagation*. We determined the total number of citations in each subject in the study period and selected the major research articles (in English) to further our discussion. Moreover, critical findings are presented when electromagnetic methods and radiological images are used to evaluate the anatomy and characteristics of meridians.

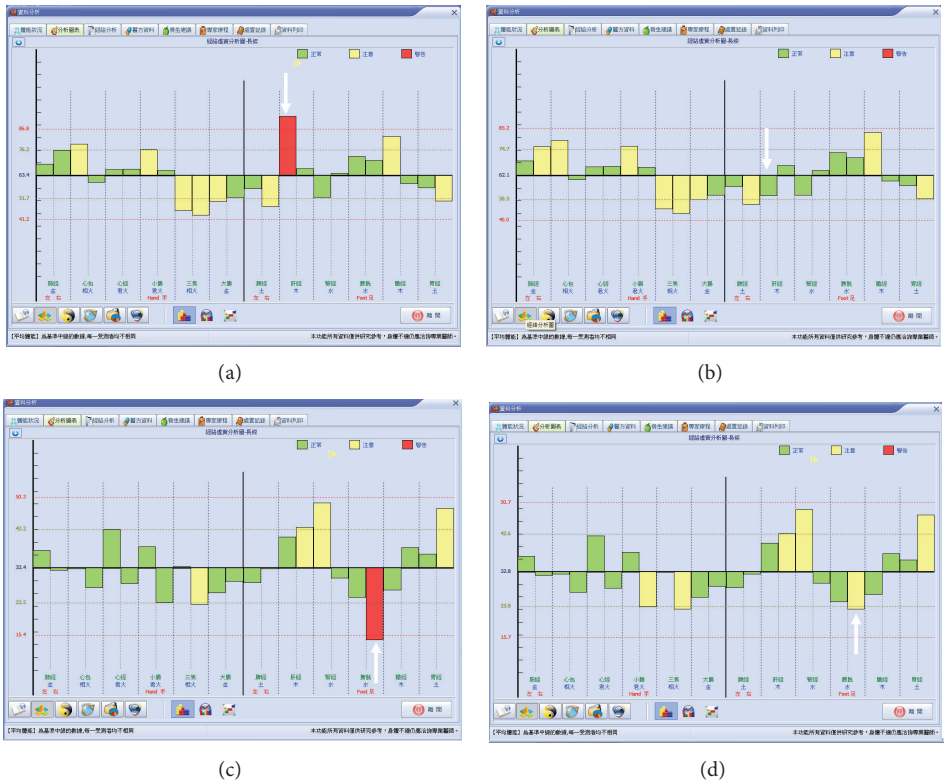
3. Results

3.1. Repeatable Results before PLB Irradiation. As previously mentioned, the MEAD Me-Pro maintains stable levels of current; a trained technician can easily repeat the results of a test (Figure 3).

3.2. Overall Meridian Current. The participants with abnormal meridian current findings such as extremely high current level or extremely low current level received PLB irradiation on the specific corresponding acupuncture points and then remeasured after 15 minutes. The results showed significant effect and tendency of their normalization of abnormal meridian currents (Figure 4). Besides, there is also observed tendency of interaction between specific meridian channels (Figure 5). There are totally 147 meridian points on 76 candidates that received PLB irradiations on specific corresponding acupuncture points. Amongst them, 69 meridian points were measured as extremely high or moderately high current levels. There were 78 meridian points measured as extremely low or moderately low current levels.

3.3. Results of Mean Current Level Change and Normalizing Ability after PLB Irradiations. Table 1 shows that there is no significant difference (P-value = 0.054) of the candidates (n = 76) after PLB irradiations on their mean current levels (averaging from their 24 Ryodoraku meridian points). But there are significant normalizing ability of PLB irradiations on extremely high and moderately high current level group (n = 69; positive current values above the mean current level); and on extremely low and moderately low current level group (n = 78; negative current values below the mean current level). Therefore, the PLB irradiation significantly regulated the meridian points and tended to normalize the current flow of abnormal meridians approaching their normal current level.

3.4. Results of One Specific Meridian Channel Indirectly to Affect Another Meridian Channel (Crossover Effect). There is about 73.7% (56/76) of testing candidates that revealed "crossover" influence of one specific meridian to another meridian channels such as "lung," "liver," "gall bladder,"



(a) (b)

(c) (d)

Figure 4: (a, c) Two examples of candidates with extremely high and low current levels measured by instrumental measurements; (b, d) after PLB irradiation of 15 minutes, there are remarkable normalization of current levels on the two specific meridian channels.

(a) (b)

Figure 5: (a) One example of candidate with six different extremely high and low current levels of meridian channels; (b) After PLB irradiation of corresponding acupuncture points of the two of the abnormal meridian channels, there are remarkable normalization of current levels on all of the six specific meridian channels.

"spleen," "triple energizer," "heart," and "pericardium." Among the positive "crossover" effect (change of current level over 50% or change of its current level category) of meridian by PLB treatment is the following, (the most explicit findings on PLB treatment in decreasing order) (Table 2): "lung" to affect "liver" (86%) > "gall bladder" to affect "lung" (71%) = "lung" to affect "spleen" (71%) > "triple energizer" to affect "spleen" (60%) > "gall Bladder" to affect "pericardium" (54%) > "gall Bladder" to affect "heart" (50%). Therefore, the PLB irradiation had a tendency to provoke intermeridian interaction.

3.5. Review of Literature. We located scant publications related to the topic of the current study. We selected a time range (2000–2013) and the English language, searching for studies regarding electromagnetic methods, radiological images, and the characteristics of meridians. Table 3 lists the papers selected for discussion.

Table 1: Mean current level change and normalizing ability after PLB irradiations.

	Before PLB (mean ± SD[#])	After PLB (mean ± SD)	Statistic calculation P value by t-test
Mean current level of 24 meridian channels (candidates number = 76)	33.2 ± 15.9	29.6 ± 18.1	0.054 (>0.05)
Positive current value above mean current level (sampled meridian number = 69) for testing the normalizing ability of PLB irradiations on extremely high/moderately high current level (HCLMM)	22.8 ± 12.5	7.67 ± 13.8	<0.0001*
Negative current value below mean current level (sampled meridian number = 78) for testing the normalizing ability of PLB irradiations on extremely low/moderately low current level (LCLMM)	−25.8 ± 9.53	−7.30 ± 17.3	<0.0001*

[#]SD is standard deviation. *Mean significant difference.

Table 2: The overall percentage of one specific meridian channel indirectly affecting another meridian channel according to the change of current level over 50% or change of its current level category[#].

Meridian channel Treated by PLB (candidates number)	Positive influence on "lung"	Positive influence on "pericardium"	Positive influence on "heart"	Positive influence on "spleen"	Positive influence on "liver"
"Gall bladder" (24)	71%	54%	50%	—	—
"Lung" (7)	—	—	—	71%	86%
"Triple energizer" (5)	—	—	—	60%	—

[#]Current level categories of extremely high current level, moderately high current level, normally high current level, extremely low current level, moderately low current level, and normally low current level.

4. Discussion

In Table 1, we prove that the 15 minutes of PLB irradiation exhibits a reliable complementary effect on LCLMM, rehabilitating to or approaching the normal current level; a 15-minute PLB irradiation also tended to suppress the HCLMM.

In Table 2, we show that the specific meridian channel current is indirectly affected by another meridian channel which has been treated by the PLB irradiation. Because the meridian channels and their corresponding acupuncture points are located in distinct locations, typical light energy irradiation should not be able to affect the electrical resistance of the skin or other meridian channels if no interconnecting network exists. Our previous water-based experiment proved that PLB weakens the hydrogen bonds and modifies the characteristics of liquid water [13, 14]. In this study, we applied the PLB technique to the Ryodoraku meridian point irradiation, attempting to detect its effects on the meridian current flow by the possible alteration of liquid characteristics in the meridian channels. Table 1 (Figure 4 as one example) shows that PLB irradiation has complementary effects on the current flow of abnormal meridians returning to its normal current level. Table 2 (Figure 5 as one example) shows that there are evident interactions between the current flows of relative meridians. Based on results of the current study, we suggest that the meridian channels are interconnected and acupuncture point stimulation induces a systematic wave-induced flow as shown in Figure 6.

To strengthen our hypothesis, we searched for support in the relevant literature. Although numerous studies of TCM are published each year, the results and corresponding discussions are typically TCM theories, which are rarely proven through scientific methods. In order to decrease the amount of bias and avoid referring to unproven TCM theories, our discussion is based exclusively on the results of evidence-based publications.

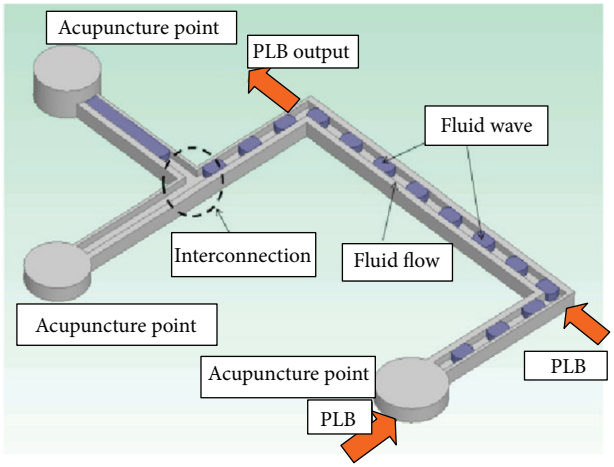


Figure 6: PLB irradiation of the specific meridian (acupuncture) points may induce a wave-induced flow in the meridians, by using the interconnection of various meridians.

After reviewing recent studies of meridians, acupuncture, propagation, stimulation, current, water, fluid, anatomy, energy, transport, flow, and propagation (Table 3), we discuss

Table 3: Meridian-related publications (2000–2013) that used evidence-based scientific methods.

Researchers	Method	Finding or hypothesis
Zhang et al. [30]	Single-power alternating current (SPAC) instrument	The subcutaneous impedance is lower at the low-impedance points as measured by the SPAC, two-electrode method.
Langevin and Yandow [31]	Ultrasound and postmortem tissue sections	The sites of acupuncture points are at locations of intermuscular or intramuscular connective tissue planes
Ahn et al. [32]	Ultrasound and electrical impedance instrument	The acupuncture point probably located on the skin overlying the fascial plane separating the muscles.
Brătilă and Moldovan [33]	Harmonic medicine: harmonic sounds stimulate the lung (LU; shoutaiyin feijing) and kidney (KI; zushaoyin shenjing) meridians	Resonance can be developed using music to stimulate acupuncture points. In this kind of acupunctural stimulation, a symphony may act and play a listening role.
Choi and Soh [34]	Optical fiber from a tungsten-halogen lamp	Light propagates more efficiently along the meridian than the reference path, demonstrating a 20% or greater difference among all tested participants.
Schlebusch et al. [35]	(1) Moxibustion (2) Similar light stimulation (3) Infrared detector within 3–5 μm range	Light channels appear within the body, seemingly identical to meridians.
Schlünzen et al. [36]	Positron emission tomography (PET); Cerebral blood flow in healthy humans	The penetration of the skin by using needles affects the medial frontal gyrus, whereas acupuncture of the LI-4 influences the putamen.
de Souza et al. [37]	Bioavailability of radio-pharmaceutical sodium pertechnetate	Uptake of the radiopharmaceutical in organs.
Zhang et al. [38]	Highly sensitive CO_2 instruments	The high correlation of transcutaneous CO_2 emissions along the meridian may illustrate that the metabolism on the meridian has similar changes or relationships. A strong correlation of energy metabolism activity exists among the body surfaces along the meridian, and an even stronger correlation exists among the acupoints on the meridian.
Lee et al. [39]	Optimal stimulator frequency of 40 Hz through the pericardium meridian; Hydrodynamic analysis	The mean transfer speed in the meridian (4 m/s) was significantly lower than it was in the adjacent control region (8.5 m/s, $P < 0.001$). Significant differences existed between the meridian and control points in attenuation rate ($P < 0.001$) and peak amplitude ($P < 0.001$). This implies that the composition of the meridian differs from that of the adjacent control regions.
Zhang et al. [40]	(1) Hydromechanic model (a) Guyton's method (b) Single pressure transducer (c) Two pressure transducers provided more stable measurement (2) The transmission of interstitial fluid pressure wave (3) Presentation of the channel by isotopic migration	The findings support the hypothesis that the interstitial fluid channels form the physiological and morphological basis of the acupuncture meridians described in detail by the ancient Chinese more than 2000 years ago.

the progression of scientific evidence regarding meridians and the possibility of wave-induced flows.

4.1. Current and Low Impedance Characteristics of Meridians. After using a single-power alternating current (SPAC) instrument to measure low-impedance acupuncture points, it was determined that the mean subcutaneous impedance at the acupuncture points was significantly lower than it was at the impedance of control points; subcutaneous impedance was lower at the low-impedance points measured using the SPAC two-electrode method. This suggests that a high amount of interstitial fluid lies beneath the low-impedance acupuncture points [30]. Previous studies have suggested that the acupuncture meridians are physiologically characterized by low electrical impedance and anatomically associated with the planes of connective tissue.

4.2. Anatomy of Meridians. Regarding the possible location of meridians, previous publications have suggested collagenous bands and the fascial plane. Collagenous bands, which can be detected by increasing the echogenicity of an ultrasound, are significantly associated with lower electrical impedance and may explain the reduced impedance that was previously reported at the acupuncture meridians. This finding provides critical insights about acupuncture meridians and the relevance of collagen in bioelectrical measurements.

Acupuncture points are likely located on the skin overlying the fascial planes that separate muscles; thus, acupuncture meridians may be located along the fascial planes between muscles or between a muscle and bone or tendon [5, 32]. Magnetic resonance imaging suggests that acupuncture points are located at connective tissue sites and cleavage planes [31].

4.3. Energy Consumption of Meridians. When a highly sensitive CO_2 instrument was used to measure the transcutaneous CO_2 emissions at the meridian lines, it showed that the level of the emission was highly related to the positions of acupuncture points and meridian lines on the body. It was concluded that a strong correlation exists in energy metabolism activity among the body surfaces along the meridian [38]. After moxibustion (or similar light stimulation) of the body in the $3\,\mu$m–$5\,\mu$m range, light channels appear on the body, demonstrating the existence of the acupuncture meridian structure.

It was proven that high temperature responses can occur along the meridians in physiological and pathological conditions, suggesting that meridians have infrared or near infrared radiation characteristics. These findings appear to confirm the existence of acupuncture meridians, suggesting that living matter is not in the ground state, but rather permanently excited [35, 41].

4.4. Light Propagation of Meridians. Previous studies have used non-invasive methods to detect the human meridian system. When the optical transport properties of visible laser lights and halogen lamps were used to irradiate meridian and nonmeridian pathways, it was suggested that the optical properties of the human meridian significantly differ from the surrounding tissues [42, 43]. The study concluded that the strong light propagation and optical properties along the meridian channel comprised a histological structure correlated with interstitial fluids [42, 43].

4.5. Radioactive Isotopes Pass through the Meridian Channel. Numerous experiments have proven that a radioactive tracer inserted at an acupuncture point follows a course corresponding to the meridians described by TCM. According to human anatomy, these pathways are neither part of the vascular system, nor the lymphatic ducts, and the velocity of the radioactive message suggests that they are not transferred along the nervous system. Thus, the meridian channels are likely individual pathways, separated from the microcirculation, vessels, lymphatic ducts, and nervous system [36, 37, 39, 44–47].

4.6. Flow Channel Characteristics of Meridians. A hydrodynamic analysis of the waveforms stimulated by vibration stimuli at meridian and nonmeridian points was conducted by using the optimal stimulator frequency at the pericardium meridian. It was determined that the mean transfer speed in the meridian was significantly lower than in the adjacent control region, and differences in the attenuation rate and peak amplitude were also noted [39]. Zhang et al. [40] conducted a hydromechanic study, exploring the fundamentals of acupuncture points and meridians, and measuring the transmission of artificial interstitial fluid pressure waves to examine their connection with the low resistance points; a strong connection was confirmed between the points. This indicates that the points form channels along the meridians (low-hydraulic resistance channels), corresponding with the meridian channels described in TCM. Interstitial fluid is an essential body fluid, which connects blood vessels, lymphatic ducts, and intracellular spaces; however, modern physiology pays little attention to interstitial fluid, and some clinicians debate whether interstitial fluid actually flows freely [48–50]. Their results showed that a lower hydraulic resistance channels (LHRC) existed along the meridians. The discovery of LHRCs provides the first physiological explanation for meridians, and the flow channel could interpret as the movement of isotope tracks. Another human study using an isotope tracing method showed that isotopes migrate along the meridian lines, deducing that this movement represented the flow of interstitial fluid along the LHRCs. Combining Zhang et al. [40] and other findings confirms that the meridian channels exist among the subcutaneous tissues and demonstrate the characteristics of fluid flow [51–61].

4.7. Contributions and Limitations. In our opinion, meridian lines are interstitial microscopic fluid channels and fulfilling most of the previously mentioned characteristics [62]. Although pure water containing no electrolytes or ions is an excellent electrical insulator, water is an effective solvent and always contains some dissolved solutes such as sodium chloride or other salts; water containing few impurities is a strong conductor of electricity [63–65]. In typical circumstances, water is able to propagate or transfer sound [66], visible light [67], heat (infrared) [68], and radioactive isotopes [69].

Based on the results of the current study and our review of the literature, we suggest that the hydrodynamic of waveforms fluid flow and interstitial fluid concepts [70] of the meridians and acupuncture points explains the reported transmission of current [30, 32], acoustic responses [33, 71], thermal responses [34, 35], optical transmissions [34, 43], isotope passages [36, 37, 72], hydrodynamic analysis [40, 73], and PLB stimulation [13, 14] in meridians. The hypothesis that meridians are open channels of interstitial fluid seems to be accepted, based on evidence-based research. Some limitations must be considered. In the future, we plan to create methods of observing and measuring the wave movement pattern and direction of induced flow within the meridian channels (Figure 6). Demonstrating the objective existence and 3D network of meridians requires combining various technologies including biophysics, biochemistry, molecular biology, and radiological imaging.

5. Conclusion

In this study, we applied the PLB technique to the Ryodoraku meridian point irradiation, attempting to detect its effects on the meridian current flow by the possible alteration of liquid characteristics in the meridian channels. Our data show that PLB has complementary effects for current flow of abnormal

meridians returning to its normal current level, and there are significant interactions between the current flows of relative meridians. In the future PLB can be used to regulate meridian current flow and provoke the intermeridian interactions.

Authors' contribution

Both authors C. Will Chen and Chau-Yun Hsu contributed equally to this work.

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References

[1] S.-M. Huang, L.-Y. Chien, C.-C. Chang, P.-H. Chen, and C. J. Tai, "Abnormal gastroscopy findings were related to lower meridian energy," *Evidence-based Complementary and Alternative Medicine*, vol. 2011, Article ID 878391, 7 pages, 2011.

[2] G.-J. Wang, M. H. Ayati, and W.-B. Zhang, "Meridian studies in China: a systematic review," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 3, no. 1, pp. 1–9, 2010.

[3] M. L. Yeh, H. H. Chen, and I. H. Lin, *Contemporary Meridians and Acupoints in Practice*, Farseeing publications, Taipei, Taiwan, 2004.

[4] C. Z. Liu and Y. H. Chang, "Channel medicine and meridian doctrine," *Journal of Chinese Medical Association of Acupuncture*, vol. 6, pp. 5–18, 2003 (Chinese).

[5] W. S. Yang, "Acta scieentarium naturalium Unviersitati Pekinensis, Investigation of the lower resistance meridian IV," *Speculation on the Physiological Functions of Acpuncture Meridians*, vol. 44, no. 2, 2008.

[6] Y. Nakatani, *A Guide for Application of Ryodoraku Autonomous Nerve Regulatory Therapy*, Chan's Books and Products, Alhambra, Spain, 1972.

[7] Y. Nakatani, "Skin electric resistance and Ryodoraku," ,*Journal of Autonomic Nerve*, vol. 6, article 52, 1956.

[8] Y. Nakatani and K. Yamashita, *Ryodoraku Acupuncture*, Ryodoraku Research Institute, Tokyo, Japan, 1977.

[9] C. N. Wang, C. S. Weng, W. C. Hu, Y. H. Chang, and J. G. Lin, "The development of new Ryodoraku neurometric patterns," *Journal of Medical and Biological Engineering*, vol. 22, pp. s99–s106, 2002 (Chinese).

[10] C.-C. Hsu, C.-S. Weng, T.-S. Liu, Y.-S. Tsai, and Y.-H. Chang, "Effects of electrical acupuncture on acupoint BL15 evaluated in terms of heart rate variability, pulse rate variability and skin conductance response," *The American Journal of Chinese Medicine*, vol. 34, no. 1, pp. 23–36, 2006.

[11] A. White, "A cumulative review of the range and incidence of significant adverse events associated with acupuncture," *Acupuncture in Medicine*, vol. 22, no. 3, pp. 122–133, 2004.

[12] H. Yamashita, H. Tsukayama, N. Hori, T. Kimura, and Y. Tanno, "Incidence of adverse reactions associated with acupuncture," *Journal of Alternative and Complementary Medicine*, vol. 6, no. 4, pp. 345–350, 2000.

[13] T. K. Leung, J. C. Yang, and Y. S. Lin, "The physical, chemical and biological effects by room temperature ceramic far-infrared ray emitting material irradiated water: a pilot study," *Journal of the Chinese Chemical Society*, vol. 59, no. 5, pp. 589–597, 2012.

[14] T.-K. Leung, P.-J. Huang, Y.-C. Chen, and C.-M. Lee, "Physical-chemical test platform for room temperature, far-infrared ray emitting ceramic materials (cFIR)," *Journal of the Chinese Chemical Society*, vol. 58, no. 5, pp. 653–658, 2011.

[15] T. W. Wong and K. P. Fung, "Acupuncture: from needle to laser," *Family Practice*, vol. 8, no. 2, pp. 168–170, 1991.

[16] T.-K. Leung, Y.-S. Lin, Y.-C. Chen et al., "Immunomodulatory effects of far-infrared ray irradiation via increasing calmodulin and nitric oxide production in raw 264.7 macrophages," *Biomedical Engineering*, vol. 21, no. 5, pp. 317–323, 2009.

[17] T. K. Leung, Y. S. Lin, C. M. Lee et al., "Direct and indirect effects of ceramic far infrared radiation on hydrogen peroxide-scavenging capacity and on murine macrophages under oxidative stress," *Journal of Medical and Biological Engineering*, vol. 31, no. 5, pp. 345–351, 2011.

[18] T.-K. Leung, H.-F. Shang, D.-C. Chen et al., "Effects of far infrared rays on hydrogen peroxide-scavenging capacity," *Biomedical Engineering*, vol. 23, no. 2, pp. 99–105, 2011.

[19] T.-K. Leung, C.-H. Chen, C.-H. Lai et al., "Bone and joint protection ability of ceramic material with biological effects," *The Chinese Journal of Physiology*, vol. 55, no. 1, pp. 47–54, 2012.

[20] T.-K. Leung, C.-M. Lee, S.-Y. Tsai, Y.-C. Chen, and J.-S. Chao, "A pilot study of ceramic powder far-infrared ray irradiation (cFIR) on physiology: observation of cell cultures and amphibian skeletal muscle," *Chinese Journal of Physiology*, vol. 54, no. 4, pp. 247–254, 2011.

[21] T. K. Leung, C. H. Chen, S. Y. Tsai, G. Hsiao, and C. M. Lee, "Effects of far infrared rays irradiated from ceramic material (bioceramic) on psychological stress-conditioned elevated heart rate, blood pressure, and oxidative stress-suppressed cardiac contractility," *Chinese Journal of Physiology*, vol. 55, no. 5, pp. 323–330, 2012.

[22] T. K. Leung, C. H. Kuo, C. M. Lee, N. W. Kan, and C. W. Hou, "Physiological effects of bioceramic material on human, assessment by 'Harvard Step', 'Resting Metabolic Rate' (RMR) and 'Treadmill Running' tests," *Chinese Journal of Physiology*, vol. 56, no. 6, 2013.

[23] T.-K. Leung, C.-M. Lee, M.-Y. Lin et al., "Far infrared ray irradiation induces intracellular generation of nitric oxide in breast cancer cells," *Journal of Medical and Biological Engineering*, vol. 29, no. 1, pp. 15–18, 2009.

[24] B. Y. Liau, T. K. Leung, M. C. Ou, C. K. Ho, and Y. S. Lin, "Inhibitory Effects of far-infrared ray generating belt on primary dysmenorrheal," *International Journal of Photoenergy*, vol. 2012, Article ID 238468, 6 pages, 2012.

[25] T. K. Leung, C. F. Chan, P. S. Lai, C. H. Yang, C. Y. Hsu, and Y. S. Lin, "Inhibitory effects of far-infrared irradiation generated by ceramic material on murine melanoma cell growth," *International Journal of Photoenergy*, vol. 2012, Article ID 646845, 8 pages, 2012.

[26] T. K. Leung, C. M. Lee, C. H. Wu et al., "The protective effect of non-ionized radiation from far infrared ray emitting ceramic material (cFIR) against oxidative stress on human breast epithelial cell (MCF-10A)," *Journal of Medical and Biological Engineering*. In press.

[27] Y. S. Lin, M. Y. Lin, T. K. Leung, C. H. Liao, T. T. Huang, and H. S. Huang, "Property characterization and biological function

of high far-infrared emitting ceramic powders," *Instruments Today*, vol. 28, no. 158, pp. 60–67, 2007.

[28] T.-K. Leung, J.-M. Lin, H.-S. Chien, and T.-C. Day, "Biological effects of melt spinning fabrics composed of 1% bioceramic material," *Textile Research Journal*, vol. 82, no. 11, pp. 1121–1130, 2012.

[29] T. K. Leung, Y. C. Liu, C. H. Chen, H. N. Fang, K. C. Chen, and C. M. Lee, "In vitro cell study of the possible anti-inflammatory and pain relief mechanism of far-infrared ray emitting ceramic material," *Journal of Medical and Biological Engineering*, vol. 33, no. 2, pp. 179–184, 2013.

[30] W.-B. Zhang, D.-M. Jeong, Y.-H. Lee, and M. S. Lee, "Measurement of subcutaneous impedance by four-electrode method at acupoints located with Single-Power Alternative Current," *The American Journal of Chinese Medicine*, vol. 32, no. 5, pp. 779–788, 2004.

[31] H. M. Langevin and J. A. Yandow, "Relationship of acupuncture points and meridians to connective tissue planes," *Anatomical Record*, vol. 269, no. 6, pp. 257–265, 2002.

[32] A. C. Ahn, M. Park, J. R. Shaw, C. A. McManus, T. J. Kaptchuk, and H. M. Langevin, "Electrical impedance of acupuncture meridians: the relevance of subcutaneous collagenous bands," *PLoS ONE*, vol. 5, no. 7, Article ID e11907, 2010.

[33] F. Brătilă and C. Moldovan, "Music acupuncture stimulation method," *Romanian Journal of Internal Medicine*, vol. 45, no. 4, pp. 407–411, 2007.

[34] C. H. Choi and K. S. Soh, "Study of propagation of Light along an acupuncture meridian," *Journal of the Optical Society of Korea*, vol. 7, no. 4, pp. 245–248, 2003.

[35] K.-P. Schlebusch, W. Maric-Oehler, and F.-A. Popp, "Biophotonics in the infrared spectral range reveal acupuncture meridian structure of the body," *Journal of Alternative and Complementary Medicine*, vol. 11, no. 1, pp. 171–173, 2005.

[36] L. Schlünzen, M. S. Vafaee, and G. E. Cold, "Acupuncture of LI-4 in anesthetized healthy humans decreases cerebral blood flow in the putamen measured with positron emission tomography," *Anesthesia and Analgesia*, vol. 104, no. 2, pp. 308–311, 2007.

[37] D. E. de Souza, V. Senna-Fernandes, L. D. C. Brito et al., "Acupuncture stimulation at Sanyinjiao: effect on the sodium pertechnetate bioavailability in rats," *The American Journal of Chinese Medicine*, vol. 35, no. 6, pp. 977–986, 2007.

[38] W.-B. Zhang, Y.-Y. Tian, Z.-X. Zhu, and R.-M. Xu, "The distribution of transcutaneous CO_2 emission and correlation with the points along the pericardium meridian," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 2, no. 3, pp. 197–201, 2009.

[39] M. S. Lee, Y.-C. Kim, S.-R. Moon, B.-C. Shin, and D.-M. Jeong, "Hydrodynamic analysis of waveforms induced by vibrational stimuli at meridian and non-meridian points," *The American Journal of Chinese Medicine*, vol. 32, no. 6, pp. 977–984, 2004.

[40] W.-B. Zhang, Y.-Y. Tian, H. Li et al., "A discovery of low hydraulic resistance channel along meridians," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 1, no. 1, pp. 20–28, 2008.

[41] J. Lin, Q. Wang, H. Liang et al., "Biophysical characteristics of meridians and acupoints: a systematic review," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 793841, 6 pages, 2012.

[42] H.-Q. Yang, S.-S. Xie, S.-H. Liu, H. Li, and Z.-Y. Guo, "Differences in optical transport properties between human meridian and non-meridian," *The American Journal of Chinese Medicine*, vol. 35, no. 5, pp. 743–752, 2007.

[43] S. Kaznachiev and L. Mochanova, "Light from living and human," *Novosibirsk Russia*, pp. 101–105, 1998.

[44] P. de Vernejoul, P. Albarède, and J. C. Darras, "Nuclear medicine and acupuncture message transmission," *Journal of Nuclear Medicine*, vol. 33, no. 3, pp. 409–412, 1992.

[45] J. C. Darras, P. Albarède, and P. de Vernejoul, "Nuclear medicine investigation of transmission of acupuncture information," *Acupuncture in Medicine*, vol. 11, pp. 22–28, 1993.

[46] P. de Vernejoul, P. Albarède, and J. C. Darras, "Etude des meridiensd'acupuncture par les traceurs radioactifs," *Bulletin de L'Académie Nationale de médecine*, vol. 169, pp. 1071–1075, 1985.

[47] J. B. Meng, H. H. Gang, B. Q. Chang, C. Weng, J. H. Tian, and F. L. Xu, "Radionuclide scintigraphy of the meridian system in normal subjects," *Acupuncture Research*, supplement 4, pp. 1–6, 1989 (Chinese).

[48] J. R. Levick, "The influence of hydrostatic pressure on transsynovial fluid movement and on capsular expansion in the rabbit knee," *Journal of Physiology*, vol. 289, pp. 69–82, 1979.

[49] K. Aukland and R. K. Reed, "Interstitial-lymphatic mechanisms in the control of extracellular fluid volume," *Physiological Reviews*, vol. 73, no. 1, pp. 1–78, 1993.

[50] J. R. Levick, "Flow through interstitium and other fibrous matrices," *Quarterly Journal of Experimental Physiology*, vol. 72, no. 4, pp. 409–437, 1987.

[51] H.-R. Xie, F.-C. Li, and W.-B. Zhang, "Observation and analysis on the meridian-collateral running track-related anatomical structure in the human body," *Zhen Ci Yan Jiu*, vol. 34, no. 3, pp. 202–206, 2009 (Chinese).

[52] J. B. Men, *Discovery of Fourteen Meridian Channels Image*, Science and Technology Publishing Company, 1998.

[53] D. X. Zhou, P. Yan, M. Zheng, P. F. Zhang, and S. Y. Ma, "A study on the transmission route of three Yin meridian in the foot using 32P radioautography study," *Chinese Journal of Anatomy*, vol. 22, pp. 307–308, 1999 (Chinese).

[54] J. B. Meng, H. H. Gao, and P. Wang, "A primary study of meridian circulation by isotopic migration," *Acupuncture Research*, vol. 1, pp. 77–80, 1987.

[55] W. B. Zhang, X. H. Jing, C. H. Li, Y. T. Lui, L. J. Zhang, and Z. C. Li, "The transport of interstitial fluid pressure wave in subcutaneous tissue on and beside the stomach meridian in rats," *Biomedical Engineering*, vol. 14, pp. 40–43, 1995 (Chinese).

[56] W. B. Zhang, Y. Y. Tian, H. Li, Y. J. Zeng, and F. Y. Zhuang, "A method to continuously measure hydraulic resistance of subcutis and the study of low hydraulic resistance points," *Acta Biophysica Sinica*, vol. 14, pp. 373–378, 1998 (Chinese).

[57] P. C. W. Fung, "Probing the mystery of Chinese medicine meridian channels with special emphasis on the connective tissue interstitial fluid system, mechanotransduction, cells durotaxis and mast cell degranulation," *Chinese Medicine*, vol. 29, no. 4, pp. 10–15, 2009.

[58] W. B. Zhang, F. Y. Zhuang, H. Li, Y. Y. Tian, Y. C. Wang, and B. J. Zhu, "An improved Guyton's method to measure hydraulic conductance and its use in measuring conductance in meridian tissue of animals," *Biomedical Engineering*, vol. 16, pp. 199–204, 1997 (Chinese).

[59] W. B. Zhang, H. Li, Y. C. Wang, B. J. Zhu, H. T. Dai, and Q. Y. Xu, "The wave propagation of interstitial fluid pressure along the Ren Mai and the mechanism of acupoint injection," *Zhongguo Zhen Jiu*, vol. 18, pp. 60–61, 1998 (Chinese).

[60] W. Zhang, F. Zhuang, Y. Tian, and H. Li, "A simulating study of biophysical features along meridians on a gel model," *Journal*

of Biomedical Engineering, vol. 18, no. 3, pp. 357–361, 2001 (Chinese).

[61] W. B. Zhang, X. H. Jing, R. M. Xu, Z. X. Zhu, C. H. Li, and H. Li, "The study on specificity of transcutaneous CO2 emission in large intestine meridian and stomach meridian," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 15, pp. 625–627, 1995 (Chinese).

[62] F. Q. Zhu, *Theoretical a Computational Studies of Microscopic Water Channels [Dissertation]*, University of Illinois Urbana-Champaign, 2004.

[63] G. M. Liu and J. S. Yang, "Study on the correlation of soil salt content with electric conductivity and soil water content," *Chinese Journal of Soil Science*, vol. 1, pp. 85–87, 2001 (Chinese).

[64] F. Ozcep, E. Yildirim, O. Tezel, M. Asci, and S. Karabulut, "Correlation between electrical resistivity and soil-water content based artificial intelligent techniques," *International Journal of Physical Sciences*, vol. 5, no. 1, pp. 47–56, 2010.

[65] J. A. Widegren, E. M. Saurer, K. N. Marsh, and J. W. Magee, "Electrolytic conductivity of four imidazolium-based room-temperature ionic liquids and the effect of a water impurity," *Journal of Chemical Thermodynamics*, vol. 37, no. 6, pp. 569–575, 2005.

[66] F. Sciortino and S. Sastry, "Sound propagation in liquid water: the puzzle continues," *The Journal of Chemical Physics*, vol. 100, no. 5, pp. 3881–3893, 1994.

[67] C. J. Funk, "Multiple scattering calculations of light propagation in ocean water," *Applied Optics*, vol. 12, no. 2, pp. 301–313, 1973.

[68] W. Qu, G. M. Mala, and D. Li, "Heat transfer for water flow in trapezoidal silicon microchannels," *International Journal of Heat and Mass Transfer*, vol. 43, no. 21, pp. 3925–3936, 2000.

[69] W. J. Kaufman and G. T. Orlob, "Measuring ground water movement with radioactive and chemical tracers warren," *Journal of American Water Works Association*, vol. 48, no. 5, pp. 559–572, 1956.

[70] M. Jakobsen, "The interacting inclusion model of wave-induced fluid flow," *Geophysical Journal International*, vol. 158, no. 3, pp. 1168–1176, 2004.

[71] X.-M. Wang, "Analysis on the mechanism of acupuncture infrasound energy in treatment of diseases," *Zhongguo Zhen Jiu*, vol. 29, no. 3, pp. 223–226, 2009 (Chinese).

[72] X. Shen, G. Ding, J. Wei et al., "An infrared radiation study of the biophysical characteristics of traditional moxibustion," *Complementary Therapies in Medicine*, vol. 14, no. 3, pp. 213–219, 2006.

[73] W. K. Wang, T. L. Hsu, H. C. Chang, and Y. Y. Wang, "Effect of acupuncture at Tsu San Li (St-36) on the pulse spectrum," *The American journal of Chinese medicine*, vol. 23, no. 2, pp. 121–130, 1995.

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Research Article

Effect of Low-Level Laser Stimulation on EEG Power in Normal Subjects with Closed Eyes

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In a previous study, we found that the low-level laser (LLL) stimulation at the palm with a frequency of 10 Hz was able to induce significant brain activation in normal subjects with opened eyes. However, the electroencephalography (EEG) changes to LLL stimulation in subjects with closed eyes have not been studied. In the present study, the laser array stimulator was applied to deliver insensible laser stimulations to the palm of the tested subjects with closed eyes (the laser group). The EEG activities before, during, and after the laser stimulation were collected. The EEG amplitude powers of each EEG frequency band at 19 locations were calculated. These power data were then analyzed by SPSS software using repeated-measure ANOVAs and appropriate posthoc tests. We found a pronounced decrease in the EEG power in alpha-bandwidth during laser simulation and then less decrease in the EEG power in delta-bandwidth in normal subjects with laser stimulation. The EEG power in beta-bandwidth in the right occipital area also decreased significantly in the laser group. We suggest that LLL stimulation might be conducive to falling into sleep in patients with sleep problems.

1. Introduction

The EEG activity can be affected by different stimulation modalities [1–8], including visual, auditory, and somatosensory stimulation. It has been proposed that, through the intermediary of adequate and effective electrocerebral modification (e.g., EEG activity), an appropriate sensory stimulation is able to induce a desired mental state, such as a relaxed or sleep state [9–12]. Such sensory stimulation can be applied to the fore or back of the head, scalp, eyes, nose root, temples, and also the specific acupoints. For example, Yasushim invented an apparatus to induce brain wave changes by an optically stimulating signals at a frequency close to actual human brain waves [9]. Siever designed a technique to activate the central nervous system (CNS) by auditory stimulations at different frequencies in the right or left brain hemisphere [10]. Sunnen proposed a method to generate sleep-inducing stimuli with a transducer [11]. Flagg et al. proposed an apparatus which can deliver a plurality of magnetic pulses from the nuchal region to influence the brain centers to obtain a desired mental state [12]. We reckon that low-level laser (LLL) stimulation should also be a good alternative to have the similar effect.

In our previous study, we found that the EEG activities of normal subjects with opened eyes could be affected by LLL stimulation at the left palm. With stimulation, the EEG powers in alpha- and theta-bandwidths in the posterior head regions increased, while the EEG power in beta-bandwidth in the frontal head regions decreased [13]. In that study, we required the tested subjects to keep eyes opened during the whole 30-minute-recording period, in order not to become drowsy or even fall into sleep. The aim of the present study is to investigate if there are any significant changes in EEG power to LLL stimulation in the normal subject with closed eyes.

2. Subjects and Methods

Prior to the trial, the study protocol was approved by the Institutional Ethics Committee of Min-Sheng General

Hospital. Each participant was required to give a written informed consent. This study was directed in conformity with the guidelines in the Helsinki Declaration.

2.1. Participants. Twenty normal healthy subjects were included (mean age 21.0 ± 1.2 years, 14 males, 6 females) in the present study. Each subject received two trials: one trial with the laser stimulator being turned on (the laser group) and the other trial with the stimulator being not turned on (the control group). In the first trial, each subject was randomly and blindly assigned to either laser or control group. Several days later in the second trial, the tested subject was then arranged to enter into the laser or the control group, just different to that in his or her first trial. As we had to match the operation schedule of the examination room as well as the free time of the tested subjects, the intervals between two trials were not the same and ranged from 3 to 7 days.

Exclusion criteria included (a) having a history of psychiatric disorders, for example, major depression, substance abuse, schizophrenic, or paranoid disorder, (b) having cardiopulmonary disease, and (c) receiving medication currently.

2.2. Laser. In this study, the same laser stimulator in our previous study was used [13]. The LAS consists of 6 laser diodes (LDs). Each LD was set at 7 mW output for minimum stimulation in this study, and the operational frequency was set at 10 Hz, duty cycle 50%. The light of laser diode without any collimated lens was a stripe shape due to the different divergence angle in horizontal (10°) and vertical (30°) directions. The area of the laser light was approximately equal to 14.8 mm^2 at 10 mm distance. Thus, the dosage would be approximately 20 joules/cm^2 for 10 minutes treatment; it is insensible on operation.

2.3. Procedure. The "double-blind randomized trial" was used in this study. In each trial, the subjects did not know which group they were in. The subject sat in an armchair and was then required to put the left palm on the LAS device. He or she was instructed to relax, follow the eyes-closed directive, and withhold any movements. In the laser group, the laser diodes were turned on for 10 minutes and not turned on in the control group. In the beginning, each subject was required to relax for five minutes in order to be in a stale physiological state. The ongoing EEG was recorded with closed eyes in three stages (6 sessions): before stimulation (baseline 5 min, session 1), during stimulation (laser stimulation, 10 min, session 2 and session 3), after stimulation (poststimulation, 15 min, and session 4, 5, and 6). This procedure was similar to that in our previous study [13], but the patients kept their eyes closed. The EEG technologist was required not to disturb the tested subject even evidence of drowsiness or sleep emerged out of the ongoing EEG.

2.4. Control. The low-level infrared laser diode is invisible and emits no heat or any other detectable indication; therefore, it is ideal for a double-blind study. When the subjects received a sham laser stimulation in the control group, they underwent the same procedure as in the laser group, but the laser stimulator was not turned on.

2.5. EEG Recording and Measurement. During experiment, the tester was required to put his or her left palm on the LAS. An electroencephalograph (Neurofax model EEG-1000, NIHON KOHDEN) was used in this study. The band pass was set at 0.5–70.0 Hz. The variation of EEG potential was recorded on the scalp with Ag/AgCl recording electrodes. Electrode placement was arranged following international 10–20 system. A quantitative referential (monopolar montage) EEG was recorded with 19 electrodes with linked earlobe references. The sampling rate was set at 256 samples per second. An ECG was recorded by placing electrodes on both hands. EEG data were analyzed to provide power data for the 19 recording locations in each of the four bandwidths (delta, 0.5–3.5 Hz; theta, 4–7 Hz; alpha, 8–13 Hz; beta, 13–50 Hz) with the software Neurofax version 05–80. The mean and standard deviation of calculated values were expressed as "Mean ± SD".

2.6. Statistical Analysis. A one-way repeated-measure ANOVA and appropriate posthoc tests were used to compare the differences of EEG band power before and after LAS stimulation from 19 recording locations in each of four band passes. Two-tailed paired t-test was applied to compare the difference of EEG band power before and after LAS stimulation from F4, C4, P4, O2, F3, C3, P3, and O1 electrodes. All the statistical analyses were executed with SPSS software (version 11). A statistical significance was recognized as P value <0.05.

3. Result

For the laser group, the ANOVA analysis indicated a total of 14 significant locations with $P < 0.05$. The locations, by band pass, were as follows: delta: Fp2, alpha: Fp1, Fp2, F3, Fz, F4, T3, P3, P4, T6, O1, and O2; beta: T6, O2; theta: no significant ANOVA results. For the placebo group, the ANOVA analysis indicated a total of 14 significant locations with $P < 0.05$ too but in different places. The locations, by band pass, were as follows: delta: no significant ANOVA results, theta: F7, alpha: F3, Fz, F4, F8, T3, T4, P3, Pz, T6, O1, and O2; beta: T5, T6. Locations indicating significant changes from baseline power during and after the 10 min LAS stimulation session are presented in Figure 1 for the laser group and in Figure 2 for the placebo group. Even the differences between these two groups are not significant at all recording locations, but it is worthy to mention that the alpha power decreased significantly in frontal regions (Fp1 and Fp2) and the beta power decreased significantly in occipital lobe (O2) in the laser group.

The ROI (regions of interest: frontal, central, parietal, and occipital regions) were analyzed with paired t-test analysis. Figure 3 shows the temporal change of the normalized intensity of EEG power in alpha-bandwidth in the six consecutive sessions in the laser (rhomboid spot) and the control (square spot) group. In this figure, the normalized intensity in

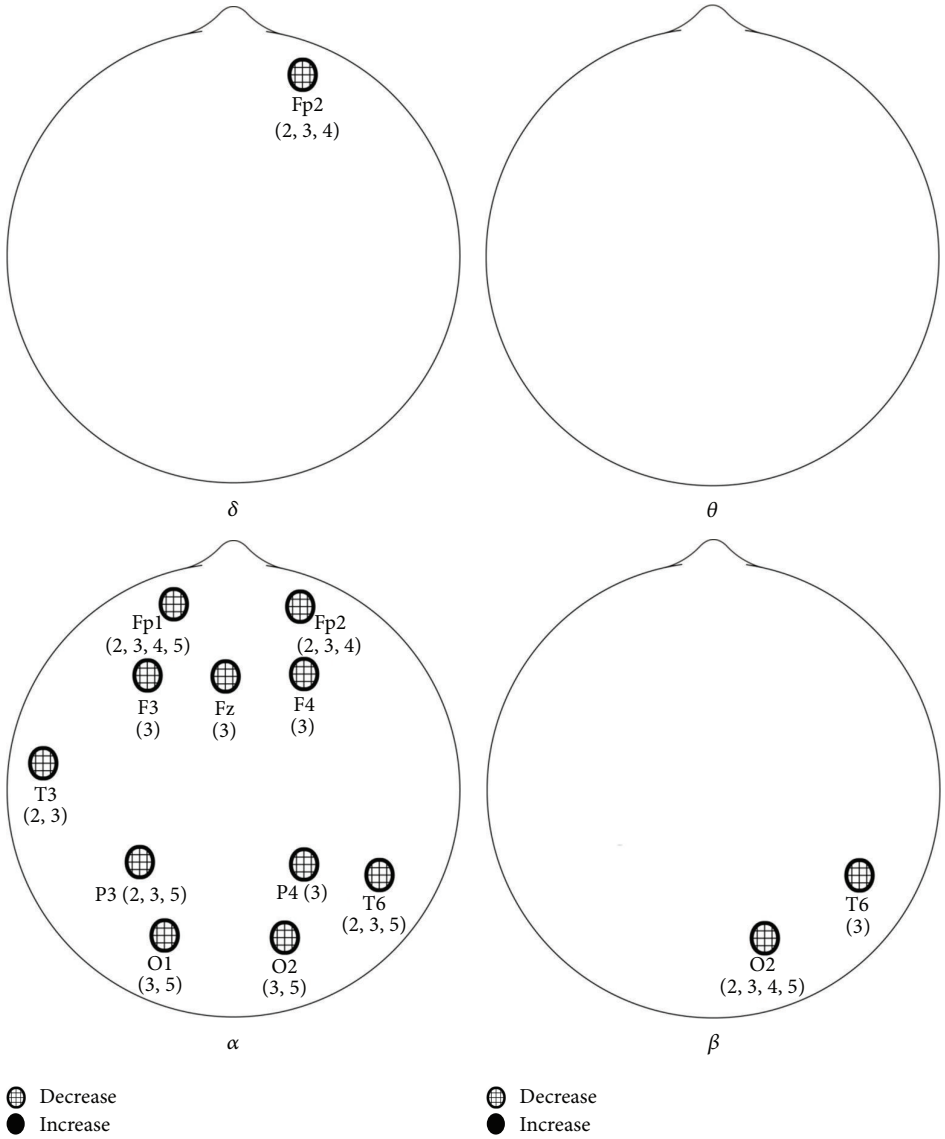

Figure 1: Significant changes from baseline EEG activity during 10 Hz stimulation. Numbers indicate time period of significance (1 first 5 min of baseline, 2 first 5 min of LAS, 3 second 5 min of LAS, 4 fourth 5 min, 5 fifth 5 min, and 6 last 5 min).

the ordinate was obtained from division of the measured alpha power by the corresponding alpha power in the first session. In either laser or control group, the alpha power significantly decreased from session 2 to session 6, especially in the posterior head region (i.e., P3, P4, O1, and O2). The decrease in alpha power in sessions 2 and 3 is more prominent in the laser group than in the control group.

The temporal changes in the normalized intensity of EEG power in beta-bandwidth are showed in Figure 4. In either laser or control group, there was a variation in beta power in the anterior head area (F3, F4, C3, and C4) from session 2 to session 6, but a tendency to decrease in beta power was seen. A decrease of beta power was seen in the posterior head region, especially the occipital area (O1 and O2). In the right occipital area, decrease in beta power in the laser group was greater than that in the control group, and it has significant meaning in session 2, $P < 0.05$.

Figure 5 shows the temporal changes in the normalized intensity of EEG power in theta-bandwidth. As compared with that in the first session, little changes in theta power were found. There were only mild decrease in the frontal area (F3 and F4) and mild decrease in the last session in nearly all the head areas. Difference in intensity changes between two groups was minimal and not significant.

The temporal changes in the normalized intensity of EEG power in delta-bandwidth are showed in Figure 6. A similar pattern of temporal changes in the delta power was found in all of the head areas; that is, the delta power decreased in the 2nd and 3rd sessions and gradually returned toward the original level (the first session) in the 4th and 5th sessions, with an exception of delta power in the frontal area (F3 and F4). The delta power dropped again in the last session. Degree of drop in the delta power in some areas was greater in the control than in the laser group.

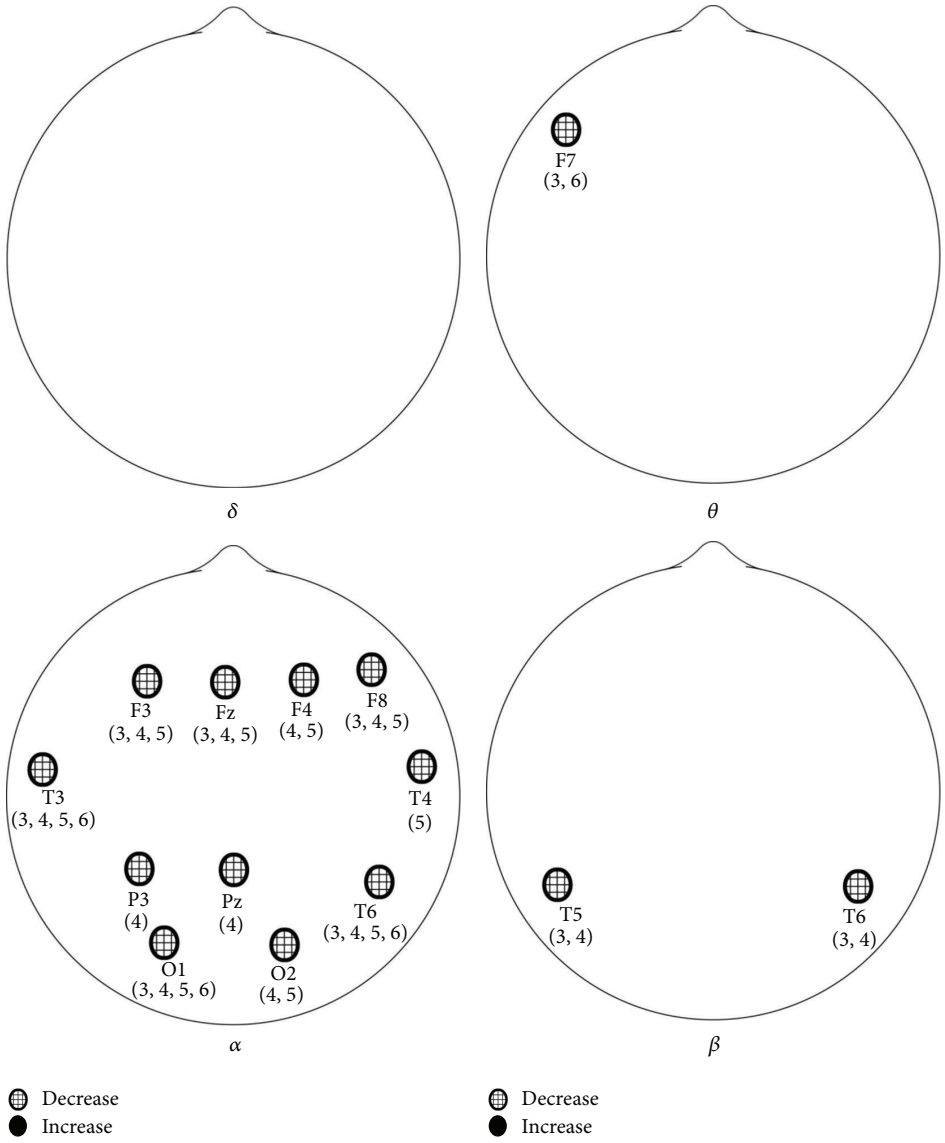

Figure 2: Significant changes from baseline EEG activity in placebo group. Numbers indicate time period of significance (1 first 5 min of baseline, 2 first 5 min of LAS, 3 second 5 min of LAS, 4 fourth 5 min, 5 fifth 5 min, and 6 last 5 min).

4. Discussion

When taking routine awake EEG in the EEG laboratory, the patient is instructed to sit quietly, to relax himself, and to keep eyes closed. In such a circumstance, the patient not surprisingly easily becomes drowsy or even falls into sleep. Therefore, the EEG technologist has to watch the ongoing EEG tracings carefully and to awake the patient if EEG evidence of drowsiness appears. In our previous study, we required the tested subjects to keep eyes opened and found LLL stimulation able to induce brain activation in normal awake subjects. In the present study, we let the tested subjects close their eyes and relax completely. We did not prevent them from becoming drowsy or falling into sleep. Electroencephalographically, almost all the tested subjects could not maintain awake during the whole experiment period.

In normal, awake, relaxed adults with closed eyes, the dominant brain waves are alpha rhythms distributed mainly in the posterior head region. The alpha rhythms are attenuated by visual attention and mental efforts [14]. The typical EEG changes in drowsiness in normal adults are gradual or brisk dropout of alpha rhythms, appearance of desynchronized low-voltage slow waves (2–7 Hz), and emergence of vertex sharp waves. Anterior diffusion of alpha rhythms and increased beta activities (mainly 18–25 Hz) in the frontocentral areas are occasionally noted. Then, sleep spindles, vertex sharp waves, and K-complexes may probably emerge from a background with low amplitude and mixed frequency. High-voltage rhythmic theta or delta waves are very rare [15].

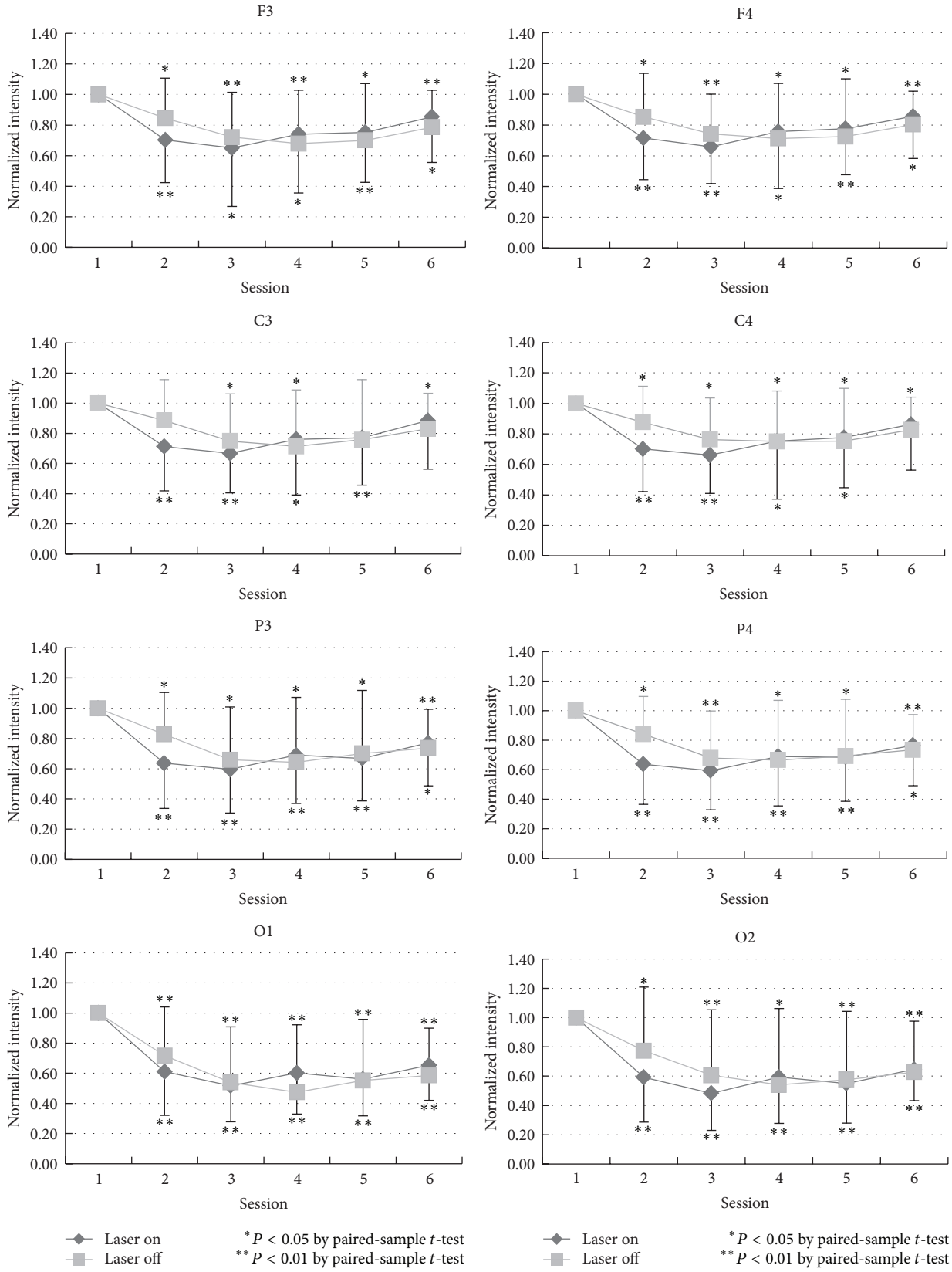

Figure 3: The statistical analysis of the alpha-band by comparing the baseline and each session in laser and placebo group is shown in different locations: F3, C3, P3, O1, F4, C4, P4, and O2.

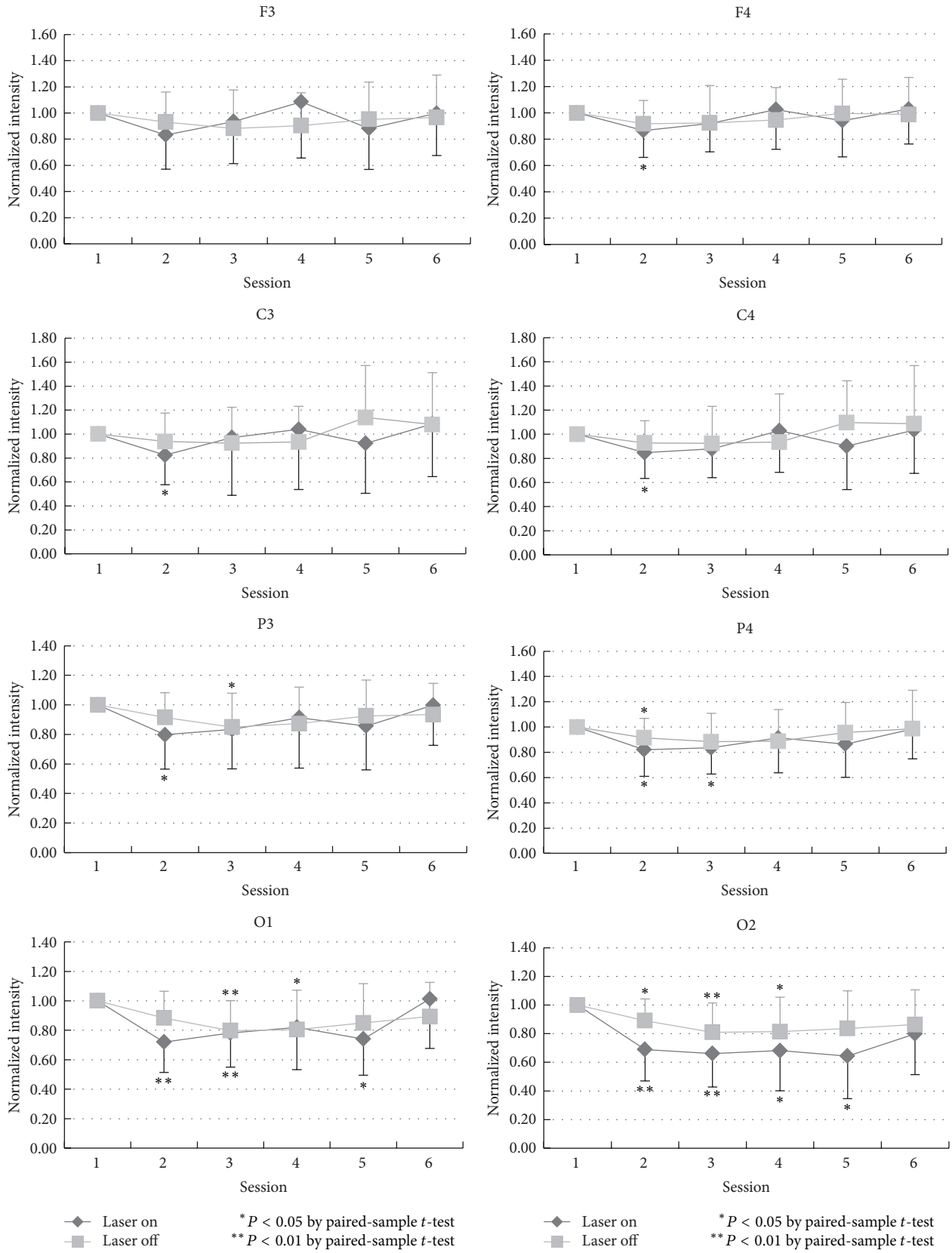

Figure 4: The statistical analysis of the beta-band by comparing the baseline and each session in laser and placebo group is shown in different locations: F3, C3, P3, O1, F4, C4, P4, and O2.

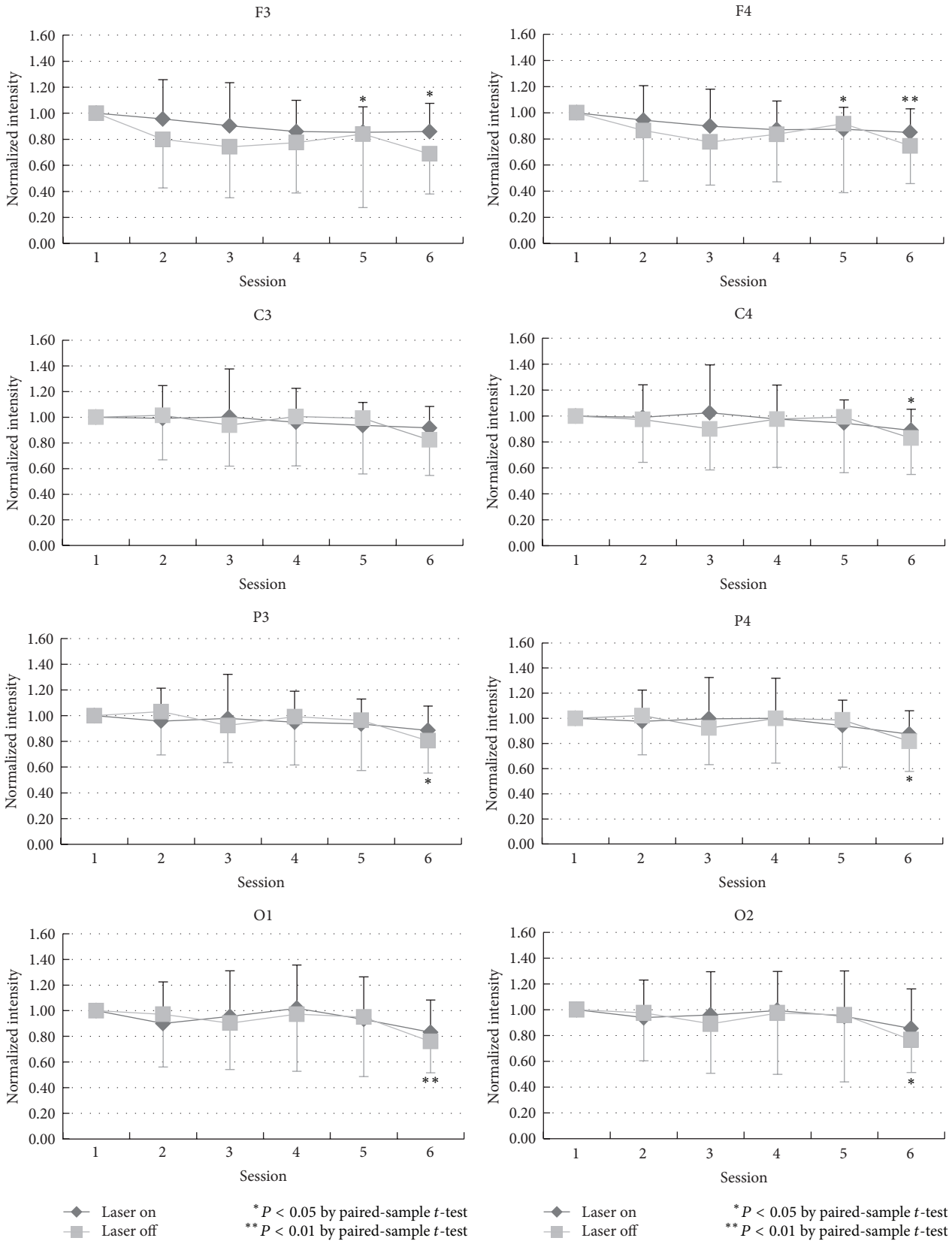

Figure 5: The statistical analysis of the theta-band by comparing the baseline and each session in laser and placebo group is shown in different locations: F3, C3, P3, O1, F4, C4, P4, and O2.

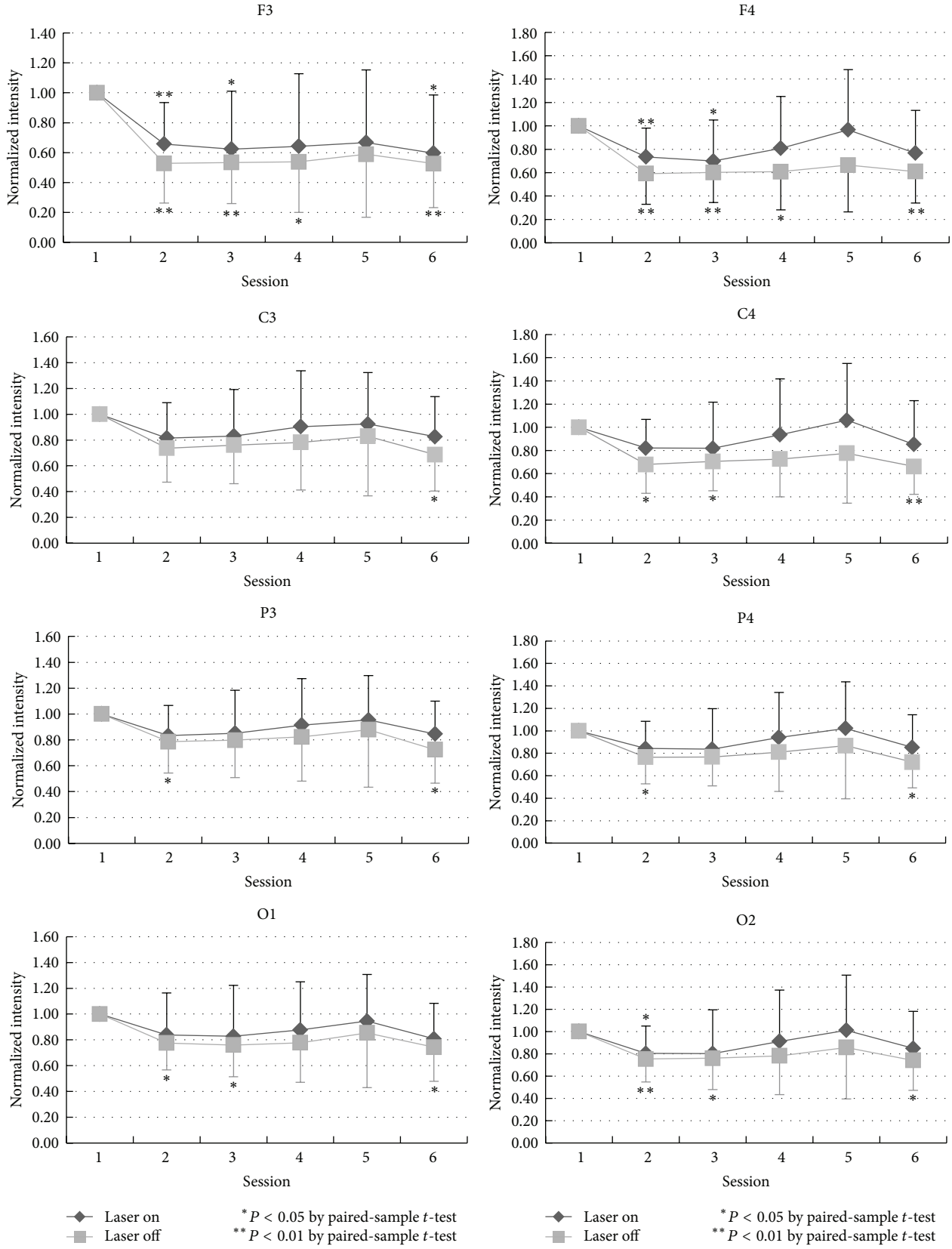

Figure 6: The statistical analysis of the delta-band by comparing the baseline and each session in laser and placebo group is shown in different locations: F3, C3, P3, O1, F4, C4, P4, and O2.

Curves in Figure 3 to Figure 6, which showed the temporal changes of EEG power in four different bandwidths, reliably reflect the awake-drowsy-sleepy state of the tested subjects. In a restful situation and a relaxed body/mind, they almost unavoidably entered a drowsy and sleepy state. Dropout of alpha rhythms in drowsiness can result in a decreased EEG power in alpha-bandwidth and the degree of decrease is reasonably more prominent in the posterior head region (Figure 3). The rebound of EEG power in alpha-bandwidth to some extent in the latter sessions (sessions 5 and 6) may be related to anterior diffusion of alpha rhythms in drowsiness and emergence of sleep spindles (12–14 Hz, part of them being in the alpha range) in the light sleep.

Similar to other EEG activities, beta waves in drowsiness also become less prominent. The temporal curve of EEG power in beta-bandwidth therefore decreased in the earlier sessions (Figure 4). However, occasional enhancement of beta activity in light sleep is the most likely reason to cause the normalized intensity of EEG power in beta-bandwidth undulated gently around the original level.

In awake adults, theta waves are abnormal if occurring excessively. However, desynchronized low-voltage theta waves are normally seen in drowsiness or sleep. The appearance of theta waves is one important hallmark of onset of drowsiness [16]. In this study, there were no significant changes in EEG power in theta-bandwidth (Figure 5). All of the tested subjects were university students and nearly all of them were used to stay up late. Hence, sitting in a comfortable armchair with closed eyes and doing nothing, almost all of the tested subjects drowsed even early in the first session in EEG recording. The EEG power in theta-bandwidth therefore was maintained rather stable in the whole recording periods (Figure 5). A mild drop of the normalized intensity in the last session (session 6) is of uncertain significance. The reason for decreased theta power in the frontal region (F3 and F4) is considered similar to that for EEG power in delta-bandwidth (see below).

In visual analysis of EEG recording, delta waves are not seen in the normal awake adults. They are the main EEG activities in deep sleep. With power spectrum analysis, EEG power in the delta-bandwidth is present, probably related to slow waves subharmonic to alpha or other rhythms. Artifacts arising from blinking or eyeball movements usually resemble EEG waves in the delta (sometimes theta) range, especially in the anterior head region. Under power spectrum analysis, they are not possibly differentiated from genuine EEG delta waves and they also play an important role in EEG power in delta-bandwidth (and also theta). The slow-rolling eye movements in light sleep or slow-wave sleep did not contribute significantly to the delta power in the present study, for their frequency is usually below 0.5 Hz and outside the range of spectrum analysis. Temporal changes in EEG power in delta-bandwidth (Figure 6) are compatible with appearance of desynchronized low-voltage slow waves in drowsiness or light sleep. In the frontal areas (F3, F4), more prominent drop in delta power is considered caused by less or no eyeball movement artifacts in drowsiness or light sleep. Similar to the theta power, there is also a mild drop of the normalized intensity in the last session (session 6) of uncertain significance.

Main differences in EEG power in different bandwidths between the laser and the control group include more prominent decreased EEG power in the alpha-bandwidth during LLL stimulation (sessions 2 and 3 in the laser group), more decrease in the beta-bandwidth at O2 in the laser group, and less decrease in the delta-bandwidth in the latter sessions in the laser group especially in the right hemisphere. Although the actual and detailed mechanisms of LLL-induced physiological changes in the brain are not well known, on the basis of sensory physiology, we deem that such effects should be more pronounced in the hemisphere contralateral to side of stimulation, that is, the right hemisphere in the present study. Side-to-side differences in LLL-induced change in the EEG power in the present study (Figure 3 to Figure 6) are considered related to laser stimulation at the left palm.

Transcranial electric stimulation to evoke generalized convulsions (electroconvulsive therapy) is a well-known technique to treat schizophrenia and depression. Transcranial magnetic stimulation has been tried for the treatment of major depression [17]. In addition to direct brain stimulation, stimulation at the peripheral nerve has also been applied to the treatment of some neurological or psychiatric diseases, such as vagus nerve stimulation in intractable epilepsy and resistant depression [18, 19] and occipital nerve stimulation for cluster headache and other types of headache [20, 21]. We consider LLL stimulation in the present study to be comparable with a kind of peripheral nerve stimulation.

In view of more decrease in the alpha power during LLL stimulation (earlier sessions in the present study), we infer that LLL stimulation is helpful in sleep induction. According to less decrease in the delta power in the latter sessions in the laser group, we postulate that LLL stimulation can lead to a deeper drowsiness or sleep state. So, we suggest that LLL stimulation can be one of the nonpharmacological solutions for patients with sleep problems. However, further studies are necessary.

5. Conclusion

The effects of low-level laser stimulation on the EEG power in normal subjects with closed eyes were investigated. Nearly all the tested subjects were found to fall into drowsiness or light sleep easily. Pronounced decrease in the EEG power in alpha-bandwidth during laser simulation and then less decrease in the delta power were found in normal subjects with laser stimulation. We suggest that low-level laser stimulation is probably useful for patients with sleep problems.

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References

[1] N. Salansky, A. Fedotchev, and A. Bondar, "Responses of the nervous system to low frequency stimulation and EEG rhythms: clinical implications," *Neuroscience and Biobehavioral Reviews*, vol. 22, no. 3, pp. 395–409, 1998.

[2] C. N. A. Chen, F. J. Liu, L. Wang, and L. Arendt-Nielsen, "Mode and site of acupuncture modulation in the human brain: 3D (124-ch) EEG power spectrum mapping and source imaging," *NeuroImage*, vol. 29, no. 4, pp. 1080–1091, 2006.

[3] M. Teplan, A. Krakovská, and S. Štolc, "Direct effects of audio-visual stimulation on EEG," *Computer Methods and Programs in Biomedicine*, vol. 102, no. 1, pp. 17–24, 2011.

[4] E. B. Lyskov, J. Juutilainen, V. Jousmäki, J. Partanen, S. Medvedev, and O. Hänninen, "Effects of 45-Hz magnetic fields on the functional state of the human brain," *Bioelectromagnetics*, vol. 14, no. 2, pp. 87–95, 1993.

[5] D. Lewis, *The Alpha Plan: Making the Most of Your Mind*, Methuen, London, UK, 1986.

[6] D. J. Anderson, "The treatment of migraine with Variable Frequency Photo-stimulation," *Headache*, vol. 29, no. 3, pp. 154–155, 1989.

[7] D. L. Timmermann, J. F. Lubar, H. W. Rasey, and J. A. Frederick, "Effects of 20-min audio-visual stimulation (AVS) at dominant alpha frequency and twice dominant alpha frequency on the cortical EEG," *International Journal of Psychophysiology*, vol. 32, no. 1, pp. 55–61, 1999.

[8] G. Litscher, "Cerebral and peripheral effects of laser needle-stimulation," *Neurological Research*, vol. 25, no. 7, pp. 722–728, 2003.

[9] M. Yasushim, "Brain wave inducing apparatus," US Patent 5, 330, 414, 1994.

[10] D. Siever, "Stimulation of central nervous system," US Patent 0, 149, 144, 2005.

[11] G. V. Sunnen, "Vibrational delta and theta brain wave induction apparatus and method for the stimulation of sleep," US Patent 0, 168, 503, 2010.

[12] R. H. Flagg, B. Barham, D. A. Stokes, and E. Kotapish, "Method and apparatus for magnetic brain wave stimulation," US Patent 6, 978, 179, 2005.

[13] J. H. Wu, W. D. Chang, C. W. Hsieh et al., "Effect of low level laser stimulation on EEG," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 951272, 11 pages, 2012.

[14] E. Niedermeye and F. L. da Silva, "The normal EEG of the waking adult," in *Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*, E. Niedermeyer and F. L. da Silva, Eds., pp. 149–173, Lippincott Williams and Wilkins, Baltimore, Md, USA, 1999.

[15] D. L. Schomer and F. L. da Silva, *Niedermeyer's Electroencephalography*, Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 6th edition, 2011.

[16] M. H. Libenson, *Practical Approach to Electroencephalography*, Saunders, Philadelphia, Pa, USA, 2010.

[17] C. Cassels, "FDA clears TMS device for resistant depression," Medscape Medical News, 2013, http://www.medscape.com/viewarticle/777357.

[18] R. P. Saneto, M. A. Sotero de Menezes, J. G. Ojemann et al., "Vagus nerve stimulation for intractable seizures in children," *Pediatric Neurology*, vol. 35, no. 5, pp. 323–326, 2006.

[19] D. Christmas, J. D. Steele, S. Tolomeo, M. S. Eljamel, and K. Matthews, "Vagus nerve stimulation for chronic major depreesive disorder: 12-month outcome in highly treatment-refractory patients," *Journal of Affective Disorders*, 2013.

[20] B. Burns, L. Watkins, and P. J. Goadsby, "Treatment of medically intractable cluster headache by occipital nerve stimulation: long-term follow-up of eight patients," *The Lancet*, vol. 369, no. 9567, pp. 1099–1106, 2007.

[21] R. L. Weiner, "Occipital neurostimulation (ONS) for treatment of intractable headache disorders," *Pain Medicine*, vol. 7, no. 1, pp. S137–S139, 2006.

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Research Article

Effect of Electroacupuncture at ST36 on Gastric-Related Neurons in Spinal Dorsal Horn and Nucleus Tractus Solitarius

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The aim of this study was to observe the effect of electroacupuncture (EA) at the ST36 acupoint on the firing rate of gastric-related neurons in the spinal dorsal horn (SDH) and nucleus tractus solitarius (NTS). There were different effects of gastric distention in SDH and NTS in 46 male Sprague-Dawley rats. In 10 excitatory neurons in SDH, most of the neurons were inhibited by homolateral EA. The firing rates decreased significantly ($P < 0.05$) in 10 excitatory gastric-related neurons in NTS; the firing rates of 6 neurons were further excited by homolateral EA, with a significant increase of the firing rates ($P < 0.05$); all inhibitory gastric-related neurons in NTS were excited by EA. The inhibition rate of homolateral EA was significantly increased in comparison with contralateral EA in gastric-related neurons of SDH ($P < 0.05$). There was no significant difference between homolateral and contralateral EA in gastric-related neurons of NTS. EA at ST36 changes the firing rate of gastric-related neurons in SDH and NTS. However, there are some differences in responsive mode in these neurons. The existence of these differences could be one of the physiological foundations of diversity and complexity in EA effects.

1. Introduction

Electroacupuncture (EA), a new and modern type of traditional acupuncture, is widely used in treating various types of diseases in a clinical setting with the alterations of peripheral electrical stimulation rather than hand manipulation [1]. Conventional acupuncture or "manual acupuncture" involves the manipulation of the inserted needles by hand, such as lifting, thrusting, twisting, twirling, or other complex combinations. EA is a modification of this technique that stimulates acupoints with electrical current instead of manual manipulations and appears to have more consistently reproducible results in both clinical and research settings [2, 3].

With the excellent pain relief efficacy profile and significant effects in some clinical symptoms, EA is being increasingly accepted by practitioners and patients in the West as well [4, 5]. During the last two decades, a considerable number of studies have investigated the efficacy of EA for the treatment of functional gastrointestinal disorders. Human and animal studies were conducted to explore the effects of EA on gastrointestinal secretion, sensation, motility, myoelectrical activity, and molecular neurobiology [6–8]. The ST36 acupoint (Zusanli), the most important and most frequently used acupoint on the stomach meridian, is considered to be the main point of regulation of gastrointestinal function, promoting gastrointestinal peristalsis and detoxification and protecting the mucosal barrier [9].

The transmission of nociceptive signals can be modulated by powerful controls at the first spinal relays including both the segmental mechanism and systemic mechanism that involve supraspinal structures [10]. It is generally accepted that multiple supraspinal sites of the descending pain modulatory system exert powerful effects on the inhibitory response of acupuncture to the visceral nociceptive messages

at the spinal level [11, 12]. It was reported that nociceptive visceral inputs could be inhibited by acupuncture at ST36, and the spinal dorsal horn played a significant role in this process [13]. Many reports suggest that regulation of gastric sensation and motility induced by stimulating ST36 seems to be mediated via vagal reflex in the supraspinal pathway [14–17]. In this study, we focused on these two levels of the central nervous system and observed possible effects of EA at ST36 on the firing rate of gastric-related neurons in SDH and nucleus tractus solitarius (NTS).

2. Materials and Methods

2.1. Animal Preparations. Experiments were carried out on 46 adult male Sprague Dawley (SD) rats (weight: 250–300 g), which were purchased from the Institute of Laboratory Animal Sciences, China Academy of Chinese Medical Sciences (CACMS), and PUMC (Beijing, China). All manipulations and procedures were carried out in accordance with The Guide for Care and Use of Laboratory Animals issued by USA National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of CACMS. Rats were housed (23±1°C) in groups and maintained under a 12-hour light/dark cycle with food and water available ad libitum. The rats were fasted overnight with free access to water and anesthetized about 4-5 hours with an intraperitoneal injection of urethane (1.0 g/kg, Sigma-Aldrich, St. Louis, MO, USA). All the experiments were done in the daytime; a 2 mm diameter polyurethane tube attached to a 1 cm diameter latex balloon was inserted into the stomach through the small longitudinal incision, which was made in the duodenum about 2-3 cm from the pylorus. A syringe was attached to the cannula to inflate and deflate the balloon. The balloon could be filled with 5 mL air, which is equal to 10 cm H_2O pressure [18].

2.2. Extracellular Recording of SDH and NTS. Twenty-two rats were employed in the experiment of laminectomy, which was performed from the T10 to L1 vertebrae to expose spinal neurons for recording. Extracellular recordings were made with a Tungsten electrode (cusp: 20 μm, impedance: 1.5 MΩ; AM systems, Sequim, WA, USA) which was inserted on the left side of the spinal cord (0.5–1.0 mm lateral to the midline, depth 300–1300 μm). Stability for the recordings was achieved by placing 2% Ringer-agar gel over the surface of the medulla. Wide dynamic range (WDR) neurons of SDH were identified on the basis of characteristic responses to mechanical stimuli applied to the receptive field [19–21]. Extracellular records of WDR were continuously monitored using an MP150 data acquisition system (Biopac, Goleta, CA, USA). Both signals were analyzed offline using the PowerLab data system (PowerLab/4s, ADInstruments, Sydney, Australia) and the Spike 2 package (Cambridge Electronic Devices, Cambridge, UK).

The part of the experiment involving extracellular recording of NTS was performed on 24 rats. The extracellular signals of the NTS neurons (distance to the bregma, AP: −11.3~−14.3 mm; ML: 0~1.3 mm; DV: 4~7 mm) were recorded by glass microelectrodes (10–20 MΩ, pulled by Narishige PE-2 vertical puller from a filamented glass) which were backfilled with 2% pontamine sky blue. Firings of the NTS neurons recorded from the glass electrodes were fed through a microelectrode amplifier (MEZ-8201, Nihon Kohden, Tokyo, Japan). Signals were captured online and analyzed offline using the CED 1401-plus data acquisition system and the Spike 2 package (Cambridge Electronic Devices, Cambridge, UK).

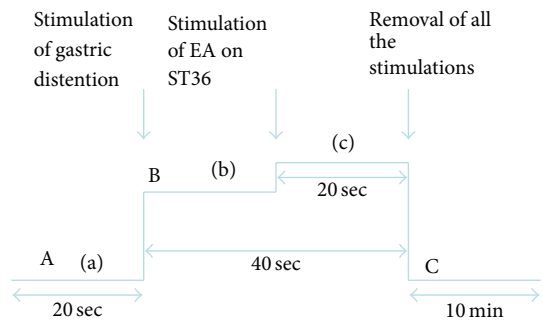

Figure 1: Measurement procedure of the study. A: before stimulation, recording the initial state of neurons (20 sec). B: recording during both kinds of stimulation (40 sec). C: recovery time after stimulation (10 min).

2.3. Electroacupuncture. The stimulation electrode was placed at ST36, a hind limb point at which EA or manual acupuncture enhances gastric motility [22]. Based on the descriptions in previous reports [23], the location is on the anterolateral side of the hind limb near the anterior crest of the tibia below the knee under the tibialis anterior muscle. This point was bilaterally stimulated with a 2-3 mA pulse of 0.5 ms duration at a frequency of 20 Hz for 40 seconds by a pair of needle electrodes inserted 3 mm deep into the skin. The electrical current for somatic stimulation was generated by a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan).

2.4. Experimental Procedure. Once the data of the extracellular recorded monitoring had reached a steady state for 5 minutes, gastric distention and EA stimulation were performed. The following measurement periods were analyzed: (a) 20 seconds before the stimulation of gastric distention, (b) 20 seconds during gastric distention stimulation, and (c) 20 seconds stimulation of gastric distention and EA on ST36 (Figure 1). There was a 10-minute recovery time after stimulation.

2.5. Data Analysis. The firing rate and the change rate of spikes in 20 seconds were analyzed. The change rate of spikes is calculated by the number of spikes before stimulation and the number of spikes after stimulation. Data are shown as mean ± standard error of the mean (SEM). For significance evaluation, data sets with normal distribution were analyzed by paired or unpaired t test for two groups or one way

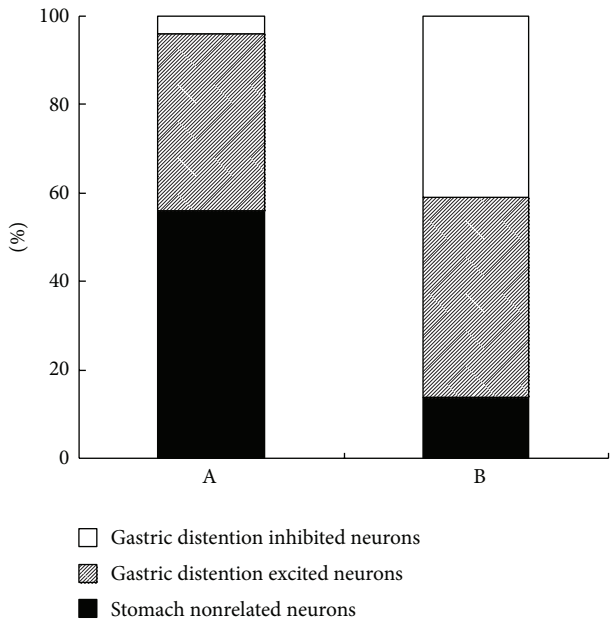

Figure 2: Different effects of gastric distention in SDH and NTS. A: 25 neurons recorded in SDH. There were 14 gastric nonrelated neurons (56%), 10 gastric distention excited neurons (40%), and 1 gastric distention inhibited neuron (4%). B: 22 neurons recorded in NTS. There were 3 gastric nonrelated neurons (14%), 10 gastric distention excited neurons (45%), and 9 gastric distention inhibited neurons (41%).

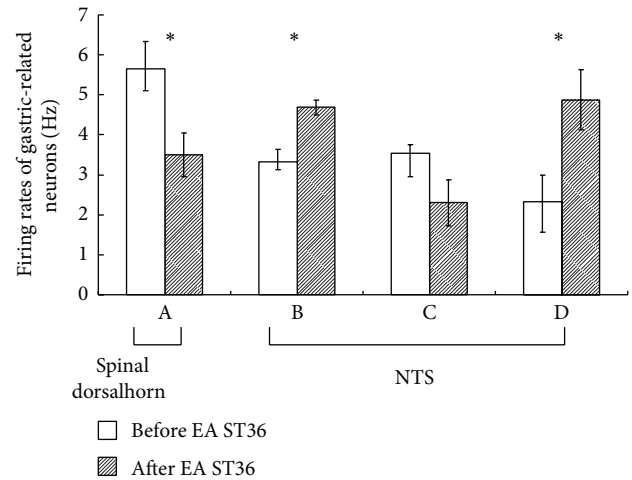

Figure 3: Effects of homolateral EA at ST36 on the firing rate of gastric-related neurons in SDH and NTS. A: stimulation of ST36 in excitatory gastric-related neurons of SDH; the firing rates were inhibited and decreased from 5.65 ± 0.68 Hz to 3.5 ± 0.54 Hz ($P < 0.05$). B: stimulation of ST36 in excitatory gastric-related neurons of NTS; the firing rates increased from 3.32 ± 0.31 Hz to 4.69 ± 0.18 Hz ($P < 0.05$). C: stimulation of ST36 in excitatory gastric-related neurons of NTS: the firing rates increased from 3.53 ± 0.22 Hz to 2.30 ± 0.58 Hz after EA, but there was no significant difference ($n = 4$, $P > 0.05$). D: stimulation of ST36 in inhibitory gastric-related neurons of NTS; the firing rates increased from 2.32±0.67 Hz to 4.87 ± 0.75 Hz ($P < 0.05$).

ANOVA followed by q test, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of Gastric Distention in SDH and NTS. Of all the 25 neurons recorded in SDH after gastric distention, 11 neurons were sensitized by the stimulation. They showed more than 15% change in the number of spikes and are called gastric-related neurons [15]. Among them, 10 were excited and 1 was inhibited by gastric distention. A total of 22 NTS neurons were recorded in the study; 19 neurons showed apparent excitatory ($n = 10$) or inhibitory ($n = 9$) responses to gastric distention (Figure 2).

3.2. Effects of Homolateral EA at ST36 on the Firing Rate of Gastric-Related Neurons in SDH and NTS. In 10 excitatory gastric-related neurons in SDH, the firing rates of 8 neurons were inhibited by EA. The firing rates decreased from 5.65 ± 0.68 Hz to 3.5 ± 0.54 Hz ($P < 0.05$; A in Figure 3). In 10 excitatory gastric-related neurons in NTS, the firing rates of 6 neurons were further excited by EA, with an increase of the firing rates from 3.32 ± 0.31 Hz to 4.69 ± 0.18 Hz ($P < 0.05$; B in Figure 3). In 4 other excitatory gastric-related neurons in NTS, the firing rates increased from 3.53 ± 0.22 Hz to 2.30 ± 0.58 Hz after EA, but there was no significant difference (C in Figure 3). All the 9 inhibitory gastric-related neurons in NTS were excited by EA. The firing rate increased from 2.32 ± 0.67 Hz to 4.87 ± 0.75 Hz ($P < 0.05$; D in Figure 3) (Figure 4).

3.3. Effects of Homolateral and Contralateral EA at ST36 on the Change Rates of Firing Gastric-Related Neurons in SDH and NTS. The inhibition rate induced by homolateral stimulation at ST36 was 30.87 ± 9.06% and significantly increased in comparison with contralateral ST36 stimulation (8 ± 3.59%) in excitatory gastric-related neurons in SDH ($P < 0.05$). There was no significant difference between stimulation of homolateral ST36 (117.03 ± 38.73%) and contralateral ST36 (78.43 ± 36.30%) with regard to change rates of firing excitatory gastric-related neurons in NTS ($P > 0.05$). There was also no significant difference between homolateral ST36 (196.51 ± 89.78%) and contralateral ST36 (217.32 ± 74.44%) with regard to change rates of firing inhibitory gastric-related neurons in NTS ($P > 0.05$) (Figure 5).

4. Discussion

The SDH is the first synaptic relay point for afferent pathways which play an important role in modifying the transmission of noxious input [24]. The integrity of the dorsolateral funiculus is necessary for EA-induced analgesia in the rat tail-flick test [25]. The NTS is a primary center not only for receiving visceral afferents but also for somatic afferents. Commonly labeled medulla oblongata regions were dorsal motor nucleus of vagus nerve (DMV), NTS, and area

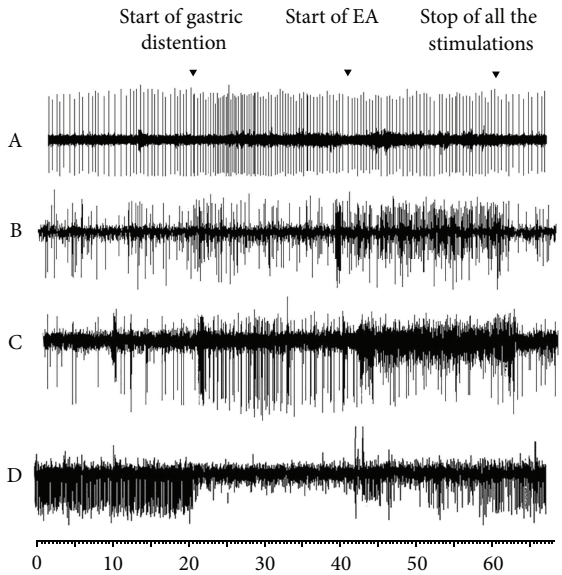

Figure 4: Examples of cells firing in SDH and NTS. A: the change in excitatory gastric-related neurons of SDH caused by EA stimulation of ST36. B and C: the change in excitatory gastric-related neurons of NTS caused by EA stimulation of ST36. D: the change in inhibitory gastric-related neurons of NTS caused by EA stimulation of ST36.

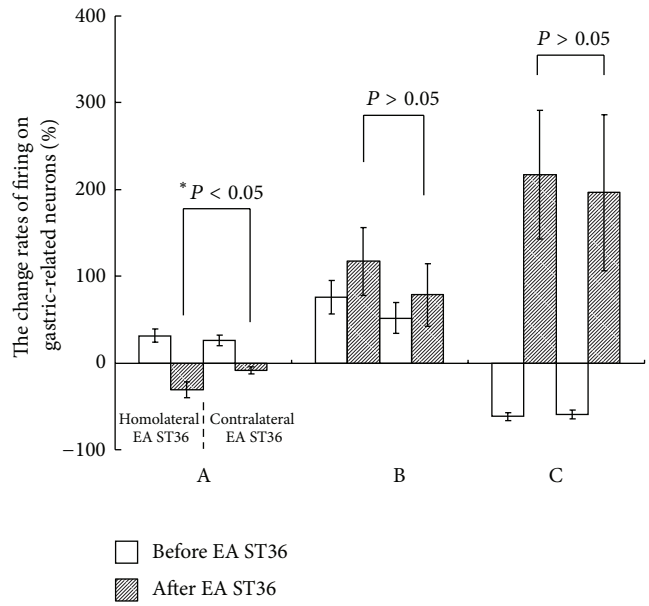

Figure 5: Effects of homolateral and contralateral EA at ST36 on the change rates of firing gastric-related neurons in SDH and NTS. A: the change of firing rate of gastric-related neurons induced by homolateral and contralateral EA at ST36 in SDH. Compared to the contralateral ST36, inhibition rate was significantly increased by homolateral ST36 EA ($^{*}P < 0.05$). B and C: the change of firing rates of gastric-related neurons caused by homolateral and contralateral EA at ST36 in NTS; there was no significant difference between homolateral and contralateral ST36 ($P > 0.05$).

postrema (AP) following injection of cholera toxin B subunit (CTB) and pseudorabies virus Bartha strain Galactosidase (PRV-Ba-Gal) into the stomach and ST36, respectively [26]. There were some varieties in the response of NTS neurons to gastric distention stimuli and acupuncture at different body surface points [27]. In this study, we observed that NTS neurons presented more diverse responsive modes and sensitivity to gastric distention than did SDH neurons.

A report by Fusumada et al. suggested that the periaqueductal gray (PAG) neurons activated by EA at ST36 might play an important role in the descending pain control system involving gamma aminobutyric acid (GABA), since the PAG has special reference to the SDH and function of pain control [28]. A bilateral microinjection of nociceptin receptor (NOP) antagonist into either the dorsal horn or the intermediolateral column at T1 partially reversed the inhibitory effect of EA at ST36, which suggests that nociceptin in the spinal cord mediates a part of the EA-related modulation of visceral reflex responses [29]. EA at ST36 could extensively regulate the information processing of SDH and induce the modulation of genes/expressed sequence tags (ESTs) in the same direction, which was correlated with neural signal transmission [30]. These results are similar to our study, in which the most popular mode of response to EA was a reduction of the number of spikes in the excited neurons.

EA at ST36 not only regulates gastric activity but also activates neurons in the NTS and DMV significantly [31]. EA at ST36 possibly regulates gastric activity through mediation of the dorsal vagal complex, similar to cardiovascular function, by activating baroreceptor sensitive neurons in the NTS [32]. According to recent evidence, acupuncture at ST 36 can regulate gastric activity, depending on the neural basis or structure and is probably related to the central neurons in the dorsal vagal complex [33]. EA-induced expression of transient receptor potential vanilloid type-1 neuronal nitric oxide synthase (TRPV1-nNOS) and the NTS/gracile nucleus is involved in the signal transduction of EA stimuli via somatosensory afferent-medulla pathways [34]. In our study, the modes of response to EA at ST36 were more complicated in gastric-related neurons of the NTS than in those of the SDH. This is based on the complex components and contact of NTS neurons.

5. Conclusion

In summary, EA at ST36 changes the firing rate of gastric-related neurons in the SDH and NTS. However, there are some differences with regard to the responsive mode in these neurons. The existence of these differences could be one of the physiological foundations of diversity and complexity in EA effects.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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References

[1] J.-S. Han, "Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies," *Trends in Neurosciences*, vol. 26, no. 1, pp. 17–22, 2003.

[2] Y. Li, G. Tougas, S. G. Chiverton, and R. H. Hunt, "The effect of acupuncture on gastrointestinal function and disorders," *American Journal of Gastroenterology*, vol. 87, no. 10, pp. 1372–1381, 1992.

[3] G. Lux, J. Hagel, P. Bäcker et al., "Acupuncture inhibits vagal gastric acid secretion stimulated by sham feeding in healthy subjects," *Gut*, vol. 35, no. 8, pp. 1026–1029, 1994.

[4] A. Goldstein and B. M. Cox, "Opioid peptides (endorphins) in pituitary and brain," *Psychoneuroendocrinology*, vol. 2, no. 1, pp. 11–16, 1977.

[5] C. H. Li and D. Chung, "Primary structure of human β lipotropin," *Nature*, vol. 260, no. 5552, pp. 622–624, 1976.

[6] D. L. Diehl, "Acupuncture for gastrointestinal and hepatobiliary disorders," *Journal of Alternative and Complementary Medicine*, vol. 5, no. 1, pp. 27–45, 1999.

[7] Y. Li, G. Tougas, S. G. Chiverton, and R. H. Hunt, "The effect of acupuncture on gastrointestinal function and disorders," *American Journal of Gastroenterology*, vol. 87, no. 10, pp. 1372–1381, 1992.

[8] X. Gao, Y. Qiao, B. Jia et al., "NMDA receptor-dependent synaptic activity in dorsal motor nucleus of vagus mediates the enhancement of gastric motility by stimulating ST36," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 438460, 11 pages, 2012.

[9] X. F. Ye, J. G. Li, Z. H. Li et al., "Effect of ST36 point of acupuncture on discharge of vagus nerve of rats," *Acupuncture Research*, vol. 31, no. 5, pp. 290–293, 2006.

[10] J. C. Willer, D. Bouhassira, and D. Le Bars, "Neurophysiological bases of the counterirritation phenomenon: diffuse control inhibitors induced by nociceptive stimulation," *Neurophysiologie Clinique*, vol. 29, no. 5, pp. 379–400, 1999.

[11] J. C. Wu, E. T. Ziea, L. Lao et al., "Effect of electroacupuncture on visceral hyperalgesia, serotonin and fos expression in an animal model of irritable bowel syndrome," *Journal of Neurogastroenterology and Motility*, vol. 13, no. 3, pp. 306–314, 2010.

[12] X. Cao, "Scientific bases of acupuncture analgesia," *Acupuncture & Electro-Therapeutics Research*, vol. 27, no. 1, pp. 1–14, 2002.

[13] P.-J. Rong, B. Zhu, Q.-F. Huang, X.-Y. Gao, H. Ben, and Y.-H. Li, "Acupuncture inhibition on neuronal activity of spinal dorsal horn induced by noxious colorectal distension in rat," *World Journal of Gastroenterology*, vol. 11, no. 7, pp. 1011–1017, 2005.

[14] J. Yin and J. D. Z. Chen, "Gastrointestinal motility disorders and acupuncture," *Autonomic Neuroscience*, vol. 157, no. 1-2, pp. 31–37, 2010.

[15] H. Ouyang, J. Yin, Z. Wang, P. J. Pasricha, and J. D. Z. Chen, "Electroacupuncture accelerates gastric emptying in association with changes in vagal activity," *American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 282, no. 2, pp. G390–G396, 2002.

[16] M. Tatewaki, M. Harris, K. Uemura et al., "Dual effects of acupuncture on gastric motility in conscious rats," *American Journal of Physiology*, vol. 285, no. 4, pp. R862–R872, 2003.

[17] J. Chen, G.-Q. Song, J. Yin, T. Koothan, and J. D. Z. Chen, "Electroacupuncture improves impaired gastric motility and slow waves induced by rectal distension in dogs," *American Journal of Physiology*, vol. 295, no. 3, pp. G614–G620, 2008.

[18] S. L. Chen, Z. G. Jin, X. H. Jing et al., "Effect of electroacupuncture of hegu (LI 4) and sibai (ST 2) on electrical activities of stomach related neurons in the nucleus of solitary tract of the rat," *Acupuncture Research*, vol. 31, no. 1, pp. 46–49, 2006.

[19] H.-J. You, C. D. Morch, J. Chen, and L. Arendt-Nielsen, "Simultaneous recordings of wind-up of paired spinal dorsal horn nociceptive neuron and nociceptive flexion reflex in rats," *Brain Research*, vol. 960, no. 1-2, pp. 235–245, 2003.

[20] J.-H. Zheng, J. Chen, and L. Arendt-Nielsen, "Complexity of tissue injury-induced nociceptive discharge of dorsal horn wide dynamic range neurons in the rat, correlation with the effect of systemic morphine," *Brain Research*, vol. 1001, no. 1-2, pp. 143–149, 2004.

[21] J.-H. Zheng, Z. Jian, and J. Chen, "Detection of deterministic behavior within the tissue injury-induced persistent firing of nociceptive neurons in the dorsal horn of the rat spinal cord," *Journal of Computational Neuroscience*, vol. 13, no. 1, pp. 23–34, 2002.

[22] X. Wang, H. Shi, H. Shang et al., "Are Primo Vessels (PVs) on the Surface of Gastrointestine Involved in Regulation of Gastric Motility Induced by Stimulating Acupoints ST36 or CV12?" *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 787683, 8 pages, 2012.

[23] S. Cheng, *Chinese Acupuncture and Moxibustion*, Foreign Language Press, Beijing, China, 1996.

[24] R. Staud, "Evidence of involvement of central neural mechanisms in generating fibromyalgia pain," *Current Rheumatology Reports*, vol. 4, no. 4, pp. 299–305, 2002.

[25] M. L. Silva, J. R. T. Silva, and W. A. Prado, "The integrity of the anterior pretectal nucleus and dorsolateral funiculus is necessary for electroacupuncture-induced analgesia in the rat tail-flick test," *European Journal of Pain*, vol. 14, no. 3, pp. 249–254, 2010.

[26] C. H. Lee, H. S. Jung, T. Y. Lee et al., "Studies of the central neural pathways to the stomach and zusanli (ST36)," *American Journal of Chinese Medicine*, vol. 29, no. 2, pp. 211–220, 2001.

[27] J. He, J. Yan, X. Chang et al., "Neurons in the NTS of rat response to gastric distention stimulation and acupuncture at body surface points," *American Journal of Chinese Medicine*, vol. 34, no. 3, pp. 427–433, 2006.

[28] K. Fusumada, T. Yokoyama, T. Miki et al., "c-Fos expression in the periaqueductal gray is induced by electroacupuncture in the rat, with possible reference to GABAergic neurons," *Okajimas Folia Anatomica Japonica*, vol. 84, no. 1, pp. 1–10, 2007.

[29] W. Zhou, A. Mahajan, and J. C. Longhurst, "Spinal nociceptin mediates electroacupuncture-related modulation of visceral sympathoexcitatory reflex responses in rats," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 297, no. 2, pp. H859–H865, 2009.

[30] K. Wang, R. Zhang, and G. P. Zhao, "Transcriptomics study of the transcriptional response of the spinal dorsal horn to electroacupuncture stimulation with different frequencies," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 32, no. 11, pp. 1508–1515, 2012.

[31] J.-J. Wang, Q. Ming, X.-D. Liu et al., "Electro-acupuncture of Foot Yangming regulates gastric activity possibly through mediation of the dorsal vagal complex," *American Journal of Chinese Medicine*, vol. 35, no. 3, pp. 455–464, 2007.

[32] X. Y. Gao, Y. H. Li, K. Liu et al., "Acupuncture-like stimulation at auricular point Heart evokes cardiovascular inhibition via activating the cardiac-related neurons in the nucleus tractus solitarius," *Brain Research*, vol. 1397, pp. 19–27, 2011.

[33] S.-Z. Wang, X.-D. Liu, Y.-X. Huang, Q.-J. Ma, and J.-J. Wang, "Disruption of glial function regulates the effects of electro-acupuncture at tsusanli on gastric activity in rats," *American Journal of Chinese Medicine*, vol. 37, no. 4, pp. 647–656, 2009.

[34] B. Ji, J. Hu, and S. Ma, "Effects of electroacupuncture Zusanli (ST36) on food intake and expression of POMC and TRPV1 through afferents-medulla pathway in obese prone rats," *Peptides*, vol. 40, pp. 188–194, 2013.

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Research Article

Effect of Laser Irradiation at Different Wavelengths (940, 808, and 658 nm) on Pressure Ulcer Healing: Results from a Clinical Study

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The aim of the study was to assess the efficacy of laser therapy (at different wavelengths: 940, 808, and 658 nm) for treating pressure ulcers. The primary endpoint in this trial included both the percentage reduction of the ulcer surface area and the percentage of completely healed wounds after one month of therapy (ulcer healing rate). The secondary endpoint was the ulcer healing rate at the follow-up evaluation (3 months after the end of the study). In total, 72 patients with stage II and III pressure ulcers received laser therapy once daily, 5 times per week for 1 month using a (GaAlAs) diode laser with a maximum output power of 50 mW and continuous radiation emission. Three separate wavelengths were used for the laser treatment: 940 nm (group I), 808 nm (group II), and 658 nm (group III). An average dose of 4 J/cm^2 was applied. In group IV, a placebo was applied (laser device was turned off). The laser therapy at a wavelength of 658 nm appeared to be effective at healing pressure ulcers. The wavelengths of 808 and 940 nm did not have any effect in our study.

1. Introduction

Of the health problems specific to frail older people, pressure ulcers are a major health disorder, and the establishment and spread of an effective treatment method for pressure ulcers are a pressing issue. Pressure ulcers are a common and costly problem in nursing home settings, with prevalence estimates varying widely from 7 to 23% [1].

Care and management can have significant economic consequences. Staff time for ongoing assessment, documentation, and dressing changes and expensive pharmaceuticals drain the available resources. Well-documented, promising, and inexpensive methods from alternative medicine are necessary.

Laser therapy has been used to accelerate wound healing since the late 1960s, but its results are still controversial. The European Pressure Ulcer Advisory Panel (EPUAP) and the National Pressure Ulcer Advisory Panel (NPUAP) published an international guideline, "Treatment of pressure ulcers: quick reference guide," in 2009 [2].

According to the guideline (content titled "Biophysical agents in pressure ulcer management"), several forms of energy have been studied for healing pressure ulcers. These include acoustic, mechanical, and kinetic energy, as well as energy from the electromagnetic spectrum (EMS). Infrared (thermal) radiation and ultraviolet light (invisible light) are all part of the EMS as is electrical/electromagnetic stimulation. The recommendation for biophysical agents is

supported by direct scientific evidence from properly designed and implemented clinical studies on pressure ulcers in humans (or on humans at risk for pressure ulcers), providing statistical results that consistently support the recommendation.

One of the proposed physical methods (alternative medicine) in this document is laser irradiation, but the guideline stated that there is insufficient evidence from research on pressure ulcers and other chronic types of wound to recommend the use of laser therapy for treating pressure ulcers. An international collaboration has recently been formed between the National Pressure Ulcer Advisory Panel (NPUAP), the European Pressure Ulcer Advisory Panel (EPUAP), and the Pan Pacific Pressure Injury Alliance (PPPIA), an alliance formed between the Australian Wound Management Association, New Zealand Wound Care Association, Hong Kong Enterostomal Therapy Association, and Wound Healing Society of Singapore. The intent of the collaboration is to develop a 2014 update of the international guidelines for the prevention and treatment of pressure ulcers, which was initially developed by the NPUAP and EPUAP in 2009. Complete clinical information must be received before creating an updated guideline and to discuss the effectiveness of controversial alternative therapies in wound healing.

The aim of this clinical study was to assess the efficacy of laser therapy (different wavelengths—940, 808, and 658 nm) for the treatment of pressure ulcers. The primary study endpoints were the percentage change in the ulcer surface area and in the number of completely healed wounds after one month of therapy (ulcer healing rate). The secondary endpoint was the follow-up ulcer healing rates (3 months after the end of the study).

2. Materials and Methods

2.1. Settings and Participants. The study was performed at the Limf-Med Clinic in Chorzow, Poland, from January 2012 to February 2013. Participating subjects met the following inclusion criteria: (1) presented with a lower extremity pressure ulcer and (2) provided written informed consent to participate in the study. There were no restrictions on gender, race, age, or ulcer duration. Subjects with the following conditions were not allowed to participate or were excluded from the study: (1) clinically detectable infection in the ulcer (critical colonization of bacteria, no signs of healing for two weeks, friable granulation tissue, foul odor, increased pain in the ulcer, increased heat in the tissue around the ulcer, an ominous change in the nature of the wound drainage, e.g., new onset of bloody drainage or purulent drainage, or necrotic tissue in the ulcer); (2) use of drugs, such as corticosteroids, that could interfere with the wound-healing process; (3) use of special dressings, such as hydrocolloids, calcium alginate, activated carbon, or any type of therapeutic procedures different from that used routinely by all groups in the study; (4) nonattendance to the therapeutic program; (5) pregnancy; (6) ankle-brachial pressure index (ABPI) <0.8; (7) diabetes mellitus; (8) systemic sclerosis; (9) cancer diagnosis; and (10) pareses and paralysis caused by injuries to the central or peripheral nervous system. Patients whose pressure ulcers required surgical intervention were also excluded from the study (Figure 1).

The body mass index (BMI) was calculated for all patients. According to international norms, a BMI higher than 30 kg/m^2 indicates adipositas. The number of smokers was recorded as well. To determine whether they met the inclusion/exclusion criteria described in the protocol, the recruited participants underwent complex tests (standard blood morphology, immunological studies, HbA_{1c}, cholesterol panel, liver enzymes, serum creatinine/glomerular filtration rate, urine testing, and ECG) twice within the three months prior to the experiment.

2.2. Randomization and Intervention. Participants were randomly allocated to the groups. Computer-generated random numbers were sealed in sequentially numbered envelopes, and the group allocation was independent of the time and person delivering the treatment. The physician (main coordinator) who allocated the patients to groups had 75 envelopes, each containing a piece of paper marked with either group I, II, III, or IV. The physician would select and open an envelope in the presence of a physiotherapist to see the symbol and would then direct the patient to the corresponding group. A clinical nurse collected the data and coded them into an Excel database. The "blinded" results were transferred to a Statistica version 10.0 (StatSoft Inc., Poland) database by a technician. The research coordinators had no contact with and could not identify the patients.

Subjects from all groups received a routine treatment, including daily simple dressings with sterile gauze after wound cleaning with a 0.9% physiologic solution, use of 1% hydrophilic silver sulfadiazine cream, and an orientation about the use of adapted footwear, self-care, and the prevention of disabilities (the drug therapy was in accordance with the TIME strategy for chronic wound treatment and the EPUAP/NPUAP recommendation from the 2009 reference guide).

Subjects from groups I, II and III received laser therapy once daily, 5 times per week for 1 month. The equipment was a Rainbow Drops (Technomex Group, Poland) gallium-aluminum-arsenide (GaAlAs) diode laser with an output power of 50 mW and continuous radiation emission at separate wavelengths of 940 nm (group I), 808 nm (group II), and 658 nm (group III). The spot size was 0.1 cm^2. The laser was wired to a cone-shaped applicator (scanner). The laser beam was scanned over the wound surface at a distance of 50 cm from the ulcer surface using a compound movement; the movement frequency was 20 Hz along the ordinate axis and 0.5 Hz along the abscissa axis. The duration of a single procedure was relative to the wound size; the therapy was adjusted to obtain an average dose of 4 J/cm^2 cm^2 (direct dose measured on a surface of the wound by Mentor MA10 device, ITAM Inc., Poland). In group IV, the placebo group, a laser was applied in the same manner, but the device was turned off during the treatment sessions (only the applicator was turned on to scan ulcers using noncoherent red visible light).

The following research was a single-blind, controlled, and randomized clinical trial. The study design, methodology,

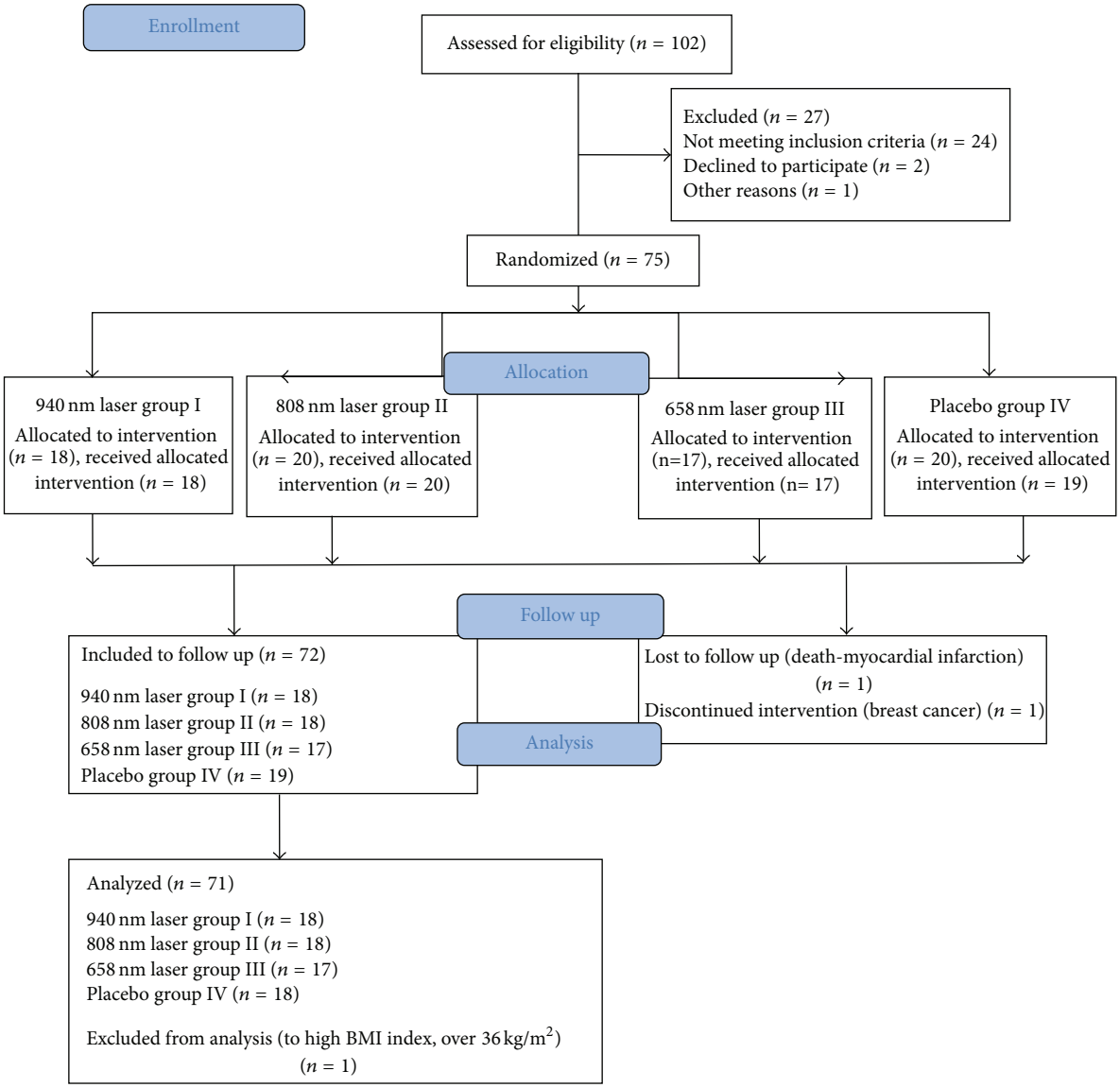


Figure 1: Flow diagram of the study.

and treatment doses were programmed by coordinators (physiotherapist, general and vascular surgeons, and an internist). Standard care, infrared camera measurements, and data collection were provided by a nurse. The laser/quasilaser therapy was performed by a physiotherapist. The final statistical analysis was performed by a technician.

2.3. Outcomes Assessment and Follow Up. The ulcer area reduction and the healing rate (number percentage of completely healed ulcers) were evaluated in this study. The healing rate was calculated as the proportion of the number of healed wounds (number of patients) to the total number of patients in that group. The criterion for complete healing was final epithelization of the skin. The nurse and physiotherapist were jointly responsible for deciding whether ulcers were healed.

A MobIR 3 (Wuhan Guide Infrared Technology, China) infrared camera was used in this study; this uncooled long-wave detector UFPA (third-generation uncooled microbolometer 8 μm–14 μm sensor) has a thermal sensitivity of 80 mK. The infrared camera was connected to a portable computer through a special interface. All images were stored on the computer for further analysis. Images were analyzed using Guide IrAnalyser V1.4 researcher software (Test-Therm Inc., Poland). Infrared thermography was used to monitor the arterial hemodynamic effects on each ulcer (Figure 2). The infrared thermography was based on an analysis of the wound surface temperatures. The reaction is a result of normal or abnormal arterial circulation in capillaries. The measurements were conducted at 24°C. The distance from the camera to the ulcer remained constant (120 cm). The computed images were based on the thermal pixels, which were used to determine the ulcer area (human skin has a thermal emissivity of 0.98 and receives a different color of pixels than open wound). The percentage change of the ulcer area was calculated by a technician as follows.

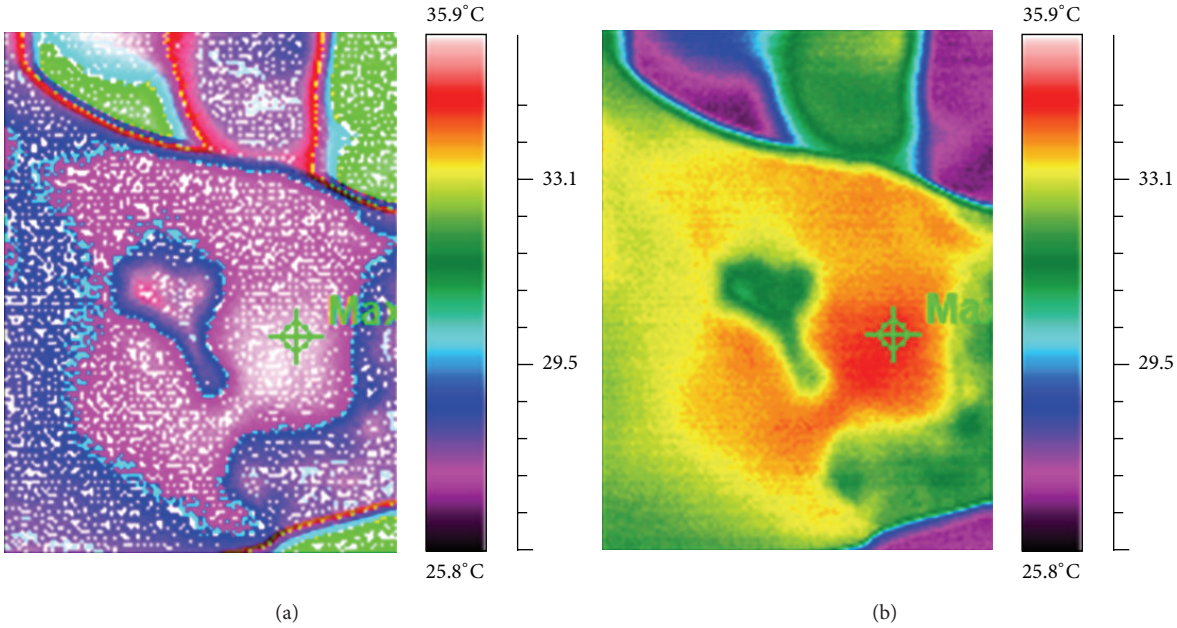


Figure 2: Ulcer area measured using an infrared camera. Example of the healing process of a chronic pressure ulcer under treatment with laser therapy (thermographic image and temperature profile across the ulcer; the difference between warmer/cooler pixels in the wound area was used to calculate the ulcer area).

$\Delta S\%$ is the percentage change of the total surface area (%):

$$\Delta S\% = \frac{(S_I - S_F) * 100\%}{S_I}, \quad (1)$$

where S_I is the initial total area (cm^2) and S_F is the final wound total area after one month (cm^2).

The laser therapy (also placebo) in all comparative groups lasted one month. Three months after the therapy, all patients were reviewed (to compare the final healing rates in the groups). During this time, the participants only received routine treatment, including daily simple dressings with sterile gauze after wound cleaning with a 0.9% physiologic solution and use of 1% hydrophilic silver sulfadiazine cream.

2.4. Statistical Analysis. To compare the individual parameters that characterized the study groups, the nonparametric Kruskal-Wallis test for countable variables and the chi-squared test (χ^2) for categorical variables were used. The nonparametric matched pair Wilcoxon test was used to compare the within-group results before and after therapy. The Kruskal-Wallis analysis of variance (*post hoc* Tukey's test) was used to evaluate differences in the percentage changes between the groups (except the healing rates, number of completely healed ulcers, which were compared using the Fisher test). Two-sided results ($P < 0.05$) were considered to be statistically significant.

3. Results

In total, 75 individuals were qualified to participate in the treatment. One patient dropped out from the study during therapy in the placebo group (patient chose to discontinue treatment and withdrew from the study for personal reasons, care in the home of her daughter suffering from scarlet fever). Two patients from group II (808 nm laser therapy) had complications unrelated to the treatment and were directed to other hospitals (one patient died) before the follow-up observation. One patient in the control group was excluded from the analysis (BMI over 36 kg/m^2, which was too high and significantly increased the SD; this increase could have seriously affected the reliability of both the nonparametric Kruskal-Wallis analysis of variance and the final conclusions).

Of the 71 patients who completed the study protocol, 32 were men and 39 were women, with ages ranging from 24 to 88 years. Twenty participants were obese (fourteen in the laser groups and six in the placebo). The body mass of the other participants was within the normal range. The patients were not addicted to either alcohol or drugs; 31 smoked cigarettes (22 in the laser groups and 9 in the placebo).

All participants had stage II and stage III pressure ulcers on the EPUAP scale [2] on their lower extremities. The pressure ulcers were located on the legs (33.3% in group I, 27.7% in group II, 35.4% in group III, and 33.3% in group IV), feet (16.6% in group I, 22.3% in group II, 23.5% in group III, and 16.6% in group IV), lateral and medial ankles (22.3% in group I, 22.3% in group II, 17.6% in group III, and 22.3% in group IV), and greater femoral trochanter (27.8% in group I, 27.7% in group II, 23.5% in group III and 27.7% in group IV).

In the first group (940 nm), four pressure ulcers occurred as a result of poorly fitting orthopedic aids (one case of wrong orthopedic footwear and three prostheses). In five patients, the ulcers developed after mechanical injuries (pressure, abrasion, and scratches), and in another five, temporary immobilization and forced positioning of the body due to surgical intervention (unconsciousness) or multiorgan injuries were considered the causative factors. In two patients,

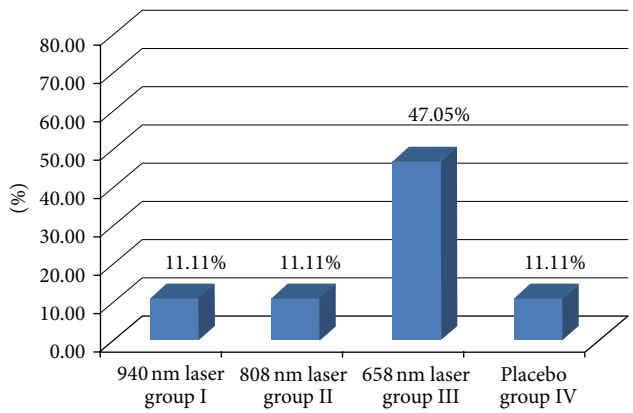


Figure 3: Healing rates (percentage number of completely healed ulcers) after therapy (1-month results). *Fisher test. Groups I and III: 11.11% versus 47.05%, $P < 0.001$, groups II and III: 11.11% versus 47.05%, $P < 0.001$, and groups III and IV: 47.05% versus 11.11%, $P < 0.001$.

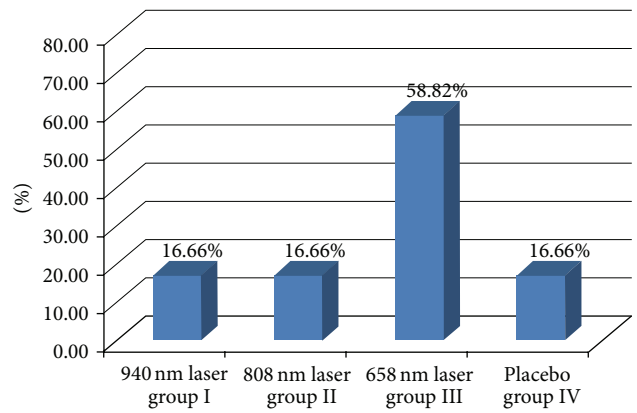


Figure 4: Healing rates (percentage number of completely healed ulcers) at the follow-up analysis (after 3 months). *Fisher test. Groups I and III: 16.66% versus 58.82%, $P < 0.001$, groups II and III: 16.66% versus 58.82%, $P < 0.001$, and groups III and IV: 58.82% versus 16.66%, $P < 0.001$.

pressure ulcers formed beneath a cast, immobilizing splint, or traction device. Two persons had pressure ulcers because of prolonged inward pressure exerted by plates and screws used for internal bone stabilization.

In the second group (808 nm), causative factors included immobilization due to postoperative positioning, multiorgan injuries, or being unconscious; poorly fitting prosthetic limbs (three patients) or footwear (one patient); mechanical injury (four); and pressure exerted by plates and screws (two patients) or pressure from a plaster cast (two) or traction device (one). In four patients, pressure ulcers appeared under postoperative dressings.

In the third group (658 nm), four pressure ulcers occurred as a result of poorly fitting orthopedic aids (two cases of wrong orthopedic footwear and two prostheses). In four patients, the ulcers developed after mechanical injuries (pressure, abrasion, and scratches), and in another five, temporary immobilization and forced positioning of the body due to surgical intervention (unconsciousness) or multi-organ injuries were considered the causative factors. In two patients, pressure ulcers formed beneath a cast, immobilizing splint, or traction device. Two persons had pressure ulcers because of prolonged inward pressure exerted by plates and screws used for internal bone stabilization.

In the fourth group (placebo), causative factors included immobilization due to postoperative positioning, multi-organ injuries or being unconscious; poorly fitting prosthetic limbs (three patients) or footwear (one patient); mechanical injury (four); and pressure exerted by plates and screws (two patients) or pressure from a plaster cast (one) or traction device (one). In five patients, pressure ulcers appeared under postoperative dressings.

The observed groups were homogenous in all participant characteristics (Table 1). After one month of therapy, the healing rate (number percentage of completely healed ulcers) was the highest in group III, 47.05% or 8/17 patients. A significantly worse rate was found in the other groups, only 11.11% or 2/18 patients in each group (Figure 3). Similar results were found in the follow-up analysis (Figure 4).

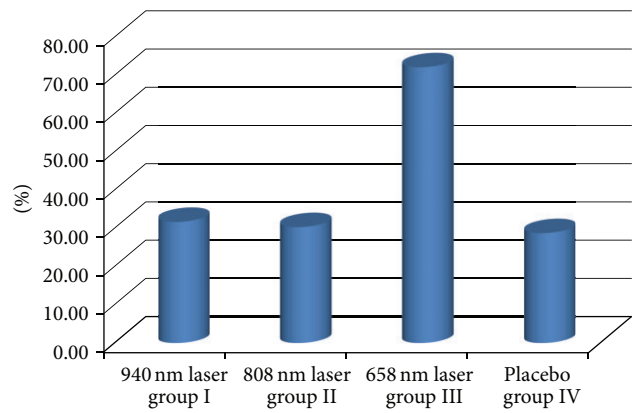


Figure 5: The decrease in the ulcer area after therapy (% of the ulcer area decrease after 1 month). *Kruskal-Wallis test. Groups I and III: 31.23% versus 71.09%, $P = 0.023$, groups II and III: 29.89% versus 71.09%, $P = 0.018$, and groups III and IV: 71.09% versus 28.34%, $P = 0.011$.

The analysis of the changes of the percentage ulcer area also confirmed that laser irradiation at a wavelength of 658 nm is the most efficient for wound healing. The wavelengths of 940 and 808 nm appeared to be much less effective (Figure 5) and were not better than those in the placebo.

Examples of healed ulcers from group III are presented in Figures 6 and 7. The healing process for all groups is shown in Table 2.

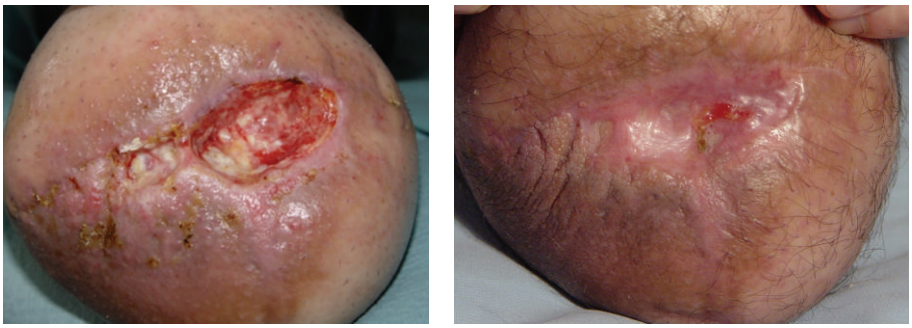
4. Discussion

Laser therapy has been suggested to be a promising treatment option for sport injuries [3], musculoskeletal disorders [4, 5], neurological problems [6, 7], and open wounds [8]. We only found a few well-documented reports (unfortunately with strongly critical remarks) in the literature about pressure ulcer management.

Table 1: Characteristics of patients.

	Group I	Group II	Group III	Group IV	P
Number of patients**	18	18	17	18	>0.05
Age (years)**					
Range	24–88	29–81	32–81	27–70	
Average	67.39	69.03	68.22	65.34	>0.05
Median	69.21	70.11	68.89	66.21	
SD	11.23	12.03	10.09	11.23	
Sex*					
Female	10	10	9	10	>0.05
Male	8	8	8	8	
Adipositas* (BMI > 30 kg/m^2)	7	7	6	6	>0.05
Smokers*	7	8	7	9	>0.05
EPUAP scale***					
IIA	5	5	4	5	
IIB	8	8	8	7	>0.05
III	5	5	5	6	
Duration of ulcers (months)**					
Range	1.2–3.6	1.3–4.6	1–3.8	2–3.6	
Average	2.12	2.78	2.03	2.01	>0.05
Median	2.02	2.52	2.67	2.39	
SD	2.56	3.01	3.02	2.89	
Initial wound size** (cm^2)					
Range	0.7–49.6	1.9–49.2	0.9–40.6	0.7–40.1	
Average	30.23	34.88	32.87	30.89	>0.05
Median	34.80	33.99	34.02	29.66	
SD	29.17	36.12	31.33	31.83	

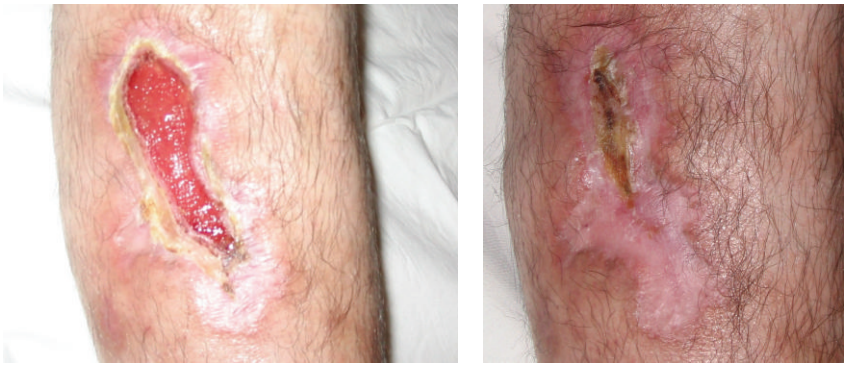
*χ^2 test.
**Kruskal-Wallis test.
***Wounds were classified based on the following criteria from the EPUAP scale. Partial-thickness loss of the dermis was considered a stage II ulcer; it was subdivided into stage IIA (shallow lesions involving only the epidermis) and stage IIB (ulcers with damaged dermis). Full-thickness tissue loss was classified as a stage III pressure ulcer and involved subcutaneous tissue and fascia.



(a) (b)

Figure 6: A 49-year-old patient (healing process after 658 nm irradiation) before and after treatment.

For example, Lucas et al. [9] assessed the efficacy of laser therapy (810 nm) in the treatment of stage III pressure ulcers. A total of 86 patients were enrolled in the study. The treatment was the prevailing consensus pressure treatment ($n = 47$); one group ($n = 39$) also received laser therapy, five times a week over a period of 6 weeks. The primary outcome measure was the absolute (mm^2) and relative (%) wound size reduction at 6 weeks compared with baseline. Secondary outcome measures were the number of patients developing a stage IV ulcer during the study period and the median change in the Norton scores at 6 weeks compared with baseline. The data were analyzed using the intention-to-treat principle and last-observation-carried-forward analyses; the Mann-Whitney U tests showed that the differences between the two



(a) (b)

Figure 7: A 53-year-old patient (healing process after 658 nm irradiation) before and after treatment.

Table 2: Results in patients with pressure leg ulcers.

	Group	Average ± SD		P
		Before therapy	After therapy	
Total ulcer surface area (cm^2)	I	30.23 ± 29.17	19.23 ± 23.88	0.005
	II	34.88 ± 36.12	21.07 ± 26.02	0.005
	III	32.87 ± 31.33	8.42 ± 14.23	<0.001
	IV	30.89 ± 31.83	20.07 ± 27.23	0.005

* Wilcoxon test.

groups in terms of absolute improvement ($P = 0.23$) and relative improvement ($P = 0.42$) were not significant. During the treatment period, 11% of the patients in the control group and 8% of the patients in the laser therapy group developed a stage IV pressure ulcer (Fisher's exact test: $P = 0.72$). The patients' Norton scores did not change during the treatment period.

Taly et al. [10] studied the efficacy of multiwavelength light therapy for treating pressure ulcers. Thirty-five subjects with spinal cord injury, with 64 pressure ulcers (stage 2, $n = 55$; stage 3, $n = 8$; stage 4, $n = 1$), were randomized into treatment and control groups. The treatment group received 14 sessions of multi-wavelength light irradiation, with 46 probes of different wavelengths from a gallium-aluminum-arsenide laser source, 3 times a week. The energy used was 4.5 J/cm^2. The ulcers in the control group underwent a sham treatment. There was no significant difference in the healing between the treatment and control groups. Eighteen ulcers in the treatment group and 14 in the control group healed completely ($P = 0.802$). The mean time for the ulcers to heal was 2.45 weeks in the treatment group and 1.78 weeks in the control group ($P = 0.330$).

Using patients with spinal cord injury, Canadian researchers [11] compared the effect on wound healing of nursing care alone with the effect on wound healing of nursing care combined with either laser treatment or a regimen of ultrasound and ultraviolet C (US/UVC). Twenty patients (22 wounds) were randomly assigned to the treatment groups. All patients received standard wound care consisting of wound cleaning twice daily, application of moist dressings, and continuous relief of pressure until the wounds were healed. The laser protocol consisted of three treatments weekly using a cluster probe with an 820 nm laser diode and 30 superluminous diodes (10 each at 660, 880, and 950 nm) and an energy density of 4 J/cm^2. The US/UVC regimen consisted of five treatments weekly, alternating the treatment modality daily. The pulsed US was applied at a frequency of 3 MHz and an average spatial-temporal intensity of 0.2 W/cm^2 (1 : 4 pulse ratio) for 5 minutes per 5 cm^2 of wound area. The UVC dosage (95% emission at 250 nm) was calculated each session according to the wound appearance. The dosage level was E1 for clean/granulating areas, E3 for purulent/slow-granulating areas, E4 for heavily infected areas, and 2E4 for wound debridement. Wounds were traced every 14 days, and surface areas were calculated using the Sigma-Scan Measurement System. The weekly percentage changes in wound area were compared. The results showed that US/UVC treatment had a greater effect on wound healing than did nursing care, either alone or combined with a laser.

Some clinical reports are promising. For example, Dehlin et al. [12] included 94 patients (76 patients were evaluated), who were pooled with 87 patients from the earlier study, bringing the total to 163. All patients were treated with monochromatic pulsating light or a placebo over the ulcerated area, according to a specified program for up to 12 weeks. The mean normalized reduction in pressure ulcer size at week 12 was 0.79 for the phototherapy group and 0.50 for the placebo group (95% confidence interval 0.01–0.53; $P = 0.039$).

In view of the absence of randomized studies with sufficiently large sample sizes, we assessed the efficacy of lasers for treating pressure ulcers. We performed a prospective, single-blinded, and randomized clinical trial to assess the effect of laser therapy as a potential alternative to standard care. We wanted to compare a few common wavelengths in pressure ulcer therapy in a well-prepared and well-planned research program.

The results of our study showed that the wavelength of the laser beam is extremely important during the wound-healing process (and perhaps this is one reason for the many controversies). In this trial, we found no evidence

that justifies using laser therapy at wavelengths of 940 and 808 nm as an adjuvant to the future consensus pressure ulcer treatment. However, in our opinion the wavelength of 658 nm is interesting, and its use yielded in promising clinical results. We cannot agree with a general statement that laser therapy does not accelerate the healing process because the correct parameter settings (wavelength, dose, and method of application) must still be demonstrated in the literature. Researchers still do not know all of the physical processes that occur at the cell or tissue level after laser irradiation. We believe that this study will be helpful in preparing the NPUAP/EPUAP 2014 update of the international guidelines for the prevention and treatment of pressure ulcers.

5. Weakness of the Study

At this moment, we are able to present the results from a small number of patients and a followup longer than three months (in future, the Kaplan-Meier survival analysis with log rank comparisons is needed). In further studies, we would like to compare the obtained results to those using other colors of lasers, especially blue, which is well known in combination with red light to be highly effective at destroying bacteria.

6. Conclusion

Laser therapy at a wavelength of 658 nm appeared to be effective for healing pressure ulcers. The wavelengths of 808 and 940 nm did not have any effect in our study. Future *in vitro* animal and clinical studies are necessary.

Ethical Approval

The Research Ethics Committee from the Medical University of Silesia in Katowice, Poland, approved this study (protocol number NN/6501/101/06).

Conflict of Interests

The authors would like to verify that they have no commercial associations with the manufacturers of the equipment described in the paper and other conflict of interests.

References

[1] M. G. Woodbury and P. E. Houghton, "Prevalence of pressure ulcers in Canadian healthcare settings," *Ostomy/Wound Management*, vol. 50, no. 10, pp. 22–38, 2004.

[2] 2. European Pressure Ulcer Advisory Panel and National Pressure Ulcer Advisory Panel, *Treatment of Pressure Ulcers: Quick Reference Guide*, National Pressure Ulcer Advisory Panel, Washington, DC, USA, 2009.

[3] S. M. J. Abrisham, M. Kermani-Alghoraishi, R. Ghahramani, L. Jabbari, H. Jomeh, and M. Zare, "Additive effects of low-level laser therapy with exercise on subacromial syndrome: a randomised, double-blind, controlled trial," *Clinical Rheumatology*, vol. 30, no. 10, pp. 1341–1346, 2011.

[4] F. Ahrari, A. S. Madani, Z. S. Ghafouri, and J. Tunér, "The efficacy of low-level laser therapy for the treatment of myogenous temporomandibular jointdisorder," *Lasers in Medicine and Science*. In press.

[5] A. Stergioulas, "Low-power laser treatment in patients with frozen shoulder: preliminary results," *Photomedicine and Laser Surgery*, vol. 26, no. 2, pp. 99–105, 2008.

[6] C. C. Shen, Y. C. Yang, T. B. Huang et al., "Low-level laser-accelerated peripheral nerve regeneration within a reinforced nerve conduit across a large gap of the transected sciatic nerve in rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 175629, 12 pages, 2013.

[7] L. V. Komel'kova, T. V. Vitreshchak, I. G. Zhirnova et al., "Biochemical and immunological induces of the blood in Parkinson's disease and their correction with the help of laser therapy," *Patologicheskaia Fiziologiia i Eksperementalnaia Terapiia*, no. 1, pp. 15–18, 2004.

[8] A. E. Saltmarche, "Low level laser therapy for healing acute and chronic wounds—the extendicare experience," *International Wound Journal*, vol. 5, no. 2, pp. 351–360, 2008.

[9] C. Lucas, M. J. C. Van Gemert, and R. J. De Haan, "Efficacy of low-level laser therapy in the management of stage III decubitus ulcers: a prospective, observer-blinded multicentre randomised clinical trial," *Lasers in Medical Science*, vol. 18, no. 2, pp. 72–77, 2003.

[10] A. B. Taly, K. P. Sivaraman Nair, T. Murali, and A. John, "Efficacy of multiwavelength light therapy in the treatment of pressure ulcers in subjects with disorders of the spinal cord: a randomized double-blind controlled trial," *Archives of Physical Medicine and Rehabilitation*, vol. 85, no. 10, pp. 1657–1661, 2004.

[11] E. L. Nussbaum, I. Biemann, B. Mustard, S. Michlovitz, and B. R. Buinewicz, "Comparison of ultrasound/ultraviolet-C and laser for treatment of pressure ulcers in patients with spinal cord injury," *Physical Therapy*, vol. 74, no. 9, pp. 812–825, 1994.

[12] O. Dehlin, S. Elmståhl, and F. Gottrup, "Monochromatic phototherapy: effective treatment for grade II chronic pressure ulcers in elderly patients," *Aging*, vol. 19, no. 6, pp. 478–483, 2007.

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Research Article

Intravenous Laser Blood Irradiation Increases Efficacy of Etanercept in Selected Subtypes of Juvenile Idiopathic Arthritis: An Innovative Clinical Research Approach

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This single-blind, placebo-controlled study assesses the efficacy of synergic administration of intravenous laser blood irradiation (ILBI) and etanercept in selected subtypes of juvenile idiopathic arthritis (JIA). Etanercept is a tumor necrosis factor alpha blocking agent with recognized importance in JIA. Laser radiation has immunomodulatory effects in animal and human studies. Fourteen patients (Group I) received ILBI and 9 patients (Group II) received placebo laser. ILBI was performed in addition to ongoing JIA medication, including etanercept. ILBI was administrated in 3 sets of 5 consecutive daily sessions, with a 7-week interval between every set of sessions. Evaluation was performed using ACR (American College of Rheumatology) Pediatric Criteria (ACR Pedi) at study enrollment and at 10 and 20 weeks, respectively. After 10 weeks, 85.7% of the patients in Group I fulfilled Pedi 30 criteria, compared to only 55.6% of the patients in Group II. After 20 weeks, all patients in both groups had a Pedi 30 response. In Group I, 92.8% of the subjects met the Pedi 50 response, compared to only 55.6% in the placebo group. One patient in Group I responded best, fulfilling Pedi 70 criteria. If applied synergistically, ILBI and etanercept would have an increased efficacy in promoting JIA remission.

1. Introduction

Juvenile idiopathic arthritis (JIA) can be defined as an inflammatory heterogeneous condition, encompassing several subtypes of disease. The term refers to all forms of arthritis which are diagnosed before 16 years of age and last more than 6 weeks [1, 2]. The prevalence of the disease varies a lot regarding the continent, study area, and population, but on average, one in 1000 children worldwide has JIA [3].

Even if there is currently no cure for JIA, in the last decade much progress has been made in the therapeutic management, especially with the introduction of biological agents which target specific inflammatory cytokines and signaling molecules [4, 5]. However, Hayward and Wallace reported that only 25% to 40% of patients with JIA achieved inactive disease on biologic medications [4].

In this scenario, additional methods which could enhance the efficacy of biological agents appear to be of high importance. It is worth mentioning that biologic medication in general and etanercept, a tumor necrosis factor alpha (TNFα) fusion protein inhibitor (see Figure 1) in particular, place a great amount of financial pressure on the health insurance

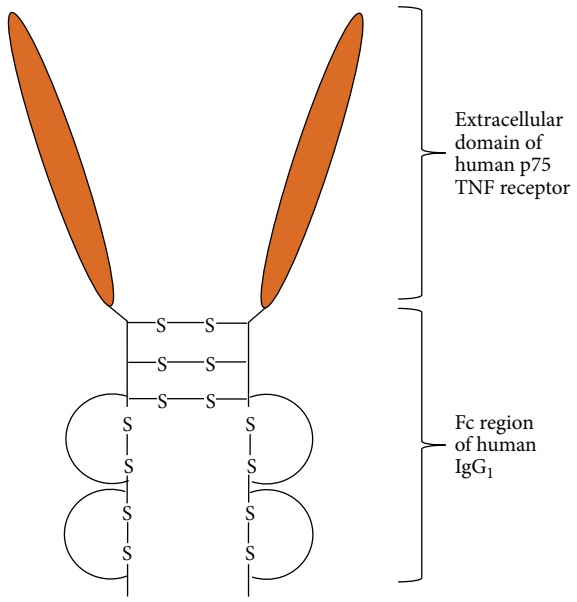

Figure 1: Schematic structure of etanercept. TNF: tumor necrosis factor; Fc: fragment crystallizable; IgG_1: immunoglobulin G1; S: sulfur.

systems, both in Europe and the United States, due to high costs [6, 7].

The importance of etanercept is a fact, being one of the first-line biological drugs to be prescribed in JIA. In 1999, it was the first anti-TNFα agent to be approved for use in JIA in the United States [4], and European approval was granted shortly after, in 2000 [8].

At the present moment, for example, in Romania, etanercept is the only biologic agent to treat JIA, which has its cost entirely reimbursed by the Romanian National Health Insurance House [9].

It was proved that laser radiation can act on the immune system and decrease serum TNFα titers [10]. There is some data regarding the positive effects of intravenous laser blood irradiation (ILBI) in immune-related diseases [11]. There are also some selected case reports and smaller studies of the authors about ILBI in JIA patients, published in proceedings of conferences.

At present, there is no randomized, placebo-controlled study of this relevance to test the efficacy of synergic administration of ILBI and etanercept in JIA.

2. Patients and Methods

2.1. Study Setup. This prospective, single-blind, placebo-controlled study was performed during a 19-month period, between November 2011 and May 2013. It was conducted at the Second Pediatric Clinic of the St. Mary Emergency Hospital for Children, Iasi, Romania, with the ethic approval of the aforementioned healthcare institution. Both patients and their families were given complete information; all their questions were answered and all of them signed a written consent.

The eligible patients were 8 to 16 years of age at study enrollment. All of them were diagnosed with JIA using the International League of Associations for Rheumatology (ILAR) criteria [2].

Altogether 23 patients (mean age ± SD: 12.3 ± 2.9 years) were included in this study, presenting moderate and severe forms of JIA, with the following subtypes: extended oligoarthritis, polyarthritis with negative rheumatoid factor (RF−), and polyarthritis with positive rheumatoid factor (RF+). The patients were randomized into two asymmetric groups, using block randomization with an allocation ratio of 3 : 2. Group I (60% of the patients) received ILBI, and Group II (40% of the patients) received placebo laser. This was also combined with stratified randomization to ensure a good balance of arthritis subtypes in each group. The initial demographic data and disease characteristics are shown in Table 1.

The enrolled patients and their parents were fully explained the possible risks and benefits of ILBI. The patients and their parents were fully aware of the existence of the placebo group and they were guaranteed that the ones who would be in the placebo group would be offered, at the end of the study, a real ILBI trial, identical with the one the patients in the laser group had, should they wish.

In this single-blind study, the qualified personnel who performed the laser treatment were aware about the patient adhesion to a specific study group. Therefore, it was of extreme importance for this category of personnel not to influence patients' outcome, and this aspect was a priority for us during the research. The evaluators, who assessed the patients' clinical condition, were not aware of the adhesion of patients to a specific treatment group, in order to avoid any bias.

The exclusion criteria were formed of three main groups. Exclusion criteria due to selected subtypes of JIA included in the study: forms of systemic arthritis, enthesitis-related arthritis, persistent oligoarthritis, psoriatic arthritis, undifferentiated arthritis, and positive diagnosis of uveitis [2]. The second group of exclusion criteria was due to contraindications and special warnings to etanercept therapy: pregnant or sexually active female patients without using effective contraception (this situation is considered to be rare below 16 years of age, but still, it should be given proper attention), active infections, risk of sepsis, history of tuberculosis and hepatitis, with B or C virus, malignancies, and lymphoproliferative disorders. These conditions represent key points, when etanercept is to be initiated, and special medical conduct is performed if one of the above occurs, according to the national guidelines and manufacturer's advice for Europe [8, 9]. The last group of exclusion criteria was related to ILBI and consisted of sensitivity to light, history of epilepsy, and age under 8 years. The last requirement was implemented due to the fact that little children have low compliance to peripheral intravenous (i.v.) line insertion and have very limited understanding of this study setup, even if their parents would have fully agreed with all the research protocols.

The main inclusion criterion was ongoing anti-TNFα therapy with etanercept for all the patients for at least 3 months, without obtaining an improvement of at least ACR

Table 1: Initial demographic data and disease characteristics.

Characteristic	Group I—ILBI (n = 14)	Group II—Placebo (n = 9)	Statistical significance
Demographic			
Male sex—no. (%)	8 (57.1)	5 (55.6)	NS
Age—years; mean ± SD	12.1 ± 3.2	12.5 ± 2.6	NS
Weight—kg; mean ± SD	30.0 ± 14.8	33.6 ± 18.6	NS
JIA characteristics			
Duration of disease—years; mean ± SD	4.1 ± 2.4	4.2 ± 3.0	NS
Extended oligoarthritis—no. (%)	6 (42.9)	4 (44.4)	NS
Polyarthritis (RF negative)—no. (%)	5 (35.7)	3 (33.3)	NS
Polyarthritis (RF positive)—no. (%)	3 (21.4)	2 (22.2)	NS

NS: nonsignificant difference between the two groups, P value ≥ 0.05. SD: standard deviation. RF: rheumatoid factor.

(American College of Rheumatology) Pediatric 30 response [12], during the last 3 months. The other inclusion criteria were active arthritis for at least 6 months, the absence of remission in the last 6 months, as defined by Wallace et al. [13], and age range between 8 and 16 years at the enrollment in the study.

Analgesics, including opiates, anti-inflammatory medication (steroidal and nonsteroidal), and disease-modifying anti-rheumatic drugs (DMARDs) were allowed. Detailed information is displayed in Table 2.

All patients were enrolled in a physical therapy program and psychological support was available throughout the whole period of the study.

2.2. Laser Equipment and Protocol. Both subjects in Group I—ILBI (14 patients) and in Group II—placebo (9 patients) received the same standard i.v. laser protocol, up to the very moment when the light radiation was delivered through optical fiber into the vein. The placebo patients did not effectively receive the laser radiation through the already connected intravenous optical fiber.

Patients did not know if they were receiving laser radiation or placebo, and confidentiality was assured with laser protective goggles worn by the patients.

Laser therapy was given in 3 sets of 5 consecutive daily sessions, with a 7-week interval between every set of sessions.

ILBI protocol consisted of 3 different wavelengths (630 nm, 536 nm, and 405 nm) with a 5 mW maximum output power in continuous mode (Figure 2). Radiation was given for 10 minutes for each wavelength, with a total duration of 30 minutes per session. The three types of radiation, regarding the wavelength, were given in the following order: red radiation (630 nm) at the beginning of the session, followed by green radiation (536 nm) and violet radiation (405 nm) at the end. This protocol was implemented for all patients and for all laser sessions performed in the study. The reason for this choice of protocol was to deliver an increasing amount of energy to the blood stream, as photons with shorter wavelengths carry a greater amount of energy compared to photons with longer wavelength. All patients were lying comfortably on a treatment bed during therapy.

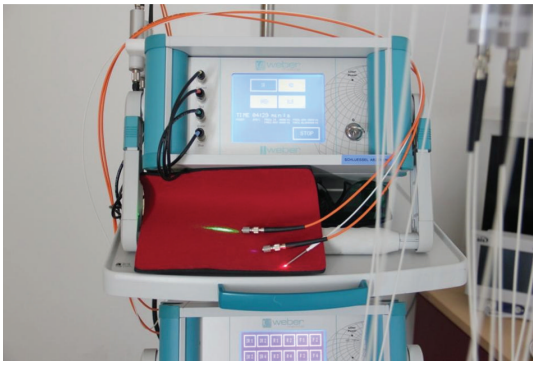

Figure 2: Weberneedle Endolaser (identical systems are available at the Medical University of Graz and the St. Mary Emergency Hospital for Children Iasi). The following wavelengths were used: 630 nm (red), 536 nm (green), and 405 nm (violet).

The site for the i.v. access was preferred at the cubital region, but if no vein could be located, alternate sites at the forearm or the dorsal region of the hand were used. Large veins at palpation were the standard of choice.

The laser radiation was delivered with a sterile optical fiber i.v. catheter, which was passing through the lumen of a butterfly needle (size 21G) into a peripheral vein. The butterfly needle and the external tip of the optical fiber catheter were immobilized to the patient's skin with adhesive tape (Figure 3).

Prior to procedure, the needle insertion site was cleansed with disinfectant solution. Approximately 2 g of topical anesthetic cream (Lidocaine 2.5%/Prilocaine 2.5%) was applied on that site to prevent the pain caused by the needle. Then, a sterile transparent film dressing was applied on the top to allow the anesthetic to work effectively, before obtaining venous access. When the ILBI procedure was finished, the butterfly and the optical fiber catheter were extracted, and an adhesive sterile dressing was applied on the site.

2.3. Assessment and Statistical Analysis. Patients were assessed initially, when enrolled in the study. Two more assessments were performed at 10 weeks and 20 weeks,

Table 2: Arthritis-related pharmacological therapy.

Characteristic	Group I—ILBI (n = 14)	Group II—Placebo (n = 9)	Statistical confidence
Current administration of DMARDs			
Methotrexate—no. (%)	12 (85.6)	7 (77.8)	NS
Methotrexate mean dose ± SD per patient—mg/m^2/week	13.7 ± 5.2	14.2 ± 4.9	NS
All patients receiving Methotrexate were also prescribed folic acid 5 mg, which was given the morning following Methotrexate administration, as per local protocol			
Other DMARDs—no. (%)	0 (0)	0 (0)	NS
Current administration of corticosteroids			
Oral corticosteroid—no. (%)	10 (71.4)	7 (77.8)	NS
Oral corticosteroid—Prednisolone equivalent mean dose ± SD per patient—mg/kg/day	0.28 ± 0.15	0.28 ± 0.18	NS
I.v. Prednisolone—no. (%)	4 (28.6)	2 (22.2)	NS
I.v. Prednisolone—no. of boluses per month per patient (30 mg/kg, max 1 g); mean ± SD	3.2 ± 1.2	3.4 ± 1.3	NS
Intraarticular Prednisolone—no. (%)	3 (21.4)	3 (33.3)	NS
Intraarticular Prednisolone—no. of joints per patient (1.5–2.5 mg/small joint; 25–50 mg/large joints); mean ± SD	5.1 ± 2.3	4.6 ± 1.9	NS
Current administration of biological agents			
Etanercept—no. (%)	14 (100)	9 (100)	NS
Etanercept mean dose ± SD per patient—mg/kg, twice a week	0.4 ± 0.0	0.4 ± 0.0	NS
Other biological agents—no. (%)	0	0	NS

NS: nonsignificant difference between the two groups, P value ≥ 0.05. DMARDs: disease-modifying antirheumatic drugs.

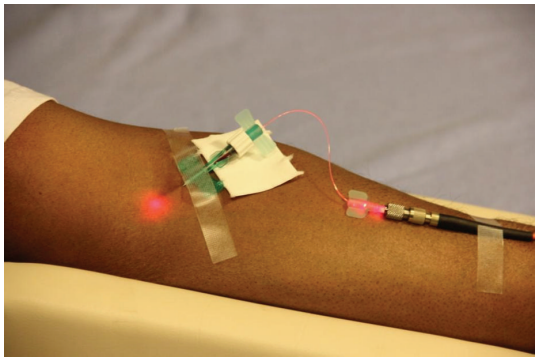

Figure 3: Application of the Weberneedle Endolaser on a patient.

respectively, from the initial time. Following the ILBI treatment schedule, it results that the second assessment was done one week after the second set of sessions, and the last evaluation was at 3-week time after the last set of sessions.

All the patients in the placebo group wanted to get benefit of verum ILBI therapy at the end of the study, and we managed to record data for five of those patients, and four patients are still undergoing the ILBI protocol at the present moment. These findings are not the objective of the present study and they will not be presented in this paper.

Each clinical assessment included the six variables of the ACR core set data for JIA [12]: the number of joints with active arthritis, the number of joints with limited range of motion, the physician's global assessment of disease activity on a visual analogue scale (VAS), the parent's or patient's global assessment of overall well-being on a VAS, physical function (assessed with the Childhood Health Assessment Questionnaire (CHAQ) [14], with scores ranging from 0 to 3 for 8 activities of daily living), and the erythrocyte sedimentation rate (ESR). The original VAS proposed by Giannini et al. [12] had a range from 0 to 100 mm, with 0 being the best score. We kept the length of the scale, but we modified its basic length unit from millimeters to centimeters, obtaining a scale ranging from 0 to 10 cm. We found this rescaling very useful, especially when the child is pointing with his finger for scoring. Not only would it have been laborious to identify the exact millimeter a child is pointing at, but it is also difficult to ask a child to adhere to a scale with so many scaling units. Approximation was performed to the closest unit in centimeters, when the patient finger was placed between two consecutive units. To maintain the uniformity, the VAS for the physician assessment was modified in the same way, and instructions for approximation were given accordingly.

Disease improvement was evaluated using the ACR Pediatric (ACR Pedi) criteria. The ACR Pedi 30 (50, 70, and 90, resp.) criteria are defined as improvement of more than 30% (50%, 70%, and 90%, resp.), in at least 3 of the 6 core set

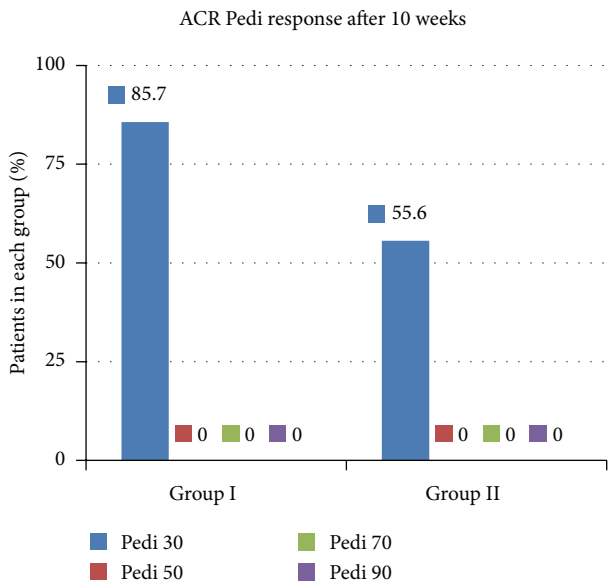

Figure 4: ACR Pedi response at the evaluation performed at 10 weeks from ILBI initiation.

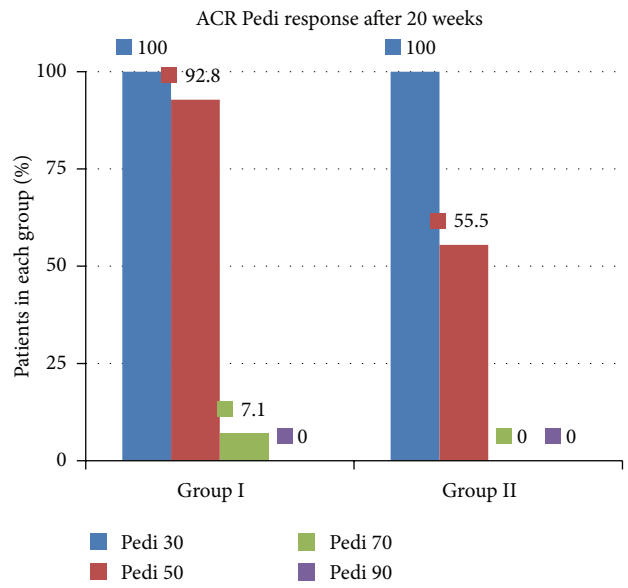

Figure 5: ACR Pedi response at the evaluation performed at 20 weeks from ILBI initiation.

variables used to assess disease activity, with no more than one variable worsening by more than 30% [12].

The median values and the standard deviation were calculated for all quantifiable data in both groups. The differences among each group regarding the initial parameters were evaluated using a 2-sample homoscedastic t-test for a confidence interval of 95%. The disease outcome for each group was tested for significant differences using a 2-sample heteroscedastic t-test. A value of $P < 0.05$ was considered significant, and a value of $P \geq 0.05$ was considered non-significant. The basic statistical analysis and chart generation were done using MS Excel 2010 software.

3. Results

The initial demographic data and disease characteristics were balanced among the two groups, with no statistically significant differences (see Table 1).

After 10 weeks, the patients treated with ILBI displayed a better improvement in all six parameters of the ACR core set data compared to the placebo group (see Table 3).

Regarding the ACR Pedi criteria, 85.7% of the patients in Group I, compared to only 55.6% of the patients in Group II, managed to meet the Pedi 30 response (see Figure 4). None of the patients in both groups met the Pedi 50, 70, or 90 responses.

At the final evaluation, the ILBI patients continued to display a more significant improvement in comparison with the patients in the control group in all aspects encompassed in the ACR core set data (see Table 4).

All patients from both groups fulfilled the Pedi 30 response. In Group I, 92.8% of the subjects met the Pedi 50 response, compared to only 55.6% in the placebo group. Only one patient in Group I had a Pedi 70 response, and none of the patients from the present research had a Pedi 90 response (see Figure 5) at the end of the study.

4. Discussion

JIA is still a pathological condition with no clear etiology, and a multifactorial approach is preferred to explain the causes of the disease [1]. The treatment evolved over time from nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids to classic DMARDs like Methotrexate and Sulfasalazine and to biological agents in the last 15 years. Biological agents target specific inflammatory cytokines involved in JIA, like TNFα, interleukin 1 (IL1), and IL6, as well as signaling molecules involved in the regulation of B-cell and T-cell lymphocyte responses [4, 5].

The main disadvantage of biological agents is their immunosuppressant effect which leads to an increased risk of opportunistic infections, from mild to severe (tuberculosis, viral hepatitis reactivation), and rarely to malignancies. However, long-term safety and effectiveness of etanercept in JIA are widely recognized [4, 5, 15].

There have been some debates over the doses of etanercept in JIA. Increased doses of etanercept may not offer any additional benefit in children with unsatisfactory response to the standard dose [16].

Due to administrative or financial reasons, it may not be possible to change the biologic agent in such cases [9], so finding a synergistic therapeutic method to increase etanercept efficacy represents a major challenge. Our results proved that ILBI had indeed a beneficial effect for the patients in Group I, who displayed a better disease improvement throughout the study. At baseline, there were no significant differences between the two groups in terms of statistical analysis. The much better outcome in the ILBI group was statistically

Table 3: Evolution of ACR core set of variables at 10 weeks from initiation of ILBI.

Variable	Group I—ILBI (n = 14)		Group II—Placebo (n = 9)		Statistical comparison among groups	
	Initial	After 10 weeks	Initial	After 10 weeks	Initial	After 10 weeks
No. of joints with active arthritis	9.9 ± 3.5	6.4 ± 2.3	10.1 ± 2.1	8.2 ± 2.4	$P = 0.890$	$P = 0.044$
No. of joints with limited range of motion	38.5 ± 5.6	29.9 ± 5.4	38.7 ± 4.8	34.6 ± 4.6	$P = 0.942$	$P = 0.021$
Score for physician's global assessment of disease activity	8.4 ± 1.2	6.1 ± 1.8	8.7 ± 0.9	6.7 ± 1.0	$P = 0.498$	$P = 0.268$
Score for parent's or patient's global assessment of overall wellbeing	8.6 ± 1.0	5.7 ± 0.8	9.1 ± 0.9	6.3 ± 0.9	$P = 0.213$	$P = 0.049$
CHAQ score	14.9 ± 2.6	9.4 ± 2.3	14.0 ± 2.2	11.3 ± 2.7	$P = 0.390$	$P = 0.041$
ESR—mm/hr	50.6 ± 24.8	30.9 ± 11.4	51.6 ± 15.6	38.8 ± 7.8	$P = 0.922$	$P = 0.042$

CHAQ: Childhood Health Assessment Questionnaire. ESR: erythrocyte sedimentation rate.

Table 4: Evolution of ACR core set of variables at 20 weeks from initiation of ILBI.

Variable	Group I—ILBI (n = 14)		Group II—Placebo (n = 9)		Statistic comparison among groups	
	Initial	After 20 weeks	Initial	After 20 weeks	Initial	After 20 weeks
No. of joints with active arthritis	9.9 ± 3.5	4.4 ± 1.5	10.1 ± 2.1	5.8 ± 2.0	$P = 0.890$	$P = 0.034$
No. of joints with limited range of motion	38.5 ± 5.6	16.3 ± 4.7	38.71 ± 4.8	24.3 ± 7.7	$P = 0.942$	$P = 0.002$
Score for physician's global assessment of disease activity	8.4 ± 1.2	3.1 ± 0.9	8.7 ± 0.9	3.9 ± 1.1	$P = 0.498$	$P = 0.046$
Score for parent's or patient's global assessment of overall wellbeing	8.6 ± 1.0	3.4 ± 1.0	9.1 ± 0.9	4.2 ± 1.3	$P = 0.213$	$P = 0.043$
CHAQ score	14.9 ± 2.6	5.7 ± 1.1	14.0 ± 2.2	6.9 ± 1.3	$P = 0.390$	$P = 0.013$
ESR—mm/hr	50.6 ± 24.8	15.9 ± 7.9	51.6 ± 15.6	22.9 ± 11.1	$P = 0.922$	$P = 0.045$

CHAQ: Childhood Health Assessment Questionnaire. ESR: erythrocyte sedimentation rate.

significant compared to the results obtained in the placebo group, while all patients continuously received etanercept.

In our study design, we excluded some subtypes of JIA. We focused on the polyarticular forms of the disease, due to their more severe evolution, excluding forms of persistent oligoarthritis. Psoriatic arthritis and enthesitis-related arthritis each represent only 1–11% of the JIA patients [1, 2]. IL6 receptor inhibition is considered more effective in systemic arthritis [17]. Patients with uveitis were excluded, as there are opinions more favorable to Infliximab [18] and Adalimumab [19] (other anti-TNFα agents), or Abatacept [20] (a cytotoxic T-lymphocyte antigen 4 fusion protein), in the treatment of JIA-associated uveitis.

Regarding steroidal medication, all patients from both groups received at least a form of corticosteroid (oral, i.v. pulsed, intraarticular) when enrolled in the study. The local practice protocol was to decrease the dose or limit the use of steroidal medication due to the associated high toxicity. When a Pedi 30 response was achieved, tapering of the steroid dose or limiting it to intraarticular administration was performed. A Pedi 50 or Pedi 70 response was a clear indication for stopping the corticosteroid medication with gradual withdrawing. Since steroid medication management and Pedi response are so closely related to one another, ILBI patients managed to reduce and avoid further corticosteroids in a much larger percent compared to placebo patients (see Figures 4 and 5).

The placebo group (after 10 or 20 weeks, resp.) achieved a disease improvement rate comparable to the data in the literature [21]. The small differences in outcome could be explained by the size of the placebo group and by the fact that not all the patients had the same length of etanercept administration period at enrolment in the study.

At both evaluations, after 10 weeks and at the end of the study, ILBI patients displayed a better improvement in all the ACR core set parameters. All the differences were statistically significant ($P < 0.05$), with the exception of the score for physician's global assessment of disease activity after 10 weeks, where P had a value of 0.268.

All patients from both groups fulfilled the Pedi 30 response at the end of the study, which certifies the value of etanercept administration. The result of 92.8% of the subjects who met the Pedi 50 response after synergistic ILBI, compared to only 55.6% patients in the placebo group, proved the importance of this new therapeutic approach in JIA. Moreover, only one patient in the ILBI group had a Pedi 70 response, whereas no patient with placebo laser obtained this outcome. None of the patients from the present research had a Pedi 90 response (see Figure 5).

No side effects to be correlated with ILBI were observed. Possible initial needle fear was overcome with thorough skin desensitization, using a topical anesthetic cream. Minor upper respiratory infections and skin reactions at the etanercept subcutaneous injection site were observed in 3 patients in Group I and in 2 patients in Group II. With appropriate

treatment (antibiotics and antihistamines, resp.), all patients could continue the study without interrupting etanercept or ILBI. These were common side effects to etanercept [4, 5, 15, 21].

Trying to explain how ILBI could promote a better outcome when given together with etanercept leads to the molecule targeted by this biologic agent. TNFα is a powerful proinflammatory cytokine and has increased titers, both in serum and in the synovial fluid of JIA patients. It exists in a soluble (sTNFα) and a membrane-attached form (mTNFα). TNF-mediated biology gains additional complexity from the distinct signaling pathways, mediated through two types of receptors, TNFR1 and TNFR2, which can also exist in circulating and membrane-attached forms. Both receptors bind all TNFα molecules plus soluble lymphotoxin alpha 3 (LTα3) and cell-surface LTα2β1, as LT, formerly called TNFβ, is structurally similar to TNFα. Moreover, mTNFα can also function as a receptor when a circulating endogenous TNFα receptor or an appropriate biological agent binds to it [22, 23].

Etanercept (Enbrel, as trade name) is a fusion protein consisting of the extracellular domain of the TNFR2 combined with the Fc portion of the human immunoglobulin IgG1. Etanercept binds to sTNFα and mTNFα and thus decreases the inflammatory TNFα-mediated signaling [4]. Because of its structure, it also binds to LTα3 and LTα2β1 [22].

Intravenous laser irradiation was reported to change physiological parameters in a rat model [24]. Laser radiation was also demonstrated to reduce sTNFα in some animal studies with experimentally induced acute inflammation in lungs [10, 25]. Mesquita-Ferrari et al. illustrated that low-level laser therapy (LLLT) caused a decrease in TNFα mRNA (messenger ribonucleic acid) expression at 1 and 7 days following the cryoinjury of tibialis anterior muscle in rats [26]. Decreasing TNFα mRNA expression in the affected muscular cells also decreases both sTNFα and mTNFα, as sTNFα results from enzymatical cleavage of mTNFα [22]. LLLT also proved to decrease the mRNA level of mTNFα in *in vitro* synoviocytes from rheumatoid arthritis patients [27].

According to these experiments, ILBI would be capable of decreasing sTNFα titers and mTNFα membrane presentation. Thus, we assume that the constant quantity of etanercept, therapeutically given to the patient, would be able to inactivate most of the TNFα molecules with a higher probability.

A pretty new research field is represented by the role played by LT family in inflammation in general and in arthritis in particular [28]. Etanercept neutralizes LTα3 and sTNFα with similar potency, and so, neutralization of sTNFα could be reduced by competition, if concentrations of LTα3 are high. There is no evidence to date that LT blockade provides etanercept with any therapeutic advantage [22].

Searching the present scientific literature, we discovered that to the best of our knowledge no one studied the interaction between laser radiation and LT. Taking into account that laser therapy is reducing the serum titers of the major pro-inflammatory cytokines [10, 25–27], it is worth raising the question if ILBI could modulate the immune response by also decreasing the LTα3 and LTα2β1 production.

In such an instance, ILBI could enhance etanercept efficacy by decreasing the competition between TNFα and the LT family, leading to a better overall patient response.

An emerging area of interest, regarding etanercept mechanisms of action, centers on the functional outcomes of its interaction with mTNFα. Current evidence suggests that etanercept acts both as an antagonist, by blocking mTNFα interaction with TNFR1 or TNFR2, and as an agonist, by initiating reverse signaling. The latter type of signaling leads to apoptosis, cytokine suppression, or cell activation [23].

Decoster et al. demonstrated that TNFα is firstly formed as a membrane-bound protein, which is responsible for receptor downmodulation [29]. Laser radiation could promote receptor down-modulation, in the case of cellular activation, due to the binding of etanercept to mTNFα [23]. This would result in a smaller probability of occurrence for further TNFα-induced reactions in those cells.

Another pathway of action for ILBI, in balancing the inflammation, is by increasing anti-inflammatory cytokines titers [30]. A recent animal *in vivo* study observed statistically significant beneficial anti-inflammatory effects of LLLT administration in induced rheumatoid arthritis in rats [31].

It is likely that several of the above mechanisms act in concert. The contribution of ILBI to the various etanercept acting mechanisms, coupled to its balancing action on enhancing the anti-inflammatory cytokines pathways, remains a focus subject for the scientific community.

ILBI, firstly discovered by Russian scientists in 1981, still represents a novel treatment modality amongst the applications of lasers in medicine. This is because the studies were published mainly in Russian and remained mostly unknown to the Western Europe and United States [11]. Within the last 10 years, ILBI started to prove its efficacy in a wide range of medical conditions like diabetes mellitus, chronic hepatitis, hepatic cirrhosis, dyslipidemia, cardiovascular diseases, and autoimmune diseases [11].

Schumm indicates that ILBI can successfully be applied in multiple sclerosis, leading to a highly significant improvement in the quality of life of the treated patients [32]. Last year, Huang et al. reported the beneficial effects of ILBI therapy on oxidative stress and mitochondrial dysfunction in subjects with chronic spinal cord injury resulting from trauma [33].

ILBI was also reported to be a valuable adjuvant in oncology due to its immunomodulatory effects [11]. Relatively new data places this therapeutic modality in the field of sports medicine, promoting significant improvement in the sleep pattern, vigilance, and overall physical performance of athletes [34].

A new challenge is represented by the transcutaneous and transmucosal (sublingual) laser blood irradiation due to the noninvasive aspect of the therapy. Under certain conditions, these noninvasive methods can have similar efficacy compared to ILBI, and it can be the only alternative to incompliant patients to vein puncture [35].

5. Conclusions

ILBI and etanercept have an increased efficacy in promoting the remission of selected subtypes of JIA, if applied synergistically. Our significant results proved the value of ILBI in cases of moderate-to-severe polyarthritis.

Further studies regarding laser therapy interaction with TNFα and the LT cytokine family might explain its anti-inflammatory effect more accurately.

Conflict of Interests

No conflict of financial interests exists. The investigations were performed using a system from weber medical GmbH; however, there was no financial support from the company.

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References

[1] A. Ravelli and A. Martini, "Juvenile idiopathic arthritis," *The Lancet*, vol. 369, no. 9563, pp. 767–778, 2007.

[2] R. E. Petty, T. R. Southwood, P. Manners et al., "International league of associations for rheumatology classification of juvenile idiopathic arthritis: second revision, edmonton, 2001," *Journal of Rheumatology*, vol. 31, no. 2, pp. 390–392, 2004.

[3] P. J. Manners and C. Bower, "Worldwide prevalence of juvenile arthritis—why does it vary so much?" *Journal of Rheumatology*, vol. 29, no. 7, pp. 1520–1530, 2002.

[4] K. Hayward and C. A. Wallace, "Recent developments in anti-rheumatic drugs in pediatrics: treatment of juvenile idiopathic arthritis," *Arthritis Research and Therapy*, vol. 11, no. 1, article 216, 2009.

[5] G. Horneff, "Update on biologicals for treatment of juvenile idiopathic arthritis," *Expert Opinion on Biological Therapy*, vol. 13, no. 3, pp. 361–376, 2013.

[6] J. Zeidler, T. Mittendorf, R. Müller, and J. von Kempis, "Biologic TNF inhibiting agents for treatment of inflammatory rheumatic diseases: dosing patterns and related costs in Switzerland from a payers perspective," *Health Economics Review*, vol. 2, article 20, no. 1, 2012.

[7] V. F. Schabert, C. Watson, S. R. Gandra, S. Goodman, K. M. Fox, and D. J. Harrison, "Annual costs of tumor necrosis factor inhibitors using real-world data in a commercially insured population in the United States," *Journal of Medical Economics*, vol. 15, no. 2, pp. 264–275, 2012.

[8] European Medicines Agency, "European Public Assessment Report (EPAR) for Enbrel (Etanercept)," 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000262/WC500027361.pdf.

[9] Order no. 220 emitted on 15 May 2007 by Romanian National Health Insurance House, "Approval of the protocol for practice, monitoring and reimbursement for medicines in cases of juvenile idiopathic arthritis for persons who are insured in the national social health insurance system" (Romanian), Monitorul Oficial al Romaniei, no. 255, part I, 2007.

[10] F. Aimbire, R. Albertini, M. T. T. Pacheco et al., "Low-level laser therapy induces dose-dependent reduction of TNFα levels in acute inflammation," *Photomedicine and Laser Surgery*, vol. 24, no. 1, pp. 33–37, 2006.

[11] M. Weber, "The intravenous laser blood irradiation. Introduction of a new therapy," in *Lasers in Medicine, Science and Praxis*, Z. Simunovic, Ed., pp. 664–706, Zrinski Printery Publishing House, Cakovec, Croatia, 2009.

[12] E. H. Giannini, N. Ruperto, A. Ravelli, D. J. Lovell, D. T. Felson, and A. Martini, "Preliminary definition of improvement in juvenile arthritis," *Arthritis and Rheumatism*, vol. 40, no. 7, pp. 1202–1209, 1997.

[13] C. A. Wallace, N. Ruperto, and E. H. Giannini, "Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis," *Journal of Rheumatology*, vol. 31, no. 11, pp. 2290–2294, 2004.

[14] N. Ruperto, A. Ravelli, A. Pistorio et al., "Cross-cultural adaptation and psychometric evaluation of the Childhood Health Assessment Questionnaire (CHAQ) and the Child Health Questionnaire (CHQ) in 32 countries. Review of the general methodology," *Clinical and Experimental Rheumatology*, vol. 19, no. 4, pp. S1–S9, 2001.

[15] E. H. Giannini, N. T. Ilowite, D. J. Lovell et al., "Long-term safety and effectiveness of etanercept in children with selected categories of juvenile idiopathic arthritis," *Arthritis and Rheumatism*, vol. 60, no. 9, pp. 2794–2804, 2009.

[16] S. Takei, D. Groh, B. Bernstein, B. Shaham, K. Gallagher, and A. Reiff, "Safety and efficacy of high dose etanercept in treatment of juvenile rheumatoid arthritis," *Journal of Rheumatology*, vol. 28, no. 7, pp. 1677–1680, 2001.

[17] F. De Benedetti, H. I. Brunner, N. Ruperto et al., "Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis," *The New England Journal of Medicine*, vol. 367, no. 25, pp. 2385–2395, 2012.

[18] R. K. Saurenmann, A. V. Levin, J. B. Rose et al., "Tumour necrosis factor α inhibitors in the treatment of childhood uveitis," *Rheumatology*, vol. 45, no. 8, pp. 982–989, 2006.

[19] P. Tynjäaldie;, K. Kotaniemi, P. Lindahl et al., "Adalimumab in juvenile idiopathic arthritis-associated chronic anterior uveitis," *Rheumatology*, vol. 47, no. 3, pp. 339–344, 2008.

[20] F. Zulian, M. Balzarin, F. Falcini et al., "Abatacept for severe anti-tumor necrosis factor α refractory juvenile idiopathic arthritis-related uveitis," *Arthritis Care and Research*, vol. 62, no. 6, pp. 821–825, 2010.

[21] P. Quartier, P. Taupin, F. Bourdeaut et al., "Efficacy of etanercept for the treatment of juvenile idiopathic arthritis according to the onset type," *Arthritis and Rheumatism*, vol. 48, no. 4, pp. 1093–1101, 2003.

[22] D. Tracey, L. Klareskog, E. H. Sasso, J. G. Salfeld, and P. P. Tak, "Tumor necrosis factor antagonist mechanisms of action: a comprehensive review," *Pharmacology and Therapeutics*, vol. 117, no. 2, pp. 244–279, 2008.

[23] N. Thalayasingam and J. D. Isaacs, "Anti-TNF therapy," *Best Practice and Research*, vol. 25, no. 4, pp. 549–567, 2011.

[24] W. He, G. Litscher, X. Y. Wang et al., "Intravenous laser blood irradiation, interstitial laser acupuncture, and electroacupuncture in an animal experimental setting: preliminary results from heart rate variability and electrocorticographic recordings,"

Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 169249, 6 pages, 2013.

[25] E. S. Boschi, C. E. Leite, V. C. Saciura et al., "Anti-inflammatory effects of low-level laser therapy (660 nm) in the early phase in carrageenan-induced pleurisy in rat," *Lasers in Surgery and Medicine*, vol. 40, no. 7, pp. 500–508, 2008.

[26] R. A. Mesquita-Ferrari, M. D. Martins, J. A. Silva Jr. et al., "Effects of low-level laser therapy on expression of TNF-α and TGF-β in skeletal muscle during the repair process," *Lasers in Medical Science*, vol. 26, no. 3, pp. 335–340, 2011.

[27] M. Yamaura, M. Yao, I. Yaroslavsky, R. Cohen, M. Smotrich, and I. E. Kochevar, "Low level light effects on inflammatory cytokine production by rheumatoid arthritis synoviocytes," *Lasers in Surgery and Medicine*, vol. 41, no. 4, pp. 282–290, 2009.

[28] F. Calmon-Hamaty, B. Combe, M. Hahne, and J. Morel, "Lymphotoxin α revisited: general features and implications in rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 13, no. 4, article 232, 2011.

[29] E. Decoster, S. Cornelis, B. Vanhaesebroeck, and W. Fiers, "Autocrine tumor necrosis factor (TNF) and lymphotoxin (LT) α differentially modulate cellular sensitivity to TNF/LT-α cytotoxicity in L929 cells," *Journal of Cell Biology*, vol. 143, no. 7, pp. 2057–2065, 1998.

[30] F. M. De Lima, A. B. Villaverde, R. Albertini et al., "Dual Effect of low-level laser therapy (LLLT) on the acute lung inflammation induced by intestinal ischemia and reperfusion: action on anti- and pro-inflammatory cytokines," *Lasers in Surgery and Medicine*, vol. 43, no. 5, pp. 410–420, 2011.

[31] A. C. Alves, P. T. de Carvalho, M. Parente et al., "Low-level laser therapy in different stages of rheumatoid arthritis: a histological study," *Lasers in Medical Science*, vol. 28, no. 2, pp. 529–536, 2013.

[32] N. Schumm, "Laser blood irradiation for multiple sclerosis: a new treatment procedure with significant improvement of the quality of life," *KIM—Komplementare und Integrative Medizin, Artztezeitschrift fur Naturheilverfahren*, vol. 49, no. 11-12, pp. 38–43, 2008.

[33] S. F. Huang, Y. A. Tsai, S. B. Wu, Y. H. Wei, P. Y. Tsai, and T. Y. Chuang, "Effects of intravascular laser irradiation of blood in mitochondria dysfunction and oxidative stress in adults with chronic spinal cord injury," *Photomedicine and Laser Surgery*, vol. 30, no. 10, pp. 579–586, 2012.

[34] F. Raggi and G. Vallesi, "Intravenous lasertherapy in sportsmedicine, a review of literature," *Photodiagnosis and Photodynamic Therapy*, vol. 5, supplement 1, p. S13, 2008.

[35] A. Wirz-Ridolfi, "Comparison between intravenous and various types of transcutaneous laser blood irradiation," *The Internet Journal of Laserneedle Medicine*, vol. 3, article 1, no. 1, 2013.

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Research Article

The Time Course Effects of Electroacupuncture on Promoting Skeletal Muscle Regeneration and Inhibiting Excessive Fibrosis after Contusion in Rabbits

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The aim of this study was to investigate the longitudinal effects of electroacupuncture (EA) on Zusanli (ST36) and Ashi acupoints in promoting skeletal muscle regeneration and inhibiting excessive fibrosis after contusion in rabbits. Sixty rabbits were randomly divided into four groups: normal, contusion, EA, and recombinant human insulin-like growth factor-I (rhIGF-I). An acute skeletal muscle contusion was produced on the right gastrocnemius (GM) by an instrument-based drop-mass technique. EA was performed for 15 minutes every two days with 0.4 mA (2 Hz), and GM injections were executed with rhIGF-I (0.25 mL once a week). Rabbits treated with EA had a higher T-SOD and T-AOC serum activities and lower MDA serum level, the blood perfusion of which was also significantly higher. In the EA group, the diameter of the myofibril was uniform and the arrangement was regular, contrary to the contusion group. The number and diameter of regenerative myofibers and MHC expression were increased in the EA group. EA treatment significantly decreased fibrosis formation and reduced both GDF-8 and p-Smad2/3 expressions in injured muscle. Our data indicate that EA may promote myofiber regeneration and reduce excessive fibrosis by improving blood flow and antioxidant capacities. Additionally, EA may regulate signaling factor expression after contusion.

1. Introduction

Skeletal muscle injuries are very common, particularly in sports. Simultaneously, muscle strains or contusions cause the majority of such damage. After muscle injuries, a healing process that involves degeneration, inflammation, regeneration, and fibrosis is initiated. Although the process of muscle regeneration is activated shortly after injury, healing can be hindered by the development of fibrosis and affects the degree of recovery. Furthermore, the development of fibrosis predisposes the muscle to further injury [1]. Additionally, incomplete functional recovery also often occurs because of fibrosis after muscle contusions [2].

Currently, treatments with therapeutic ultrasound [3–5], low-energy laser [6, 7], and cell transplantation [8–10] are new approaches for promoting skeletal muscle regeneration. However, these treatments cannot reduce excessive fibrosis according to animal experiments and clinical research. Growth factors, such as basic fibroblast growth factor (bFGF), insulin-like growth factor-I (IGF-I), and nerve growth factor (NGF), improve muscle healing, but the postcontusion healing process remains incomplete [11]. If antifibrosis agents could prevent myofibroblast formation and improve muscle healing (while at the same time antagonizing transforming growth of factor-beta 1 (TGF-β1)), gene therapy and tissue engineering may still be required [11, 12]. Therefore, this strategy will be complex in the clinic. Similarly, although studies also demonstrated that other medications including relaxin, decorin, and losartan can efficiently prevent fibrosis and enhance muscle regeneration [13–15], further research is required before they can be used in the clinic. Furthermore, the costs and side effects of drugs must be considered.

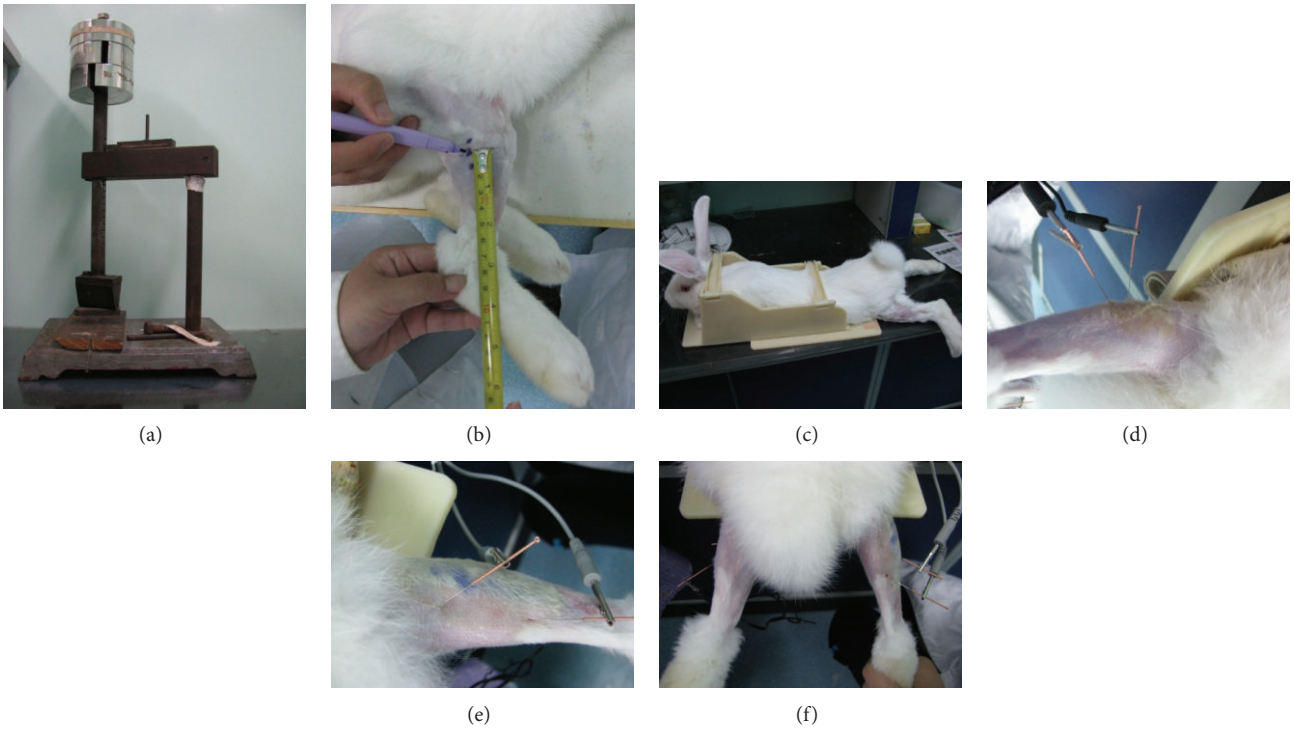


Figure 1: An acute skeletal muscle contusion model was established (a-b). EA treatment after contusion (c–f). (a) The contusion device. (b) The hit position: extending the knee and dorsi-flexing the ankle to 90°. The GM was struck 80 mm away from the rear edge of the calcaneus. The hit kinetic energy was calculated as follows: 7.5 kg × 0.13 m × 9.8 N/kg = 9.555 J. (c) The fixed position of rabbits when starting EA. (d) EA at the normal side ST36: the main needle was inserted into acupoint areas, and the auxiliary needle was placed 5 mm away from the main needle. (e) EA at Ashi acupoints of the injured side, which were located 10 mm from the proximal end (as anode) or the distal end (as cathode) at the contusion midpoint. (f) EA at ST36 and Ashi acupoints.

Complementary medicine, such as acupuncture, has been extensively used in China and other countries. It has unique clinical effects for the treatment of numerous diseases such as gouty arthritis [16], ankle sprain [17], and posttraumatic stress disorder [18]. One of the methods of acupuncture, electroacupuncture (EA), is based on traditional acupuncture but is aided by modern technology. EA shows a better curative effect and a wider range of applications than traditional acupuncture in some aspects [19, 20]. Recently, studies reported that acupuncture has positive influences on wound healing [21, 22] and functional peripheral nerve regeneration [23]. Using mRNA fingerprinting and bioinformatics approaches, Takaoka et al. [24] revealed that EA could promote cell proliferation in skeletal muscles leading to muscle repair. Our preliminary study has also demonstrated that EA not only promoted skeletal muscle regeneration but also reduced excessive fibrosis [25]. It also must be noted that the exact mechanism of EA action for both of these effects is poorly understood.

Therefore, we conducted the following experimental research to determine the mechanism of action. By performing EA on Zusanli and Ashi acupoints, we examined the repair process of acute contusion in rabbits. We observed effects of EA on myofiber regeneration, ultrastructure of injured gastrocnemius (GM), local microcirculation perfusion, oxidative stress levels, collagen deposition, and changes in myosin heavy chain (MHC), growth differentiation factor-8 (GDF-8), and p-Smad2/3. The results obtained provide certain reliable experimental evidence for explaining the effect of EA on the repair of skeletal muscle injury after contusion.

2. Materials and Methods

2.1. Animal Model and Experimental Groups. New Zealand rabbits (either male or female, weight: 2.0 ± 0.2 kg) were administered intravenous anesthesia through the marginal vein of the ear with 3% pentobarbital sodium (30 mg/kg of body weight). The animals were positioned with their right side fixed to the experiment table. The animal's hind limb was positioned by extending the knee and dorsiflexing the ankle to 90° to display the GM. After anesthetizing the animals, blunt injuries were inflicted on the GM at a location 80 mm from the rear edge of the calcaneus with a crushing machine using a drop-mass technique. The injury area was 1 cm^2 and had an energy of 9.555 J [26–28] (Figures 1(a)-1(b)). Before the animals returned to normal activities, we confirmed that the skin was intact and there were no tibia or fibula fractures. Although the anatomy, pathology, and function tests confirmed injury to the GM accompanied

by obvious systolic dysfunction, there were adjacent muscle tissues without injury. This injury is considered an acute severe GM contusion model [29, 30].

This study used 60 rabbits equally randomized into four groups: the normal group ($n = 15$, normal group), the contusion group with no treatment ($n = 15$, contusion group), the EA-treated experimental group ($n = 15$, EA group), and the recombinant human IGF-I- (rhIGF-I-) (PeproTech, NJ, USA) treated experimental group ($n = 15$, rhIGF-I group). Each group was then subdivided into three equal time point groups (days 7, 14, and 28 after contusion, $n = 5$ for each time point). All animals were reared in temperature (23 ± 1°C) and humidity (50 ± 5%) controlled rooms with 12-hour light-dark cycles. The animals had access to food and water ad libitum. All experimental procedures were approved by the Ethical Committee of the Academy of Medical Sciences and were conducted in accordance with international accepted principles for laboratory animal use and care.

2.2. Treatments. To keep the injury site dry and avoid infection, 25 g/L Entoiodine was applied topically once a day. The animals were treated with EA or rhIGF-I at 24 hours after contusion. EA was given for 15 minutes every other day with 0.4 mA (2 Hz). A needle of 0.25 mm in diameter and 25 mm in length (Zhongyan Taihe Medical Instruments Co. Ltd., Beijing, China) was used in this study. The main needle (as anode) was inserted into an acupoint area of the normal side ST36 (according to World Health Organization standards) with 15 mm. Then, the auxiliary needle (as cathode) was placed 5 mm away from the main needle. At the injured side, Ashi acupoints were located 10 mm from the proximal end (as anode) and the distal end (as cathode) at the contusion midpoint and handled with the needle liking ST36 in their acupoint areas. When all of the needles were placed, all of the electrodes were stimulated synchronously with identical parameters using Han's acupoint nerve stimulator (Han's 200E, Nanjing Jisheng Medical Co. Ltd., Jiangsu, China) (Figures 1(c)–1(f)). The rhIGF-I group was treated with 0.1 mg/mL rhIGF-I at a dose of 0.25 mL per rabbit. The rhIGF-I was injected into the GM once a week. The rabbits in the contusion group were allowed to recover naturally from the injury, which together with the normal and rhIGF-I groups received mock EA treatments (with the fixed position and time in the EA group but without EA treatments). The five rabbits from each subgroup were sacrificed on days 7, 14, or 28 after contusion.

2.3. The Evaluation of T-SOD and T-AOC Activities and MDA Level. On day 1 before contusion (as day 0) and days 1, 7, 14, and 28 after contusion blood was collected from the ear central artery and centrifuged. The blood was prepared in accordance with the requirements for each kit (Jiancheng Bioengineering, Nanjing, China). The blood was treated with the xanthine oxidase method for total superoxide dismutase (T-SOD) analysis, the iron reduction method for total antioxidant capacity (T-AOC), and the thiobarbituric acid method for malondialdehyde (MDA). The samples were assayed at 550 nm, 520 nm, and 532 nm using Varioskan Flash (Thermo, MA, USA). The spectrum scan and multifunction meter readings were performed with Plus SkanIt 2.4.3 RE software to record data.

2.4. Local Microcirculation Detection. On days 7, 14, and 28 after contusion, the animals were sedated with intravenous anesthesia, and the dorsal skin of their right hind limbs was opened surgically. Blunt dissection of the fascia tissues was performed, and the GM surfaces were scanned by a Laser Doppler Blood Perfusion Imaging (LDPI) (PeriScan PIM II type, PERIMED, Sweden). The scanning laser wavelength was 670 nm, and an NR scanning pattern with middle scanning accuracy was used. There was a 20 cm distance from the scanning head to the detected object, while the area of the scanning image was approximately 70 (width) × 70 (height) mm^2, resulting in an image with a pixel size of $0.5 \times 0.5\ mm^2$. The time used for scanning one image was 45 seconds, and each area was scanned twice to achieve a mean value. The affiliated online LDPI with a 2.5 image analysis system was used to conduct body blood flow recording, analysis, processing, and storage. Blood flow values from the surface of the GM at the contusion site were then extracted. The blood flow measurement was calculated as perfusion units (PU) in a PERIMED instrument (PU= CMBC (the concentration of measuring the volume inside the blood cells) × V (the average velocity of blood cells)).

2.5. Histological Staining and Quantitative Histological Analysis. Histological analysis in the four groups at various time points after contusion was visualized by hematoxylin-eosin (HE) staining. Regenerative myofibers were distinguished by their centralized nuclei [31]. Nuclei with no discernible surrounding cytoplasm were discarded. The total number of regenerative myofibers within the contusion site was measured in 5 random fields of each sample by using a previously described protocol [32]. To measure the diameter of the regenerative myofibers, the minor axis diameters (the smallest diameter) were measured by Image-Pro Plus Image analysis software (IPP, Version 6.0, Media Cybernetics, USA). For each sample, six sections were randomly selected from the HE sections (original magnification, ×200) for image acquisition. This technique of measuring the smallest diameters of the centronucleated myofibers is a widely used method for evaluating muscle regeneration [33]. We followed a previously established protocol [34], and the diameters of centronucleated myofibers more than 10 μm were consecutively measured in each GM. The prepared sections were observed by an investigator who was blinded to the experiment using light microscopy (ZEISS Scope. AI, Carl Zeiss, Germany).

2.6. Masson Staining and Immunohistochemistry (MHC, GDF-8, and p-Smad2/3). GM samples were fixed in 4% formalin for 3 days before being embedded in paraffin. The blocks were cut into 5 μm sections and were then dewaxed and rehydrated through a graded alcohol series. This protocol is often used for classical Masson staining and immunohistological staining.

The classical Masson staining was used. Briefly, Masson's trichrome staining was performed to quantify collagen content along the zone of injury. This process stained the skeletal muscle fibers red. The collagen is stained slightly green, and the nuclei are stained black. For each sample, six sections were analyzed by IPP, and the percentage of the total collagen-positive area relative to the total cross-sectional area was calculated to estimate fibrosis formation (×200).

Immunohistostaining: after retrieval, the sections were placed in 3% H_2O_2 for 10 minutes at room temperature (RT). After washing, the sections were blocked with blocking solution for 10 minutes at RT. Sections were incubated with the primary antibodies: MHC (1 : 50), GDF-8 (1 : 50), and p-Smad2/3 (1 : 50) rabbit polyclonal antibodies (Santa Cruz, CA, USA) overnight at 4°C. After washing, the sections were incubated at 37°C for 30 minutes with the following antibodies: polymerization HRP conjugated to anti-rabbit IgG (Wuhan Boster Biological Engineering Co. Ltd., China). After washing, the results were detected using a histochemical stain 3′-3′ diaminobenzidine (DAB, Wuhan boster biological engineering Co. Ltd., China) for 10 minutes at RT. Brown staining of the cytoplasm or nucleus was considered a positive result. When all of the staining was completed for each sample, six sections were randomly selected from immunohistochemical (×400) sections for image acquisition. Finally, the mean optical density (MOD) value was calculated by IPP. The prepared sections were observed by an investigator who was blinded to the experiment using light microscopy (ZEISS Scope. AI, Carl Zeiss, Germany).

2.7. Transmission Electron Microscopy (TEM) to Observe the Myofibril Ultrastructure. When the rabbits were sacrificed, a piece of GM was harvested ($1 \times 1 \times 1\,mm^3$). The injured GM tissues were fixed in 5% glutaraldehyde for 1–3 h, washed in buffer, and then fixed in 1% osmic acid with pH adjusted to 7.2–7.4 at 4°C. The tissues were dehydrated with an ethanol gradient and were then put in 100% acetone for 10 minutes. The tissues were then embedded in a mixture of 100% dehydrating agent and equivalent embedding medium (Epon 812) for 60 minutes, before they were finally placed in pure embedding medium overnight at 4°C.

The structures of myofibers were analyzed with an electron microscope (Hitachi Ltd., Japan). Tissue sections of 80 nm were prepared and stained with acetic acid uranium saturated aqueous solution, followed by lead citrate. The tissues were examined for morphology changes such as arrangement rules of myofibrils, position of the sarcomere, sarcomere light band (I line), and dark zones (A line). Any abnormal Z-Lines, skeletal muscle cell membrane, nucleus, mitochondria, sarcoplasmic reticulum, T small tube, satellite cell, and so forth were also observed.

2.8. Statistics. Data from this study are presented as the mean value ± standard deviation. Data from each time point were analyzed with SPSS 13.0 statistical software. The normal distribution was analyzed with single factor analysis of variance. The comparison between groups was performed using the LSD method. Outcomes were evaluated with double-sided inspection. A $P < 0.05$ was considered to have a significant difference.

3. Results

3.1. The Evaluation of Muscle Regeneration after Contusion

3.1.1. A Quantitative Histological Analysis of Muscle Regeneration. We observed that the injured gap was initially filled with a large hematoma and proliferating granulation tissue and that connective scar tissue developed in the injured site within 14 days after contusion. In this study, after contusion regenerative myofibers showed basophilic cytoplasm, and more trachychromatic nuclei were gathered in the central area (Figure 2(a)).

As the contusion healed, the centronucleated regenerative myofibers were replaced by myofibers with a larger diameter and nuclei located at the periphery. In the contusion group, regenerative myofibers were surrounded by a large number of collagen fibers. The number and diameter of collagen fibers were significantly lower than those of the normal group ($P < 0.01$). After the EA treatment, myofiber regeneration occurred and this continued until day 28. The number and diameter of regenerating myofibers were dramatically increased compared to those of the contusion group on day 7 ($P < 0.05$ or $P < 0.01$). However, there were no significant differences observed between the normal and EA groups on day 28 ($P > 0.05$). Additionally, regenerative myofibers were also found in the rhIGF-I group, and their diameter was significantly greater than that of the EA group ($P < 0.01$). However, the number of fibers was lower than that of the EA group ($P < 0.05$ or $P < 0.01$) (Figures 2(b)-2(c)).

3.1.2. The Expression of MHC in Different Groups. MHC is an important structure of myosin filaments in sarcomeres and is responsible for maintaining the structural integrity of myofibers. In the processes of skeletal muscle development and regeneration, MHC in the cytoplasm of the muscular tube marks myofiber regeneration [24]. Therefore, the expression intensity of MHC in the cytoplasm has been used to evaluate myofiber regeneration [25, 26].

We found that MHC expression in normal myofibers rarely existed and that the MOD value of MHC in the contusion group was also markedly higher than the normal group on days 7, 14, and 28 after contusion ($P < 0.01$ or $P < 0.05$). The MOD value of MHC in EA group was significantly higher than that the contusion group ($P < 0.01$) but lower than that the rhIGF-I group ($P < 0.01$) (Figure 3).

3.1.3. Ultrastructure Changes of Skeletal Muscles after Injury in Different Groups. TEM shows ultrastructural changes of myofibers, especially sarcomeres and mitochondrial changes. Sarcomere structure reflects the function of myofibers, and changes of mitochondria show the state of myofibers. In the normal group, GM sarcomeres had a normal structure, which was clear and regular, and the myofilaments were arranged in an orderly fashion. There was a small amount of mitochondria between myofibrils, and the sarcoplasmic

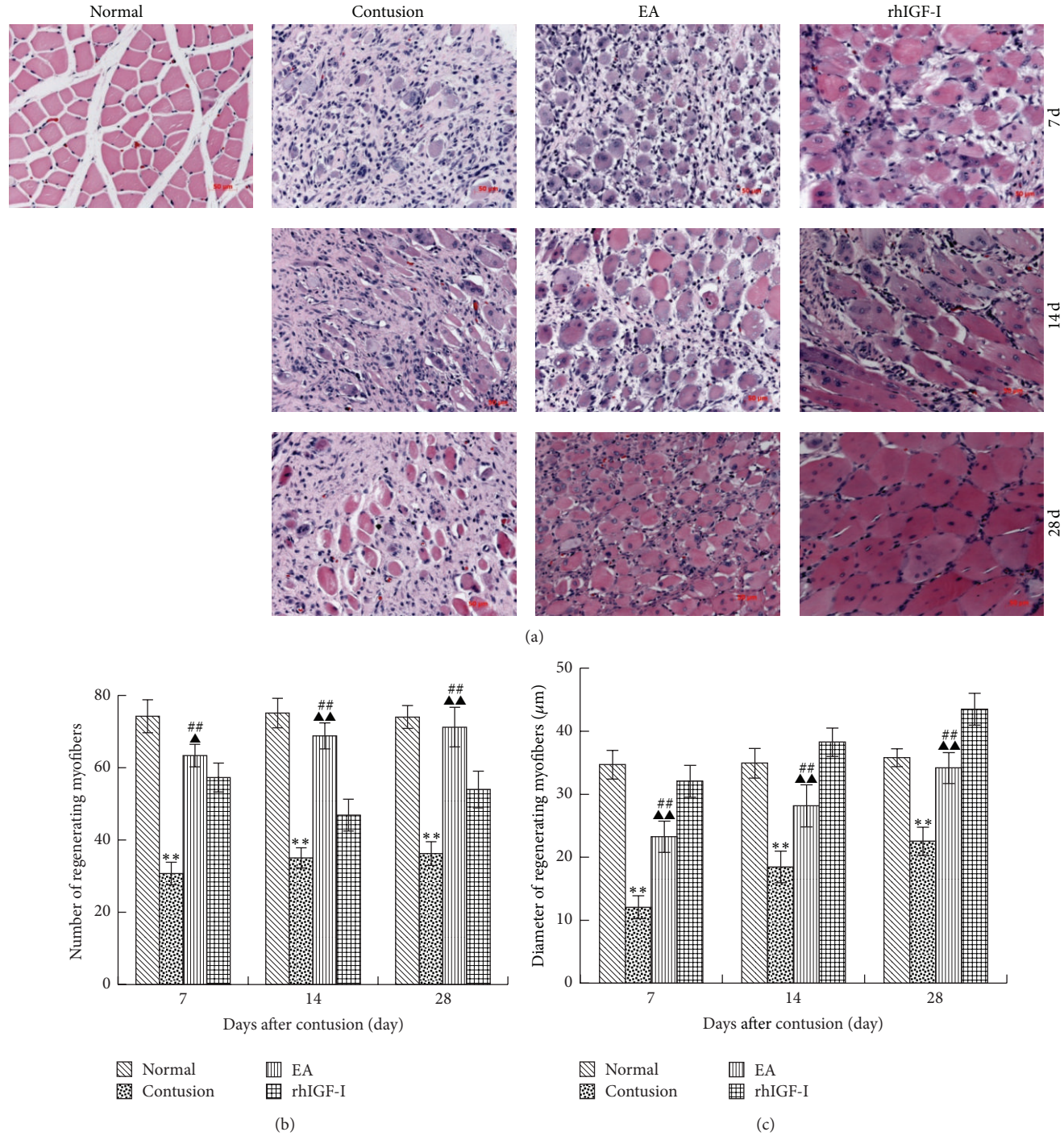


Figure 2: BBHistological analysis of the four groups at different time points after contusion was visualized by HE staining (×200) (a). A comparison of the number (b) and diameter (c) of regenerative myofibers in the different groups on days 7, 14, and 28 after contusion. The cytoplasm of regenerative myofibers was apparently basophilic, and more nuclei were located in the central area. In the contusion group, regenerative myofibers were surrounded by a mass of collagen fibers with small diameter. On day 7 in the EA group, the regeneration was stronger than that in the contusion group, but in the rhIGF-I group, the diameter of regenerative myofibers was wider than that in the normal group on day 28. In the contusion group, the number and diameter of regenerative myofibers were less than those of the normal group. The number in the EA group was markedly more than the contusion and rhIGF-I groups ($P < 0.01$ or $P < 0.05$). The diameter of fibers in the EA group was wider than that in the contusion group ($P < 0.01$) but narrower than that in the rhIGF-I group ($P < 0.01$). Contusion versus normal, $^{\star}P < 0.05$, $^{\star\star}P < 0.01$; EA versus contusion, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; and EA versus rhIGF-I, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ ($n = 5$).

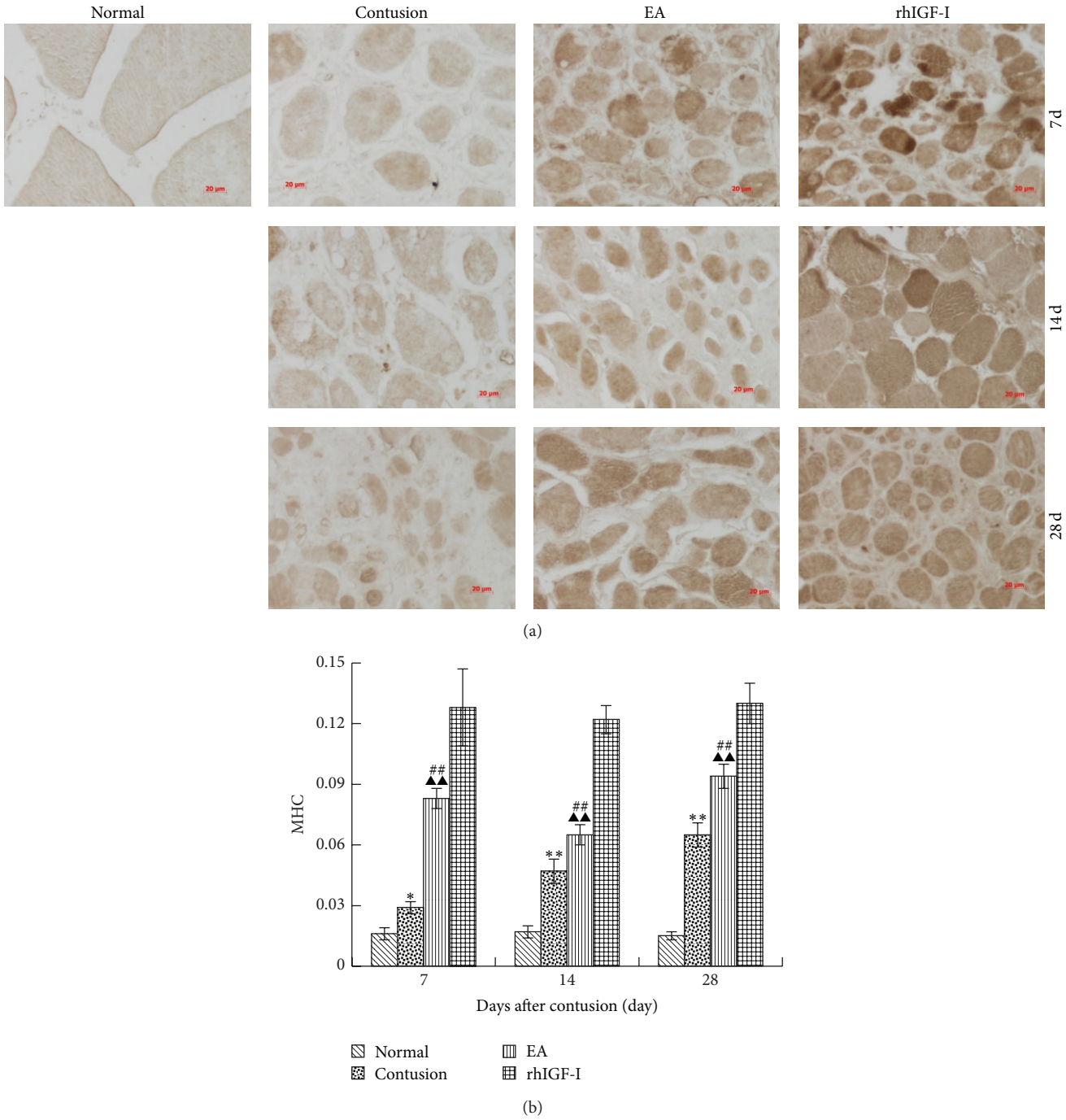


Figure 3: DAB staining of MHC on days 7, 14, and 28 after contusion (×400) (a). The comparison of the MHC levels on days 7, 14, and 28 post-contusion (b). Positive expression of MHC in the cytoplasm of regenerative myofibers stains brown. MHC staining was weak in the normal group and weaker in the contusion group. MHC staining in the EA and rhIGF-I groups was enhanced, particularly in the rhIGF-I group. In the contusion group, the MHC level was significantly higher than that in the normal group ($P < 0.01$ or $P < 0.05$), while the EA group was dramatically higher than the contusion group ($P < 0.01$) but lower than the rhIGF-I group ($P < 0.01$). Contusion versus normal, $^{\star}P < 0.05$, $^{\star\star}P < 0.01$; EA versus contusion, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; and EA versus rhIGF-I, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ ($n = 5$).

reticulum distribution was regular with an integrated muscle membrane. The number of satellite cells located in the basement membrane was reduced, and they had a spindle shape with a small amount of cytoplasm. On day 7 after contusion, the structure of myofibrils and sarcomeres in the injured groups was disordered and the Z-Line, mitochondria, and sarcoplasmic reticulum were abnormal. The satellite cells were activated after injury. In the contusion group, the Z-Line was vague and had obvious streaming. The swelling of the mitochondria and sarcoplasmic reticulum was uniform

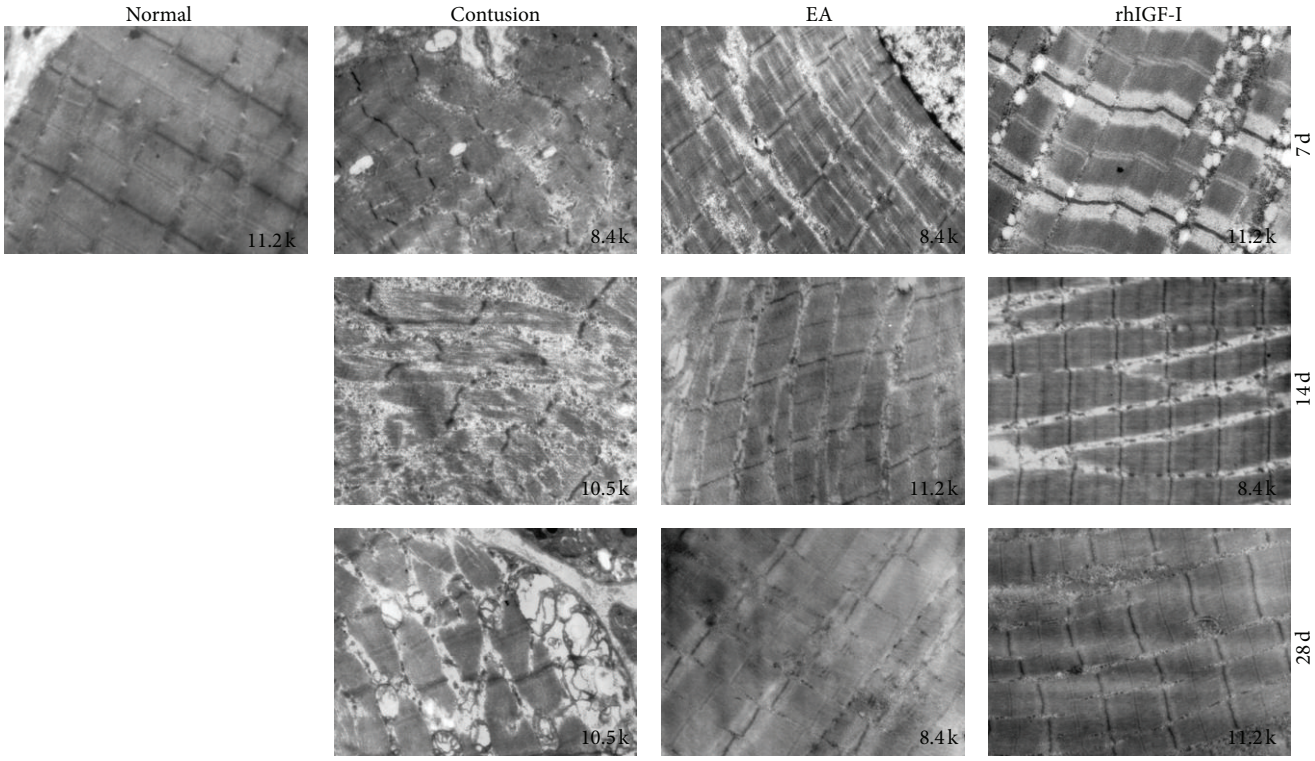


Figure 4: The ultrastructure of GM on days 7 and 14, and after contusion. On day 7 post-contusion, the structure of myofibrils and sarcomeres in the contusion groups was disordered. In the contusion group, the Z-Line was vague and streaming. The swelling of the mitochondria and sarcoplasmic reticulum was uniform in the rhIGF-I group and the number of both organelles increased. On day 14 after contusion, myofibrils were small and the diameter was different in the contusion group. In the EA and rhIGF-I groups, the arrangement between myofibrils appeared like the connection of joints: the Z-Line was clear and regular and the diameter of myofibrils was uneven. On day 28 post-contusion, the Z-Lines disappeared in some myofibrils in the contusion group and some were atrophic. The mitochondria gathered under the sarcolemma like a large bubble. The structures of the myofibril, sarcomere, and Z-Line were regular and mitochondria returned to normal in both the EA and rhIGF-I groups.

in the rhIGF-I group and both were increased in number. On day 14 after contusion, the number of swelling mitochondria in the injured groups decreased. In the contusion group, myofibrils were small and the diameters were different. In the EA and rhIGF-I groups, the arrangement between myofibrils appeared like a connection of joints, with the Z-Line being clear and regular. However, the diameter of myofibrils was uneven. On day 28 after contusion, Z-Lines disappeared in some myofibrils in the contusion group and some were atrophic with mitochondria gathered under the muscle membrane like a bubble. The structures of the myofibril, sarcomere, and Z-Line were regular, and mitochondria returned to normal in the EA and rhIGF-I groups (Figure 4).

3.2. EA Influences the Microcirculation of the GM and Antioxidant Ability

3.2.1. Microcirculation Changes of GM after Injury. LDPI can detect microcirculation status after a direct stress reaction and has been widely used in clinical diagnosis, evaluation, and research associated with microcirculation such as acupuncture [35] and skeletal muscle microcirculation [36]. We measured the number of perfusion units in this study: PU = CMBC (the concentration of measuring the volume inside the blood cells) × V (the average velocity of blood cells).

The PU values of the contusion groups were significantly lower ($P < 0.01$) than those of the normal group on days 7, 14, and 28 after contusion. The EA group was apparently more than the contusion group ($P < 0.01$) but significantly lower than the rhIGF-I group ($P < 0.05$ or $P < 0.01$). As the process of repair continued, the blood perfusion in the EA and rhIGF-I groups increased (Figure 5).

3.2.2. The Expression of T-SOD, T-AOC, and MDA in Different Groups. To investigate the protective effects of EA in oxidative stress responses after contusion, we examined the activities of serum T-SOD, T-AOC, and MDA. On day 1 after contusion, the activities of T-SOD and T-AOC decreased significantly and were lower than that of the normal group ($P < 0.01$). However, the MDA group showed the opposite results (Figure 6).

During muscle injury repair, T-SOD and T-AOC activities in each contusion group increased gradually and returned to normal by 28 days. Conversely, serum MDA level gradually decreased to normal levels. On days 7, 14, and 28 after contusion, T-SOD and T-AOC activities in the EA group were

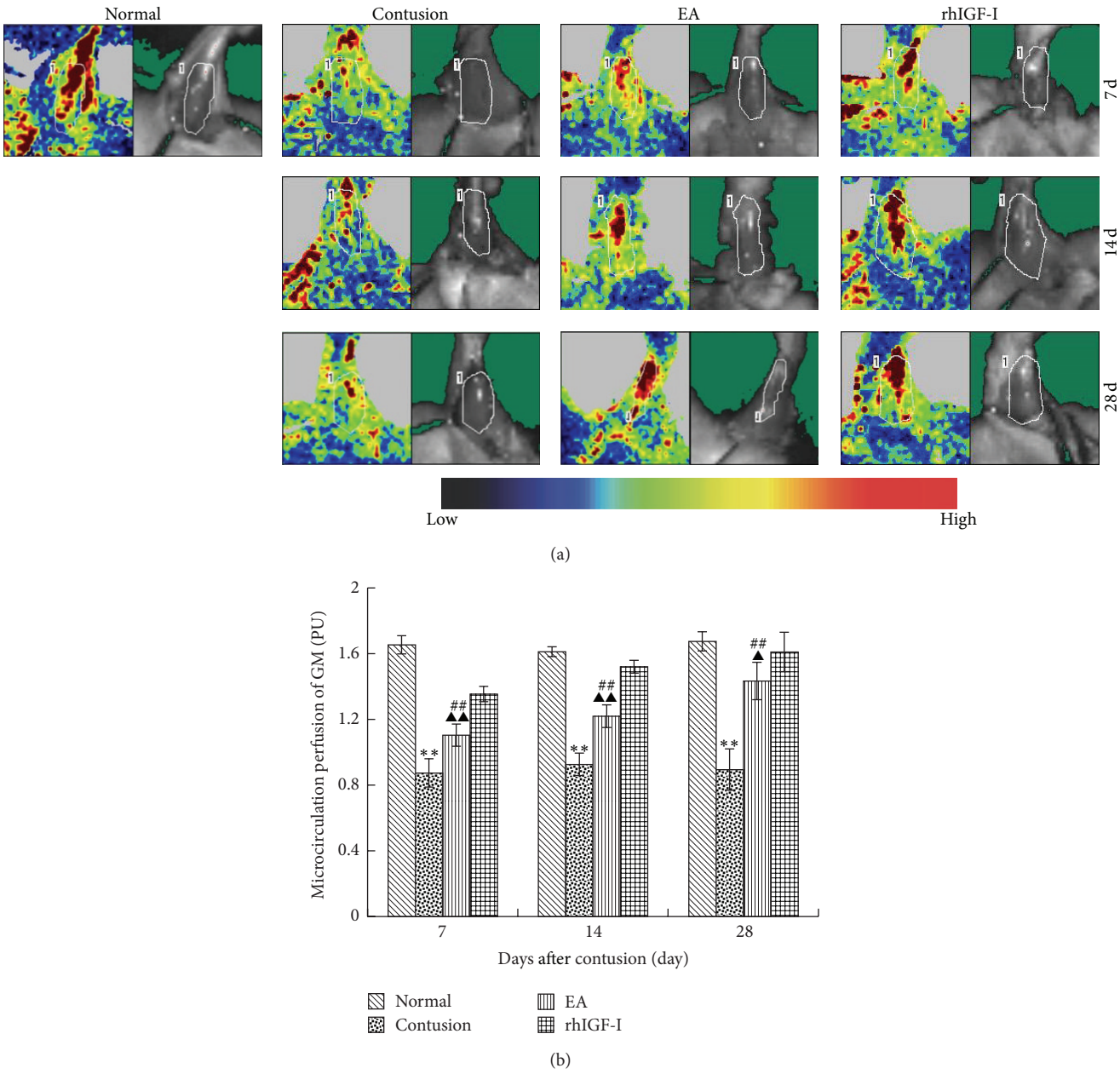


Figure 5: Repeated use of LDPI at the contusion site in different groups after contusion (a). Comparisons of the microcirculation perfusion of GM on days 7, 14, and 28 after contusion (b). In the contusion group, the contusion area was mainly blue and green, with only a small amount of yellow and red. On day 7 in the EA and rhIGF-I groups the images were mostly green, yellow, and red. The red area was expanded on days 14 and 28. The microcirculation perfusion in the contusion group was markedly lower than that in the normal group ($P < 0.01$). The EA group was apparently more than the contusion group ($P < 0.01$) but significantly lower than the rhIGF-I group ($P < 0.05$ or $P < 0.01$). Contusion versus normal, $^{*}P < 0.05$, $^{**}P < 0.01$; EA versus contusion, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; and EA versus rhIGF- I, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ ($n = 5$).

significantly higher than those of the contusion group ($P < 0.01$) but lower than those of the rhIGF-I group ($P < 0.01$ or $P < 0.05$) on day 7. There were few differences between the EA and rhIGF-I groups on day 14 ($P > 0.05$). On day 28, the EA group was significantly higher compared to the rhIGF-I group ($P < 0.01$). On days 7 and 14 after contusion, MDA level in the EA group was significantly lower than that in the contusion group ($P < 0.05$) but higher than that in the rhIGF-I group ($P < 0.05$) (Figure 6).

3.3. EA Influences the Density of Collagen Fibers, GDF-8, and p-Smad2/3

3.3.1. Density of Collagen Fibers in Different Groups. We examined the extent of fibrosis of the local extracellular matrix (ECM) after contusion. Masson trichrome staining was performed to detect the ratio of the fibrotic area to the total cross-sectional area of the muscle. There were small green stained collagen fibers around normal myofibers.

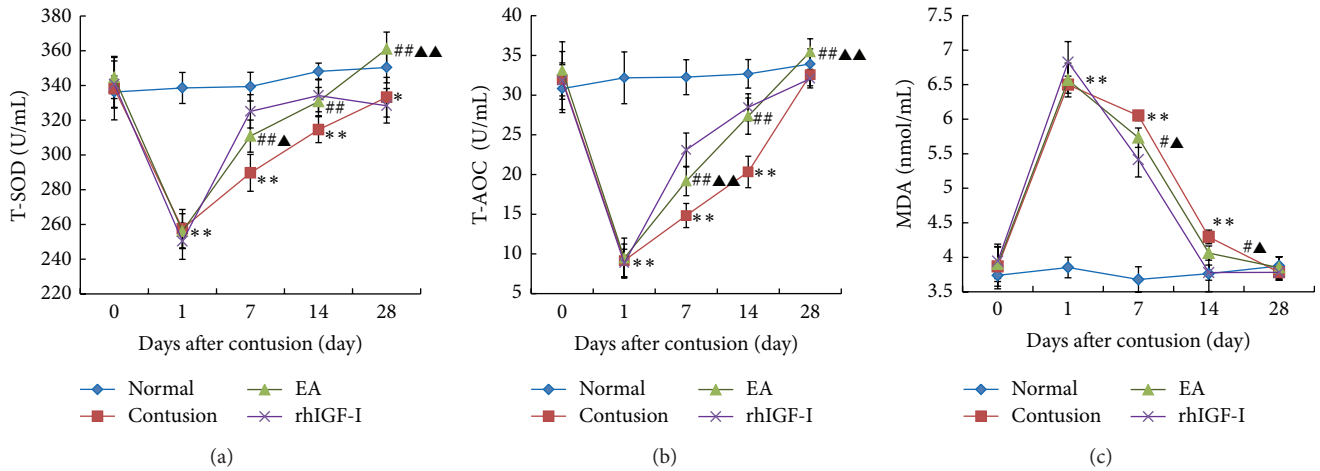


Figure 6: Comparisons of serum T-SOD (a), T-AOC (b), and MDA (c) before 1 day as 0 d and after 1d, 7d, 14d, and 28d contusion. After contusion, the activities of the T-SOD and T-AOC dramatically decreased, while the level of MDA sharply increased. The serum levels gradually returned to normal. On days 7, 14, and 28 after contusion, T-SOD and T-AOC activities in the EA group were significantly higher than those of the contusion group ($P < 0.01$) but lower than those of the rhIGF-I group ($P < 0.01$ or $P < 0.05$) on day 7. On day 28, the EA group was significantly higher compared to the rhIGF-I group ($P < 0.01$). On days 7 and 14 after contusion, MDA level in the EA group was significantly lower than that in the contusion group ($P < 0.05$) but higher than that in the rhIGFI group ($P < 0.05$). Contusion versus normal, $^{*}P < 0.05$, $^{**}P < 0.01$; EA versus contusion, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; and EA versus rhIGF-I, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ ($n = 5$).

During the repair process, the green collagen fibers around red dyed myofibers filled the area outside muscle cells. On day 7 after contusion, collagen fibers were deposited around the myofibers. On days 7, 14, and 28 after contusion, the density of collagen fibers in the contusion group was significantly higher than that in the normal and EA groups ($P < 0.01$). Additionally, the EA group had more fibers than the rhIGF-I group ($P < 0.01$) (Figure 7).

3.3.2. The Expression of GDF-8 and p-Smad2/3 in Different Groups. To analyze the mechanism how EA inhibits excessive ECM fibrosis, we investigated the GDF-8/p-Smad2/3 signaling pathway. We found that on days 7, 14, and 28 after contusion the protein expression levels of GDF-8 and p-Smad2/3 in the contusion group were significantly higher than those in the normal group ($P < 0.01$). The EA group was significantly higher than the contusion and rhIGF-I groups ($P < 0.01$). This trend was consistent with the changes in the density of collagen fibers (Figures 8 and 9).

4. Discussion

Acupuncture has a long history of use in China and throughout Asia. The curative effects of acupuncture are not well known, which limits its use globally. Therefore, it is very important to define the benefits of acupuncture. As one of the modern methods of acupuncture, EA uses the acupuncture needle as an electrode to apply low-frequency stimulation. Evidence suggests that EA is safe and effective in a wide variety of diseases such as polycystic ovary syndrome [37], dysmenorrhea [38], and autism spectrum disorder [39].

Skeletal muscle is crucial for structural support, movement, and function. One of the most common causes of muscle contusion is the impact of a nonpenetrating object [40]. Once the contusion injury occurs, the process of healing is activated and myofibers have the ability to regenerate through the activation of satellite cells [40]. However, fibrosis formation can ultimately impair the muscle healing process due to the accumulation of excessive collagen. Fibrosis can even become a self-perpetuating process, and excessive fibrosis inhibits regeneration of myofibers [41].

Based on previous studies, we investigated the mechanism of EA effects on promoting skeletal muscle regeneration and inhibiting excessive fibrosis after contusion. This study observed the healing process of rabbit GM after contusion and showed that EA treatment could significantly increase the number and diameter of regenerative myofibers and improve MHC level. Additionally, the arrangement of myofilaments within the myofibril and Z-Line distribution appeared to normalize when analyzed by TEM. The contusion group had myofibril fracture and atrophy. This result indicates that EA not only could promote myofiber regeneration but also improve the restoration of myofibril and sarcomere structure.

The natural healing area contained regions of fibrosis with a large number of collagen fibers tightly surrounding the myofibers and evidence of myofiber atrophy. The EA treatment could effectively relieve fibrosis and reduce myofiber atrophy. The surrounding tissues and blood vessels were also damaged so the supply with nutrients to nerves and myofibers was obstructed. This obstruction caused secondary damage [42], and blood circulation disturbances are an important cause of enhanced ECM fibrosis and reduced myofiber regeneration. Thus, proper blood perfusion is essential to heal injured muscle [43]. Previous studies reported that electroacupuncturing the ST36 [44] or Ashi acupoints [45] improved blood flow. We assessed microcirculation changes of GM after injury and found that the EA group was

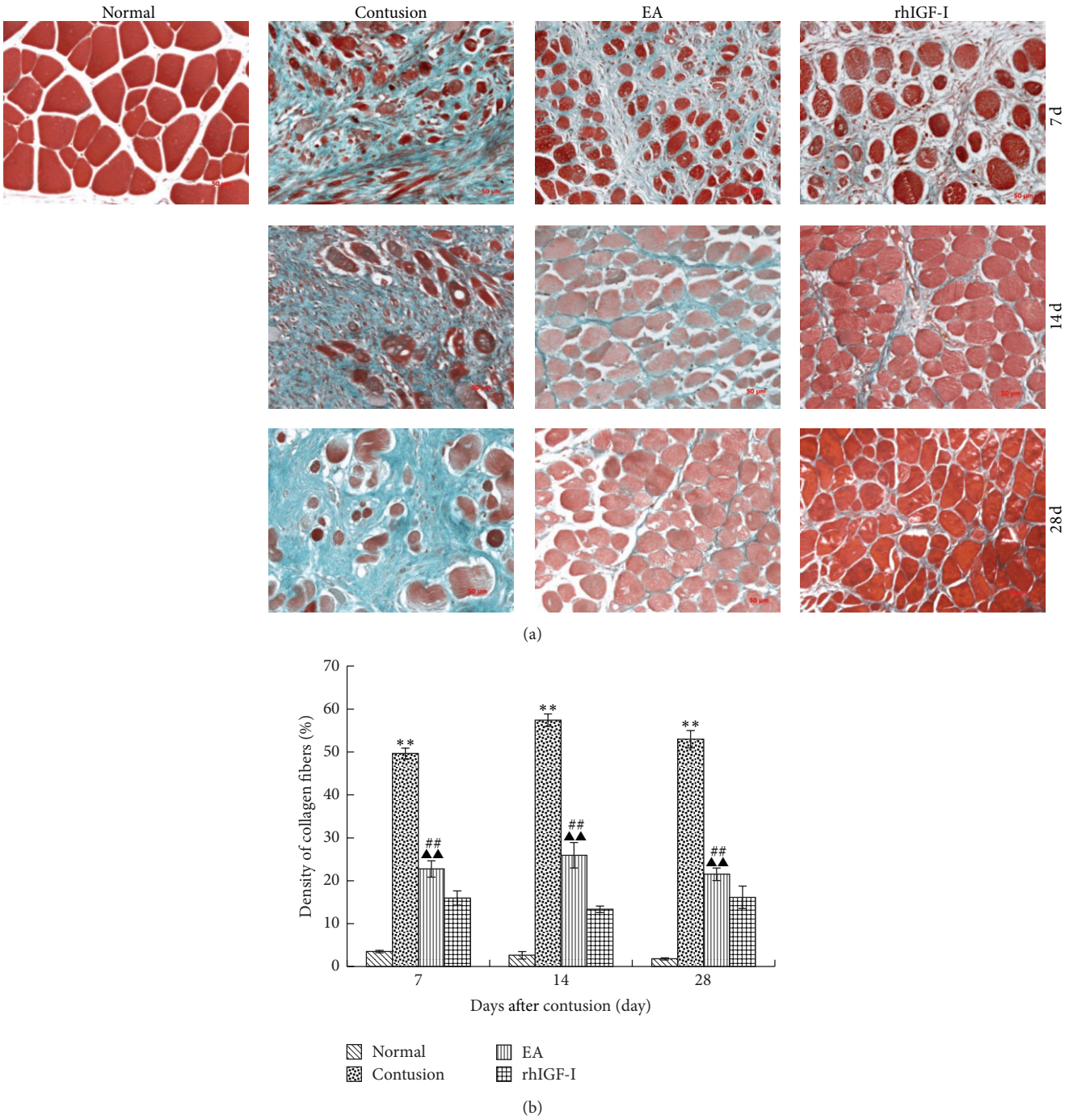
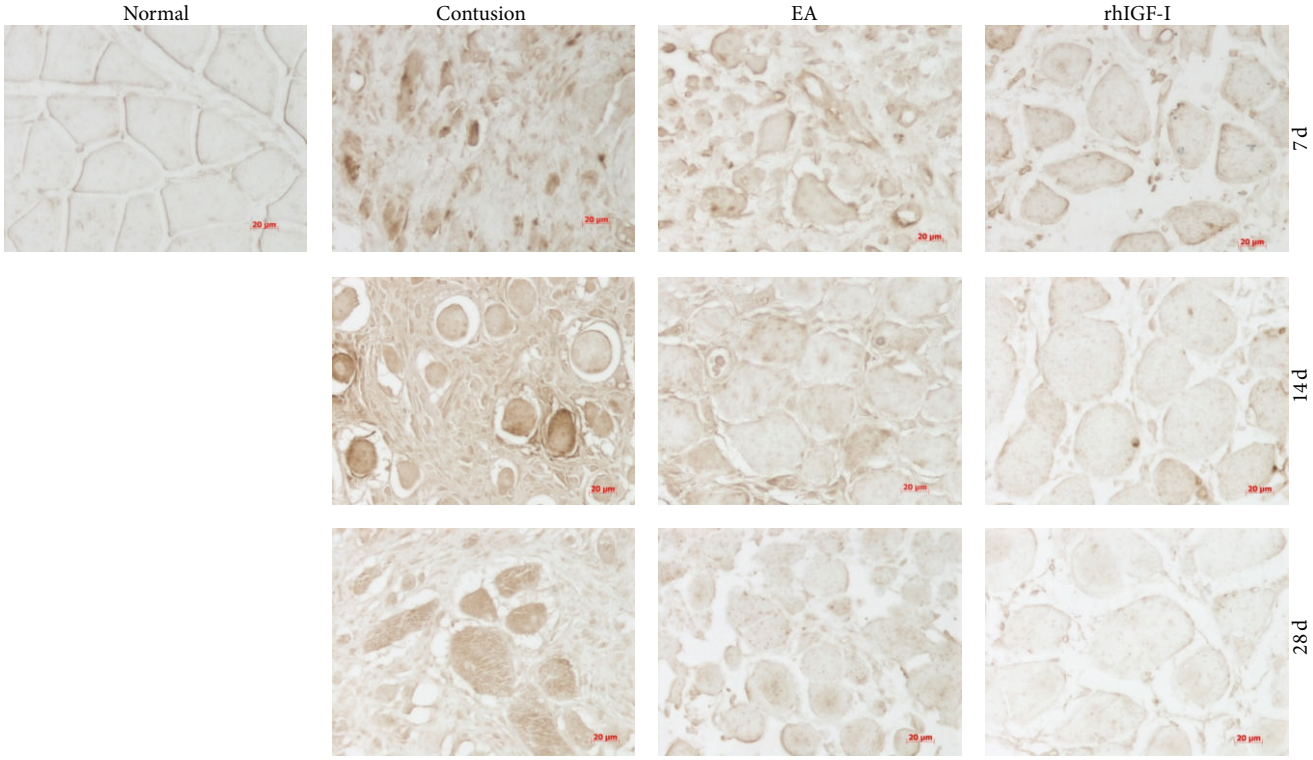


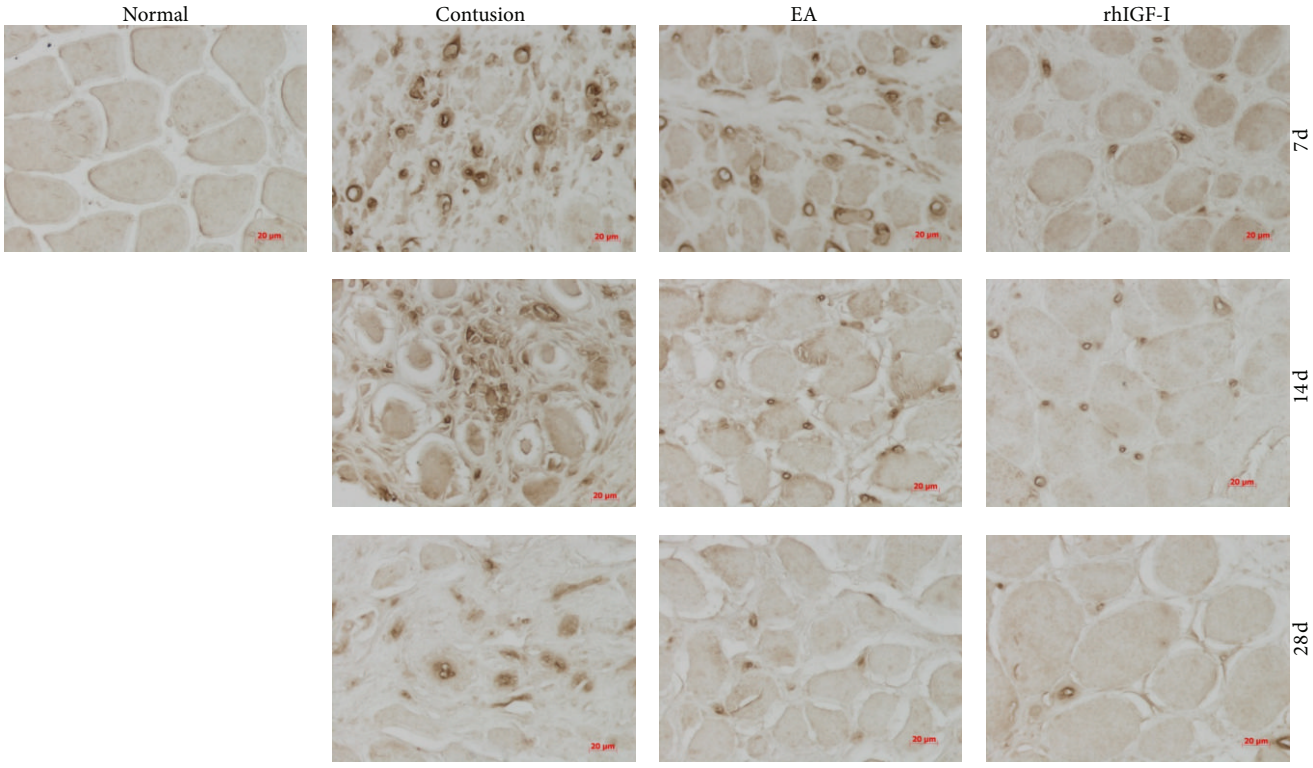
Figure 7: Collagen fibers distribution on days 7, 14, and 28 after contusion among different groups (×200) (a). By using Masson's trichrome staining, we confirmed that a large amount of collagen was deposited in the contusion site. The collagen levels were significantly decreased in both the EA and rhIGF-I groups. Masson's trichrome staining showed myofibers (red) and collagen (green) among different groups. The density of collagen (b) in the contusion was higher than that in the normal group ($P < 0.01$). The collagen in the EA group was lower than that in the contusion group ($P < 0.01$) but markedly higher than the rhIGF-I group ($P < 0.01$). Contusion versus normal, $^{\star}P < 0.05$, $^{\star\star}P < 0.01$; and EA versus contusion, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; EA versus rhIGF-I, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ ($n = 5$).

more effectively repaired than the contusion group. Additionally, there were significant differences between the EA and contusion groups. Our data demonstrated that electroacupuncturing the ST36 and Ashi acupoints improved the velocity of blood flow and promoted the absorption of hematomas. These improvements may be related to the overall effect.

Contusion can damage the integrity of plasma membranes and the basilar membrane of the skeletal muscle fibers. The damage causes extracellular calcium ion flux



(a)



(b)

Figure 8: GDF-8 (a) and p-Smad2/3 (b) staining on days 7, 14, and 28 after contusion (×400). GDF-8 in the cytoplasm and p-Smad2/3 in the nucleus appear brown. The staining was weak in the normal group and obvious in the contusion group. The staining was weaker in the EA and rhIGF-I groups, especially on day 28.

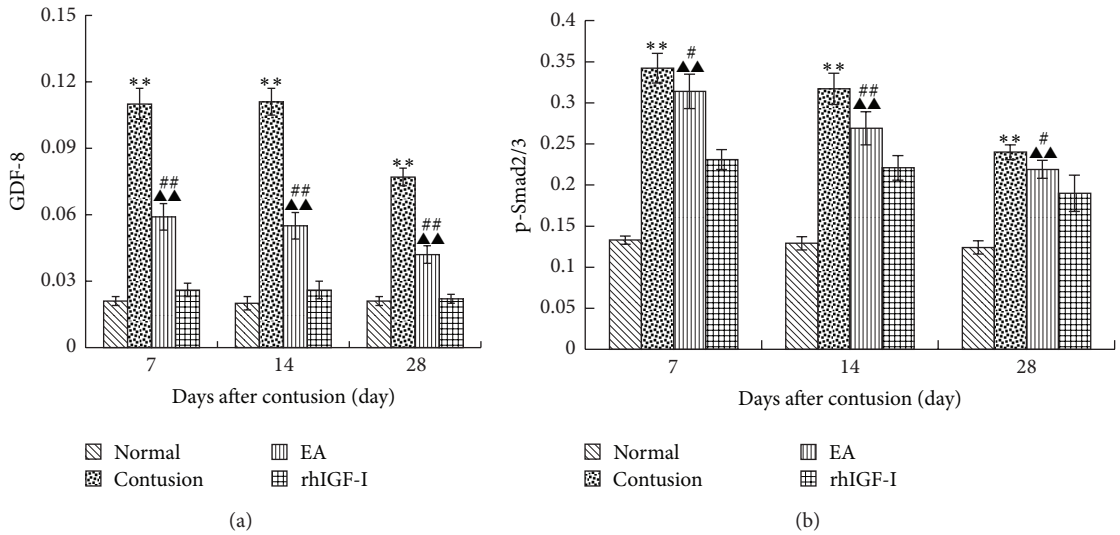


Figure 9: Comparisons of GDF-8 (a) and p-Smad2/3 (b) expression in different groups on days 7, 14, and 28 after contusion. The density of collagen and expression of GDF-8 and p-Smad2/3 in the contusion group were higher than those in the normal group ($P < 0.01$). The EA group had less expression than the contusion group ($P < 0.05$ or $P < 0.01$) but markedly higher expression than the rhIGF-I group ($P < 0.01$). Contusion versus normal, $^{\star}P < 0.05$, $^{\star\star}P < 0.01$; and EA versus contusion, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; EA versus rhIGF- I, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ ($n = 5$).

and local hematoma formation, activation of neutrophils and macrophages, and induction of "respiration burst." The respiration burst results in the formation of superoxygen ions. The oxygen free radicals damage large cellular molecules such as lipids, proteins, and DNA, ultimately leading to cell death and causing further damage of skeletal muscle [46]. Therefore, antioxidants could effectively reduce tissue fibrosis [47] and be beneficial to myofiber structure reconstruction [48] by preventing myofiber atrophy [49].

Electro-acupuncturing the ST36 acupoint protects the body through antioxidation. EA protects the hypothalamus, the liver, red blood cells [50], and the substantia nigra striatum [51], and so forth. T-AOC consists of enzymatic and nonenzymatic antioxidant defense systems and reflects the body's ability to regulate antioxidants and scavenge free radicals. Low levels of T-AOC can reduce antioxidant enzyme synthesis in the body or utilize the nonenzymatic antioxidants to control excessive reactive oxygen species (ROS) generated over the oxidation/antioxidation equilibrium. EA increased the T-AOC level in injured tissues, playing a protective role [52]. T-SOD is the main antioxidant enzyme within skeletal muscle, and it can remove free radicals and peroxides produced by tissues and cells metabolism, protecting cells against oxidative stress damage [53]. In this study, on day 1 after contusion, the activities of serum T-AOC and T-SOD decreased significantly and then increased gradually. The decreased level of serum T-SOD in our experiment might be due to the inhibition or oxidative inactivation of enzyme proteins in the serum caused by excess generation of ROS [54]. The activities of serum T-AOC and T-SOD in the EA group returned to normal gradually and were higher than those of the contusion group. The enhancement of T-AOC may increase the resistance to oxidative cellular injury or facilitate the biosynthesis in cells which receive sublethal injury [55]. These possibilities are consistent with the results of our experiments. EA could also reduce undesired oxidation by infiltrating cells and/or could facilitate tissue repair. MDA is one of the major secondary oxidation products derived from polyunsaturated fatty acids [56], so the detection of MDA level can reflect the degree of lipid peroxidation in the body and the degree of cell injury. We found that the increase of MDA level could directly reflect oxidative damage of myofibers on day 1 after contusion. After EA treatment, MDA level decreased significantly. This result shows that the removal of oxygen free radicals increased because EA improved the circulation. The improved circulation protects the cell membrane from the attack of oxygen free radicals and reduces the generation of lipid peroxidation products.

Oxidative stress causes a catastrophic cycle of mitochondrial DNA (mtDNA) damage and functional decline. This creates further reactive oxygen species generation and cellular injury [57]. This study confirmed that EA improved mitochondrial function in injured tissues [58], and electro-acupuncturing the ST36 acupoint [59] relieved the swelling of mitochondria. On day 28, the contusion group showed mitochondrial changes accompanied by myofibril fracture and atrophy. However, the EA or rhIGF-I treatment groups did not show a similar phenomenon. This phenomenon might be related to the GM microcirculation perfusion inadequacy because the contusion group had mitochondria swelling. Therefore, EA activated blood circulation and gradually improved microcirculation in the skeletal muscle after contusion. The improved circulation increased oxygen radical scavenging ability and improved the antioxidant ability. Additionally, increased circulation could also help improve injured tissue energy supplies, reduce oxidative

stress damage, relieve cellular infiltration, protect sarcomere structure, and eventually reduce fibrosis. These properties are advantageous to myofibril regeneration.

Fibroblasts were activated after injury of skeletal muscle and secreted collagen fibers for several weeks [60]. Collagen fibers in the early phase of wound healing have a positive effect and can increase the tensile strength of the wound and allow the wound on both ends of the fibers to attach to the skeleton [61]. As the repair is completed, the earliest granulation tissues are replaced by scar tissue (mainly I type collagen) and remain constant for a long time [62]. The scar tissue is an obstacle to new fibers and inhibits skeletal muscle regeneration [11]. Previous studies have shown that excessive fibrosis of ECM was an important factor that impaired myofiber regeneration [33]. Therefore, we speculated that EA could promote myofiber regeneration by inhibiting excessive fibrosis. Thorsteinsdottir et al. [63] indicated that degrading ECM and reducing fibrosis were advantageous to satellite cell migration and differentiation and promoted skeletal muscle regeneration. In organ fibrosis research, electro-acupuncturing the ST36 alleviated renal failure-induced glomerulosclerosis, tubulointerstitial fibrosis [64], and carbon tetrachloride-induced hepatic fibrosis [65]. To investigate whether EA can reduce ECM fibrosis of skeletal muscle after contusion, this study observed the fibrosis formation process in the damaged region repeatedly on days 7, 14, and 28. The results showed that fibrosis in the contusion group was obvious, but the EA group inhibited collagen fiber deposition from the 7th day and had significantly reduced overall fibrosis.

To investigate the mechanism of how EA inhibits ECM fibrosis, we analyzed effects of EA on the GDF-8/p-Smad2/3 signaling pathway. GDF-8 as one member of the TGF-β superfamily [66] is involved in accelerating the deposition of ECM by increasing the synthesis of ECM proteins and inhibiting ECM degradation [40]. It is also a negative regulator of skeletal muscle growth [66], and its mRNA and protein are highly expressed in degenerating skeletal muscle and connective tissues [67]. GDF-8 also inhibits the self-renewal of muscle satellite cells [68] by delaying myogenin expression [69] and enhancing fibrosis through the activation of the typical TGF-β/Smad signaling pathway [70]. p-Smad2/3 are profibrotic factors linked to GDF-8 in the nucleus where it induces the process of deposition of ECM fibrosis [71]. GDF-8 also has a regulatory role in the proliferation of dystrophic muscle fibroblasts [41] and myoblast differentiation [72]. TGF-β is required to activate the Smad2/3 signal transduction pathway because the effect could be blocked using a TGF-β neutralizing antibody [73]. On days 7, 14, and 28 after contusion, EA could significantly inhibit GDF-8 and p-Smad2/3 protein expression levels. EA not only reduced fibrosis by inhibiting the TGF-β/Smad signaling pathway but also improved myofiber regeneration by inhibiting GDF-8.

In conclusion, these results suggest that EA promotes skeletal muscle regeneration and inhibits excessive fibrosis after contusion, and the mechanism may be associated with several factors. These factors include its effect on improving local microcirculation perfusion with promoting blood circulation to remove blood stasis. Additionally, EA increased the ability to resist oxidative stress, which protected skeletal muscle from secondary injury. EA also inhibited ECM fibrosis and provided a good environment for skeletal muscle regeneration by enhancing collagen degradation ability and inhibiting the TGF-β/Smad signaling pathway. And some functional assessments would be added in the further design to reveal the significance of EA treatments on muscle strength and electrophysiology after contusion.

Authors' Contribution

Rongguo Wang, Dan Luo, and Cheng Xiao equally contributed to this work as joint first authors.

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References

[1] T. M. Best, B. Gharaibeh, and J. Huard, "Stem cells, angiogenesis and muscle healing: a potential role in massage therapies?" *The British Journal of Sports Medicine*, vol. 47, no. 9, pp. 556–560, 2013.

[2] S. Terada, S. Ota, M. Kobayashi et al., "Use of an antifibrotic agent improves the effect of platelet-rich plasma on muscle healing after injury," *The Journal of Bone and Joint Surgery (American Volume)*, vol. 95, no. 11, pp. 980–988, 2013.

[3] L. S. Freitas, T. P. Freitas, P. C. Silveira, L. G. Rocha, R. A. Pinho, and E. L. Streck, "Effect of therapeutic pulsed ultrasound on parameters of oxidative stress in skeletal muscle after injury," *Cell Biology International*, vol. 31, no. 5, pp. 482–488, 2007.

[4] E. S. C. Koh and E. G. McNally, "Ultrasound of skeletal muscle injury," *Seminars in Musculoskeletal Radiology*, vol. 11, no. 2, pp. 162–173, 2007.

[5] J. B. Woodhouse and E. G. McNally, "Ultrasound of skeletal muscle injury: an update," *Seminars in Ultrasound, CT and MRI*, vol. 32, no. 2, pp. 91–100, 2011.

[6] R. Lubart, M. Eichler, R. Lavi, H. Friedman, and A. Shainberg, "Low-energy laser irradiation promotes cellular redox activity," *Photomedicine and Laser Surgery*, vol. 23, no. 1, pp. 3–9, 2005.

[7] A. C. Amaral, N. A. Parizotto, and T. F. Salvini, "Dose-dependency of low-energy HeNe laser effect in regeneration of skeletal muscle in mice," *Lasers in Medical Science*, vol. 16, no. 1, pp. 44–51, 2001.

[8] R. Peçanha, L. L. Bagno, M. B. Ribeiro et al., "Adipose-derived stem-cell treatment of skeletal muscle injury," *The Journal of Bone and Joint Surgery (American Volume)*, vol. 94, no. 7, pp. 609–617, 2012.

[9] P. von Roth, G. N. Duda, P. Radojewski, B. Preininger, C. Perka, and T. Winkler, "Mesenchymal stem cell therapy following muscle trauma leads to improved muscular regeneration in both male and female rats," *Gender Medicine*, vol. 9, no. 2, pp. 129–136, 2012.

[10] P. von Roth, G. N. Duda, P. Radojewski et al., "Intra-arterial MSC transplantation restores functional capacity after skeletal muscle trauma," *The Open Orthopaedics Journal*, vol. 6, pp. 352–356, 2012.

[11] J. Huard, Y. Li, and F. H. Fu, "Muscle injuries and repair: current trends in research," *Journal of Bone and Joint Surgery A*, vol. 84, no. 5, pp. 822–832, 2002.

[12] L. Satish, P. H. Gallo, M. E. Baratz, S. Johnson, and S. Kathju, "Reversal of TGF-1stimulation of -smooth muscle actin and extracellular matrix components by cyclic AMP in Dupuytren's—derived fibroblasts," *BMC Musculoskeletal Disorders*, vol. 12, article 113, 2011.

[13] X. Mu, M. L. Urso, K. Murray, F. Fu, and Y. Li, "Relaxin regulates MMP expression and promotes satellite cell mobilization during muscle healing in both young and aged mice," *The American Journal of Pathology*, vol. 177, no. 5, pp. 2399–2410, 2010.

[14] K. Fukushima, N. Badlani, A. Usas, F. Riano, F. H. Fu, and J. Huard, "The use of an antifibrosis agent to improve muscle recovery after laceration," *The American Journal of Sports Medicine*, vol. 29, no. 4, pp. 394–402, 2001.

[15] B. Gharaibeh, Y. Chun-Lansinger, T. Hagen et al., "Biological approaches to improve skeletal muscle healing after injury and disease," *Birth Defects Research C*, vol. 96, no. 1, pp. 82–94, 2012.

[16] W. B. Lee, S. H. Woo, B. I. Min, and S. H. Cho, "Acupuncture for gouty arthritis: a concise report of a systematic and meta-analysis approach," *Rheumatology*, vol. 52, no. 7, pp. 1225–1232, 2013.

[17] J. Park, S. Hahn, J. Y. Park, H. J. Park, and H. Lee, "Acupuncture for ankle sprain: systematic review and meta-analysis," *BMC Complementary and Alternative Medicine*, vol. 13, article 16, 2013.

[18] Y. D. Kim, I. Heo, B. C. Shin, C. Crawford, H. W. Kang, and J. H. Lim, "Acupuncture for posttraumatic stress disorder: a systematic review of randomized controlled trials and prospective clinical trials," *Evidence-Based Complement Alternative Medicine*, vol. 2013, Article ID 615857, 12 pages, 2013.

[19] J. C. Deare, Z. Zheng, C. C. Xue et al., "Acupuncture for treating fibromyalgia," *Cochrane Database of Systermatic Reviews*, no. 5, Article ID CD007070, 2013.

[20] P. Tsui and M. C. Leung, "Comparison of the effectiveness between manual acupuncture and electro-acupuncture on patients with tennis elbow," *Acupuncture and Electro-Therapeutics Research*, vol. 27, no. 2, pp. 107–117, 2002.

[21] S. I. Park, Y. Y. Sunwoo, Y. J. Jung et al., "Therapeutic effects of acupuncture through enhancement of functional angiogenesis and granulogenesis in rat wound healing," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 464586, 10 pages, 2012.

[22] J. A. Lee, H. J. Jeong, H. J. Park, S. Jeon, and S. U. Hong, "Acupuncture accelerates wound healing in burn-injured mice," *Burns*, vol. 37, no. 1, pp. 117–125, 2011.

[23] N. S. Hoang, C. Sar, J. Valmier, V. Sieso, and F. Scamps, "Electro-acupuncture on functional peripheral nerve regeneration in mice: a behavioural study," *BMC Complementary and Alternative Medicine*, vol. 12, article 141, 2012.

[24] Y. Takaoka, M. Ohta, A. Ito et al., "Electroacupuncture suppresses myostatin gene expression: cell proliferative reaction in mouse skeletal muscle," *Physiological Genomics*, vol. 30, no. 2, pp. 102–110, 2007.

[25] R. G. Wang, Y. T. Wang, C. Xiao, T. T. Duan, and L. Tong, "Effects of electroacupuncture with different current intensities on microcirculation, anti-fibrosis and anti-oxidation capacity in skeletal-muscles of rabbits after acute contusion," *Chinese Journal of Rehabilitation Medicine*, vol. 27, no. 5, pp. 400–405, 2012.

[26] J. J. T. Crisco, K. D. Hentel, W. O. Jackson, K. Goehner, and P. Jokl, "Maximal contraction lessens impact response in a muscle contusion model," *Journal of Biomechanics*, vol. 29, no. 10, pp. 1291–1296, 1996.

[27] J. J. Crisco, P. Jokl, G. T. Heinen, M. D. Connell, and M. M. Panjabi, "A muscle contusion injury model. Biomechanics, physiology, and histology," *The American Journal of Sports Medicine*, vol. 22, no. 5, pp. 702–710, 1994.

[28] N. M. McBrier, T. Neuberger, N. Okita, A. Webb, and N. Sharkey, "Reliability and validity of a novel muscle contusion device," *Journal of Athletic Training*, vol. 44, no. 3, pp. 275–278, 2009.

[29] D. G. Blankenbaker and M. J. Tuite, "Temporal changes of muscle injury," *Seminars in Musculoskeletal Radiology*, vol. 14, no. 2, pp. 176–193, 2010.

[30] T. A. Jarvinen, T. L. Järvinen, M. Kääriäinen, H. Kalimo, and M. Järvinen, "Muscle injuries: biology and treatment," *The American Journal of Sports Medicine*, vol. 33, no. 5, pp. 745–764, 2005.

[31] D. A. Fischman, "The synthesis and assembly of myofibrils in embryonic muscle," *Current Topics in Developmental Biology*, vol. 5, pp. 235–280, 1970.

[32] Y. Li, W. Foster, B. M. Deasy et al., "Transforming growth factor-β1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis," *The American Journal of Pathology*, vol. 164, no. 3, pp. 1007–1019, 2004.

[33] J. Zhu, Y. Li, A. Lu et al., "Follistatin improves skeletal muscle healing after injury and disease through an interaction with muscle regeneration, angiogenesis, and fibrosis," *The American Journal of Pathology*, vol. 179, no. 2, pp. 915–930, 2011.

[34] J. Zhu, Y. Li, W. Shen et al., "Relationships between transforming growth factor-β1, myostatin, and decorin: Implications for skeletal muscle fibrosis," *The Journal of Biological Chemistry*, vol. 282, no. 35, pp. 25852–25863, 2007.

[35] D. Zhang, S. Li, S. Wang, and H. Ma, "An evaluation of the effect of a gastric ischemia-reperfusion model with laser Doppler blood perfusion imaging," *Lasers in Medical Science*, vol. 21, no. 4, pp. 224–228, 2006.

[36] J. M. B. Newman, R. M. Dwyer, P. St-Pierre, S. M. Richards, M. G. Clark, and S. Rattigan, "Decreased microvascular vasomotion and myogenic response in rat skeletal muscle in association with acute insulin resistance," *Journal of Physiology*, vol. 587, no. 11, pp. 2579–2588, 2009.

[37] E. Jedel, F. Labrie, A. Odén et al., "Impact of electro-acupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 300, no. 1, pp. E37–E45, 2011.

[38] L. L. Wu, C. H. Su, and C. F. Liu, "Effects of noninvasive electroacupuncture at hegu (LI4) and Sanyinjiao (SP6) acupoints on dysmenorrhea: a randomized controlled trial," *Journal of Alternative and Complementary Medicine*, vol. 18, no. 2, pp. 137–142, 2012.

[39] J. K. Sahu, A. Kumar, and K. Prakash, "Randomized controlled trial of electro-acupuncture for autism spectrum disorder," *Alternative Medicine Review*, vol. 15, no. 4, p. 302, 2010.

[40] H. Li, J. Chen, S. Chen, Q. Zhang, and S. Chen, "Antifibrotic effects of Smad4 small interfering RNAs in injured skeletal muscle after acute contusion," *International Journal of Sports Medicine*, vol. 32, no. 10, pp. 735–742, 2011.

[41] Z. B. Li, J. Zhang, and K. R. Wagner, "Inhibition of myostatin reverses muscle fibrosis through apoptosis," *Journal of Cell Science*, vol. 125, part 17, pp. 3957–3965, 2012.

[42] J. A. Diaz, D. A. Fischer, A. C. Rettig, T. J. Davis, and K. D. Shelbourne, "Severe quadriceps muscle contusions in athletes: a report of three cases," *The American Journal of Sports Medicine*, vol. 31, no. 2, pp. 289–293, 2003.

[43] B. A. Bryan, T. E. Walshe, D. C. Mitchell et al., "Coordinated vascular endothelial growth factor expression and signaling during skeletal myogenic differentiation," *Molecular Biology of the Cell*, vol. 19, no. 3, pp. 994–1006, 2008.

[44] Z. Dong, L. Shun-Yue, W. Shu-You, and M. Hui-Min, "Evaluation of influence of acupuncture and electro-acupuncture for blood perfusion of stomach by laser doppler blood perfusion imaging," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 969231, 6 pages, 2011.

[45] L. A. Loaiza, S. Yamaguchi, M. Ito, and N. Ohshima, "Electro-acupuncture stimulation to muscle afferents in anesthetized rats modulates the blood flow to the knee joint through autonomic reflexes and nitric oxide," *Autonomic Neuroscience: Basic and Clinical*, vol. 97, no. 2, pp. 103–109, 2002.

[46] U. Kerkweg, F. Petrat, H. Korth, and H. de Groot, "Disruption of skeletal myocytes initiates superoxide release: contribution of NAD(P)H oxidase," *Shock*, vol. 27, no. 5, pp. 552–558, 2007.

[47] J. L. Lefaix, S. Delanian, J. J. Leplat et al., "Successful treatment of radiation-induced fibrosis using Cu/Zn-SOD and Mn-SOD: an experimental study," *International Journal of Radiation Oncology Biology Physics*, vol. 35, no. 2, pp. 305–312, 1996.

[48] H. Tsutsui, S. Kinugawa, and S. Matsushima, "Mitochondrial oxidative stress and dysfunction in myocardial remodelling," *Cardiovascular Research*, vol. 81, no. 3, pp. 449–456, 2009.

[49] Y. S. Hori, A. Kuno, R. Hosoda et al., "Resveratrol ameliorates muscular pathology in the dystrophic mdx mouse, a model for Duchenne muscular dystrophy," *Journal of Pharmacology and Experimental Therapeutics*, vol. 338, no. 3, pp. 784–794, 2011.

[50] S. W. Rho, G. S. Choi, E. J. Ko et al., "Molecular changes in remote tissues induced by electro-acupuncture stimulation at acupoint ST36," *Molecules and Cells*, vol. 25, no. 2, pp. 178–183, 2008.

[51] H. Wang, Y. Pan, B. Xue et al., "The antioxidative effect of electro-acupuncture in a mouse model of Parkinson's disease," *PLoS ONE*, vol. 6, no. 5, Article ID e19790, 2011.

[52] G. Dai, Y. Chen, F. Gu, and R. Chen, "Effects of electroacupuncture on the contents of NO, ET and T-AOC in the brain tissues of the cerebral hemorrhage model rats," *Journal of Traditional Chinese Medicine*, vol. 25, no. 1, pp. 62–65, 2005.

[53] F. L. Muller, W. Song, Y. Liu et al., "Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy," *Free Radical Biology and Medicine*, vol. 40, no. 11, pp. 1993–2004, 2006.

[54] G. Li, X. Feng, and S. Wang, "Effects of Cu/Zn superoxide dismutase on strain injury-induced oxidative damage to skeletal muscle in rats," *Physiological Research*, vol. 54, no. 2, pp. 193–199, 2005.

[55] S. U. Rajguru, G. S. Yeargans, and N. W. Seidler, "Exercise causes oxidative damage to rat skeletal muscle microsomes while increasing cellular sulfhydryls," *Life Sciences*, vol. 54, no. 3, pp. 149–157, 1994.

[56] R. W. R. Dudley, G. Danialou, K. Govindaraju, L. Lands, D. E. Eidelman, and B. J. Petrof, "Sarcolemmal damage in dystrophin deficiency is modulated by synergistic interactions between mechanical and oxidative/nitrosative stresses," *The American Journal of Pathology*, vol. 168, no. 4, pp. 1276–1287, 2006.

[57] H. Tsutsui, S. Kinugawa, and S. Matsushima, "Oxidative stress and heart failure," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 301, no. 6, pp. H2181–H2190, 2011.

[58] S. Zhong, Z. Li, L. Huan, and B. Y. Chen, "Neurochemical mechanism of electroacupuncture: anti-injury effect on cerebral function after focal cerebral ischemia in rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 6, no. 1, pp. 51–56, 2009.

[59] Z. X. Yang, Y. Y. Zhuo, H. B. Yu, and N. Wang, "Effect of electroacupuncture on cellular structure of hippocampus in splenic asthenia pedo-rats," *Zhen Ci Yan Jiu*, vol. 35, no. 1, pp. 37–42, 2010.

[60] T. M. Best, S. E. Shehadeh, G. Leverson, J. T. Michel, D. T. Corr, and D. Aeschlimann, "Analysis of changes in mRNA levels of myoblast- and fibroblast-derived gene products in healing skeletal muscle using quantitative reverse transcription-polymerase chain reaction," *Journal of Orthopaedic Research*, vol. 19, no. 4, pp. 565–572, 2001.

[61] T. Hurme, H. Kalimo, M. Sandberg, M. Lehto, and E. Vuorio, "Localization of type I and III collagen and fibronectin production in injured gastrocnemius muscle," *Laboratory Investigation*, vol. 64, no. 1, pp. 76–84, 1991.

[62] Z. Yan, S. Choi, X. Liu et al., "Highly coordinated gene regulation in mouse skeletal muscle regeneration," *The Journal of Biological Chemistry*, vol. 278, no. 10, pp. 8826–8836, 2003.

[63] S. Thorsteinsdottir, M. Deries, A. S. Cachaço, and F. Bajanca, "The extracellular matrix dimension of skeletal muscle development," *Developmental Biology*, vol. 354, no. 2, pp. 191–207, 2011.

[64] Y. I. Oh, E. J. Yang, S. M. Choi, and C. W. Kang, "The effect of electroacupuncture on insulin-like growth factor-I and oxidative stress in an animal model of renal failure-induced hypertension," *Kidney and Blood Pressure Research*, vol. 35, no. 6, pp. 634–643, 2012.

[65] F. Zhang, J. Ma, Y. Lu et al., "Acupuncture combined with curcumin attenuates carbon tetrachloride-induced hepatic fibrosis in rats," *Acupuncture in Medicine*, vol. 30, no. 2, pp. 132–138, 2012.

[66] A. C. McPherron, A. M. Lawler, and S. J. Lee, "Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member," *Nature*, vol. 387, no. 6628, pp. 83–90, 1997.

[67] B. P. Pereira, H. C. Han, Z. Yu et al., "Myosin heavy chain isoform profiles remain altered at 7 months if the lacerated medial gastrocnemius is poorly reinnervated: a study in rabbits," *Journal of Orthopaedic Research*, vol. 28, no. 6, pp. 732–738, 2010.

[68] S. McCroskery, M. Thomas, L. Maxwell, M. Sharma, and R. Kambadur, "Myostatin negatively regulates satellite cell activation and self-renewal," *Journal of Cell Biology*, vol. 162, no. 6, pp. 1135–1147, 2003.

[69] B. Langley, M. Thomas, A. Bishop, M. Sharma, S. Gilmour, and R. Kambadur, "Myostatin inhibits myoblast differentiation by down-regulating MyoD expression," *The Journal of Biological Chemistry*, vol. 277, no. 51, pp. 49831–49840, 2002.

[70] Z. B. Li, H. D. Kollias, and K. R. Wagner, "Myostatin directly regulates skeletal muscle fibrosis," *The Journal of Biological Chemistry*, vol. 283, no. 28, pp. 19371–19378, 2008.

[71] X. Zhu, S. Topouzis, L. F. Liang, and R. L. Stotish, "Myostatin signaling through Smad2, Smad3 and Smad4 is regulated by the inhibitory Smad7 by a negative feedback mechanism," *Cytokine*, vol. 26, no. 6, pp. 262–272, 2004.

[72] C. McFarlane, G. Z. Hui, W. Z. W. Amanda et al., "Human myostatin negatively regulates human myoblast growth and differentiation," *American Journal of Physiology—Cell Physiology*, vol. 301, no. 1, pp. C195–C203, 2011.

[73] W. Wang, X. R. Huang, E. Canlas et al., "Essential role of Smad3 in angiotensin II-induced vascular fibrosis," *Circulation Research*, vol. 98, no. 8, pp. 1032–1039, 2006.

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Review Article

Auricular Acupuncture with Laser

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Auricular acupuncture is a method which has been successfully used in various fields of medicine especially in the treatment of pain relief. The introduction of lasers especially low-level lasers into medicine brought besides the already existing stimulation with needles and electricity an additional technique to auricular acupuncture. This literature research looks at the historical background, the development and the anatomical and neurological aspects of auricular acupuncture in general and auricular laser acupuncture in detail. Preliminary scientific findings on auricular acupuncture with laser have been described in detail and discussed critically in this review article. The results of the studies have shown evidence of the effect of auricular laser acupuncture. However, a comparison of these studies was impossible due to their different study designs. The most important technical as well as study parameters were described in detail in order to give more sufficient evidence and to improve the quality of future studies.

1. Introduction

Today, there are many publications on acupuncture but only a few on auricular acupuncture. When you search for the term "acupuncture" in the scientific database PubMed (http://www.pubmed.gov/), more than 18,300 publications are found, while only 836 publications show up when you search for the term "auricular acupuncture."

The reason for this can be found in history. Acupuncture, being a part of Traditional Chinese Medicine (TCM), has been practiced over thousands of years. From the beginning, Chinese Medicine was passed on from one generation to the next. Therefore, many books have been written—some of them more than 2,100 years ago.

Auricular acupuncture has also been practiced for about 2,500 years, but it was not passed on the same way as TCM was. Only about 60 years ago auricular acupuncture experienced its revival through the French physician named Paul Nogier.

Due to his ongoing research and successful treatments—especially in the field of pain management—auricular acupuncture developed into a distinct treatment system of its own. Currently, auricular acupuncture is used for acute as well as chronic pain associated with sciatica, osteoarthritis, headache, knee arthroscopy, hip fracture, hip arthroplasty, and even cancer [1].

As a result of the technological progress not only the range of treatment has expanded but also have the methods used for stimulating the acupuncture points developed from metal needles over electroacupuncture to laser acupuncture [2].

Research shows that laser acupuncture is still in its early stage of development and up to now only a small amount of different studies have been published.

The results of these studies, however, show that there is high potential in the field of laser acupuncture. Among others there are two main reasons why laser acupuncture should be considered as an alternative treatment to needle acupuncture: first it offers a noninvasive treatment for children or patients who are afraid of needles and second the laser treatment requires less time.

2. Classical Acupuncture

Classical acupuncture has its origin in TCM and it is believed to have been practiced for about 2,500 years. It is described

as a medical treatment by inserting needles into the skin at specific points on the body.

Acupuncture is based on the principle of Qi. Qi can be described as a vital life force. According to TCM, you inherit Qi when you are born and you obtain Qi by breathing and eating during your lifetime. Qi flows in energetic pathways or channels in the human body—the so-called meridians. They form a meridian system which partially relates to the internal organs and their physiological and pathological conditions. Along these meridians, energy comes to the surface through a series of acupuncture points. They serve as tunnels or access routes to the deeper meridian system. A dysfunction of the Qi can be restored by stimulating these acupuncture points [3–5].

It should not remain unmentioned that in 1991 a mummy named Iceman or Ötzi was found in the Ötztaler Alps in South Tyrol. Scientists believed that the Iceman was about 5,200 years old. His body showed tattoos on the locations of classical acupuncture points. Due to the strong evidence that was found Dorfer et al. [6] hypothesized that a medical system similar to Chinese acupuncture already existed in Central Europe over 5,200 years ago. This leads to the conclusion that acupuncture originated in the Eurasian continent and is nearly 2,000 years older than it was believed to be.

3. Auricular Acupuncture

For a better understanding of auricular acupuncture, we will look at its history and development from two different perspectives:

(i) *ancient auricular acupuncture* referring to the time before the 20th century and up to the 1950s,

(ii) *modern auricular acupuncture* referring to the time when Paul Nogier rediscovered auricular acupuncture.

Again this term can be divided into

(i) the *Chinese system* of auricular acupuncture and

(ii) the *Western or European system* of auricular acupuncture, which further developed into auriculotherapy and auriculomedicine [7].

3.1. Ancient Auricular Acupuncture

3.1.1. Definition. Ancient auricular acupuncture is a treatment based on the stimulation of acupuncture points on the auricle. These points were believed to be connected to the meridians and used empirically for certain treatments mainly for pain relief. They, however, were not part of an acupuncture system limited to the ear but part of a system covering the whole body [8, 9].

3.1.2. History. There is only little evidence from the past to prove which culture ear acupuncture originates from. According to Oleson [7] and Chen [8] a relationship between the ear, the meridians, and the viscera has already been mentioned in the most famous and over 2,100-year old book *Huang Di Nei Jing*—the *Yellow Emperor's Classic of Internal Medicine*. Some of the auricular points which are referred to in this and other books are still used today. They, however, have no connection to the meridians and no logical order.

There has also been historical reference to the use of auricular treatments by Hippocrates, the "father of the western medicine" and paragon of ancient physicians, who lived around 460 BC. During his studies in Egypt, Hippocrates learned about the treatment of impotence and how to facilitate ejaculation by practising phlebotomy on the veins of the posterior surface of the ear. He kept on practising and teaching this method at his medical school on the island of Kos. Today, scientists assume that the therapeutic effect was not necessarily caused by phlebotomy itself but simply by stimulating the ear.

Another example is Mediterranean sailors who wore their golden earrings on the central lobule of the ear not only for decoration but also for improving their eyesight [7–11].

In 1636 Lusitanus Zactaus, a Portuguese doctor, described the treatment of low back pain and sciatica by cauterizing the earlap. About sixty years later, in 1717, the Italian doctor Antonio Maria Valsalva found new areas on the ear to relieve dental pain by cauterization. He described this in his famous book *De Aure Humana Tractatus*. In 1810 Professor Ignazio Colla of Parma reported a patient who claimed that after being stung by a bee in the antihelix, all the pain in his legs was gone immediately. From around 1850 onwards, there was a big hype about ear cauterization. It was mainly used as relief from dental pain or during dental extraction entirely. Sometimes it was even used to prevent dental extraction. Dysfunctions of the facial nerve were also treated this way. Due to scientific inexplicability, however, the methods began to be forgotten [9, 10].

3.2. Modern Auricular Acupuncture

3.2.1. Definition. According to Gori and Firenzuoli [9], modern auricular acupuncture is defined as "a diagnostic and treatment system based on normalizing the body's dysfunction by stimulating acupuncture points on the external ear." It is not based on TCM, but on the presumption of Paul Nogier that a somatotopic organization of the body is represented on the human auricle.

3.2.2. History. It was not until 1950 when the French physician and later founder of auriculotherapy Dr. Paul Nogier discovered peculiar scars on the antihelix of his patient, who had been successfully treated for sciatic pain syndrome. These scars were caused by cauterization. Presuming it was something new, he started research and discovered that the pain ceased within a couple of hours or sometimes even minutes after being cauterized.

Following a series of his clinical studies, Paul Nogier replaced cauterizing with needling the acupuncture points. Despite the lack of scientific validation, Paul Nogier kept on investigating this phenomenon. In his research he theorized that a somatotopy, as already known of the sensory and motor cortices of the brain, could also be presented on the ear.

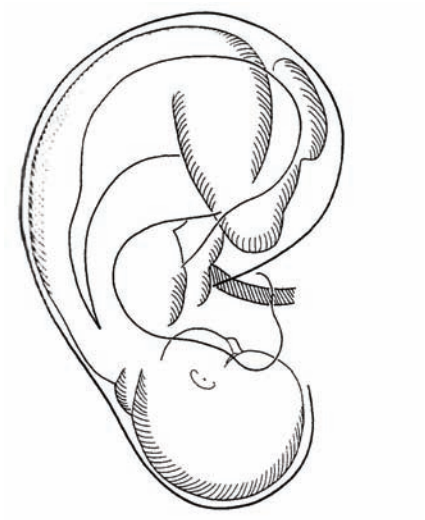


Figure 1: Inverted fetus. Modified from [10].

This resulted in his discovery of the body's anatomy displaying itself on the ear as an inverted fetus (see Figure 1).

In 1956 Dr. Niboyet, France's most famous acupuncturist at the time, took notice of Paul Nogier's work and invited him to speak at the Congress of the Société Mediterranéenne in Marseille. The presentation was later published in the German acupuncture journal "Deutsche Zeitschrift für Akupunkteure." Eventually, Paul Nogier's discovery found its way to China where a group of Chinese acupuncturists conducted a study on over 2000 patients which allowed them to verify his findings. In 1959 an article about French auricular acupuncture was published in a Chinese journal called "Popular Medicine" in which Paul Nogier was credited for his discovery. At the same year the Chinese acknowledged Paul Nogier as "the Father of Auricular Acupuncture" [7, 10, 12].

3.3. The Chinese and the Western System of Auricular Acupuncture. Like his Chinese colleagues, Paul Nogier carried on investigating. While Paul Nogier continued his work by looking at the auricular points from an anatomical point of view, Chinese scientists developed the Chinese system by looking at the functional connection between the auricular acupuncture points and their effects on the body.

In 1966, Paul Nogier discovered that the radial artery pulse showed a reaction to the stimulation of the auricle. Assuming that this response involved the ear and the heart, he called this response "Reflexe Auriculocardiaque." Later when he realized that this response was caused by the autonomic nervous system, he changed its name to vascular autonomic signal (VAS). This reflex or signal can be explained as a vascular reaction to the stimulation of the skin. With this unique vascular reflex auricular therapy advanced into auricular medicine, a system for advanced diagnosis and treatment.

In the late 1950s Paul Nogier introduced a small number of 42 auricular acupuncture points to Chinese scientists. Only 20 years later, in the late 1970s, Chinese acupuncturists had already increased the number to over 1,000 points most of which were empirically found.

In 1982, the World Health Organization (WHO) launched international working groups with the aim to facilitate teaching, research, and clinical practice of auricular acupuncture all over the world. In 1990, a standardization of auricular acupuncture was established by introducing 3 criteria. An auricular point had to have an international and a common name, its therapeutic value had to be proven, and its location on the auricle had to be generally accepted. Thirty nine auricular points fulfilled all 3 criteria; 36 points failed one or more of the criteria. Finding an agreement on these issues was a very important step in the development of auricular acupuncture. Due to different approaches of French and Chinese auricular acupuncturists, there is still a lot of discrepancy on certain auricular points and their mappings [8, 10, 13, 14].

4. Paul Nogier

Paul Nogier was born in Lyon in 1908. He was the son of a tenured professor at the Medical University of Lyon. After 3 years of studying physics, Paul Nogier went on to study medicine.

In 1938, he established his general medicine practice in Lyon where he also practiced homeopathy, manipulative medicine, and body acupuncture. To pursue these methods, Paul Nogier started a monthly study session with his students in 1942, which he kept going until he died in 1996. In 1969, he published his first book called *Traité d'Auriculothérapie*.

Furthermore, he received some of France's most honorable awards, and in 1990 the WHO honored him for his great contribution in medicine and recognized him as the founder of auricular therapy and auricular medicine. Only a few months before his death in 1996 the *Ècole Internationale Paul Nogier* was founded [12, 15].

Raphael Nogier, Paul's son, pursued his father's work and described him as "a man of innovative thoughts and productive action who listened to his patients, respected what they had to say and thoroughly investigated their maladies. He tirelessly examined his patients from Monday morning to Saturday evening" [7].

5. The Auricle

5.1. Microsystem of the Ear. A microsystem is the projection of the whole body in its function and structure on certain parts of the body. In addition to the auricle microsystems can also be found on the scalp, the feet, the hands, and the iris.

Thanks to Paul Nogier's rediscovery, auricular acupuncture has developed into one of the most commonly used and explored microsystems in the last 60 years [15, 16].

5.2. Anatomy of the Auricle. The ear is divided into 3 anatomic parts: the external ear (lat.: auris externa), the middle ear (lat.: auris media), and the internal ear (lat.: auris interna).

The external ear consists of the pinna or auricle (lat.: auricular), the external auditory canal (lat.: meatus acusticus

externus), and the tympanic membrane (lat.: membrane tympanica).

In auricular acupuncture the most important anatomic structure is the auricle. The ear cartilage (lat.: cartilage auricularis) provides a supporting framework for the auricle and gives the auricle its funnel shape, while the ear lobe (lat.: lobulus auricularis) has no elastic cartilaginous part at all.

The outer part of the ear cartilage is called the helix. The helix crus divides the concha, the deepest point of the auricle, into the cymbia conchae or hemiconcha superior and the cavity of conchae or hemiconcha inferior.

At the opposite of the helix, one finds the antihelix. In between lies the triangular fossa. The sulcus between the antihelix and helix, is called the scapha or scaphoid fossa. Together with the helix it disembogues in the ear lobe.

The cartilaginous bead that lies in front of the external auditorial canal is called tragus. Opposite of it is the antitragus. Between the tragus and the antitragus lies the incisura intertragica (see Figure 2) [10, 17, 18].

The posterior auricle can be divided into 5 main parts of the back of the ear: the posterior groove behind the antihelix, the posterior lobe, the posterior concha, the posterior triangle behind the triangular fossa, and the posterior periphery behind the scapha and the helix [19].

5.3. Innervation of the Auricle (See Figure 3). The auricle is innervated by spinal and cranial nerves (CN). The facial nerve is responsible for the motoric innervation of the outer ear muscle.

Responsible for the sensitive innervation are the greater auricle nerve (GAN), the lesser occipital nerve, the auriculotemporal nerve—the third division and mandibular branch of the trigeminal nerve—and the auricular branches of the vagus nerve (CN X), the glossopharyngeal nerve (CN IX), and the facial nerve (CN VII) [18].

The studies of He et al., Ueno et al. [20, 21], and Peuker and Filler [17] do not coincide on the areas of innervation.

According to He et al. and Ueno et al. [20, 21] the greater auricle nerve (GAN) innervates both surfaces of the lower part of the auricle whereas Peuker and Filler [17] find additional innervation of the GAN in the tail of the helix and the scapha.

He et al. and Ueno et al. [20, 21] suggest that the lesser occipital nerve supplies the skin of the upper and back parts of the auricle with fibers. Peuker and Filler [17] do not mention this nerve in their studies.

According to He et al. and Ueno et al. [20, 21] the auriculotemporal nerve innervates the anterosuperior and the anteromedial areas of the auricle (see also Table 1). This involves the crus and the upper part of helix body, the antihelix, the triangular fossa, and the tragus. Peuker and Filler [17] found the auriculotemporal nerve solely in the crus and the upper part of the helix.

The auricle branch of the vagus nerve (ABVN) supplies the concha, mainly the cavity of the conchae, and most of the region around the external auditorial canal according to He et al. and Ueno et al. [20, 21]. Peuker and Filler [17] found the ABVN also in the concha but mainly in the cymbia of the conchae and also in the antihelix.

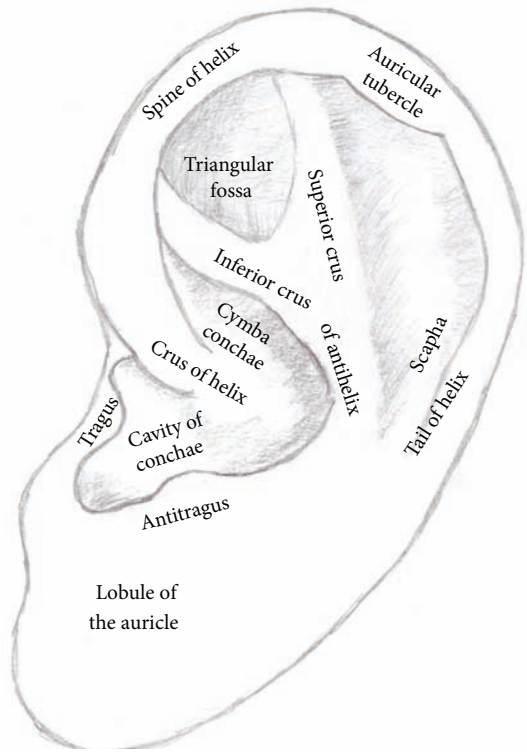


Figure 2: Anatomy of the auricle.

Figure 3: Green marks the branch of the vagus nerve, red marks the auriculotemporal nerve, blue marks the lesser occipital nerve, and yellow marks the greater auricular nerve. Modified from [20, 21].

6. Modern Auricular Acupuncture

6.1. Somatotopy. Paul Nogier's discovery of the inverted fetus being projected on the auricle is the basis of modern auricular acupuncture. As it is shown in Figure 4 the head with

Table 1: Innervation pattern (modified from [17]); auricle branch of the vagus nerve (ABVN), greater auricular nerve (GAN), auriculotemporal nerve (ATN).

	ABVN	GAN	ATN
Crus of helix	20%		80%
Spine of helix		9%	91%
Tail of helix		100%	
Scapha		100%	
Crura of antihelix	9%	91%	
Tragus	45%	46%	9%
Cymba conchae	100%		
Cavity conchae	45%	55%	
Lobule of auricle		100%	

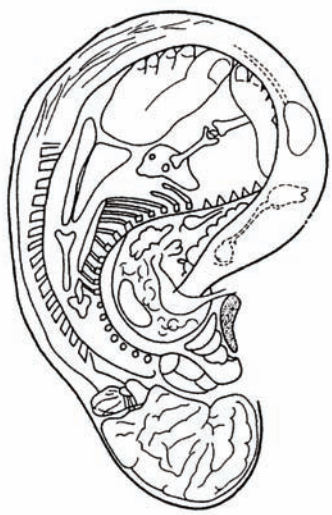


Figure 4: Somatotopy. Modified from [10].

the brain structures are represented on the ear lobe; the spine runs along the antihelix. The inner organs are represented in the concha. The legs and the arms are represented towards the upper rim of the auricle. This somatotopic representation is often seen as a homunculus like it is known of the sensory cortex in the brain [16, 22].

6.2. Embryological Aspects. The somatotopic mapping also reveals an embryological pattern of the organs. As you can see in Figure 5, organs originating from the ectoderm are presented on the ear lobe and the tragus, organs originating from the endoderm on the concha, and mesodermal organs on the remaining part of the auricle [16].

6.3. Neurological Aspects. The mechanism of auricular acupuncture is believed to work through the autonomic nervous system. By stimulating the auricle, the information travels through sympathetic and parasympathetic nerve fibers from the ear to the brain and from the brain through the spinal cord to specific areas in the body [16, 19].

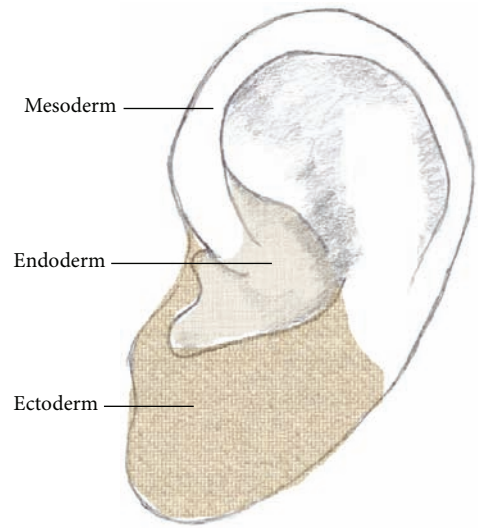


Figure 5: Auricular embryology.

Heine [23] stated in his survey that auricular acupuncture points are dot-like structures with a diameter of one-tenth millimetres at the most. These structures consist of a combination of collagen and elastic fibers which are pervaded by nerve endings, arterioles, venules, and capillaries.

According to Soliman and Frank [16], the vagal nerve carries the parasympathetic fibers and the branch of the trigeminal nerve carries the sympathetic fibers to the reticular formation where the information gets distributed to the corresponding brain structures.

So far there is no evidence in the literature that auricular acupuncture has a direct influence on the sympathetic system. However, numerous studies have shown that auricular acupuncture influences the parasympathetic activity, thus, also the autonomic nervous system which can have an impact on the cardiovascular system, the endocrine system, the respiratory and gastrointestinal system, the urinary system, and in the treatment of epilepsy and depression or even an anti-inflammatory effect [19, 20, 24–31].

With a 75% share of all parasympathetic fibers, the vagus nerve has the highest influence on the parasympathetic system [32].

The afferent fibers of the vagus nerve synapse in structures of the medulla in particular in the nucleus tractus solitarius (NTS), the nucleus of the spinal tract of the trigeminal nerve, medial reticular formation of the medulla, the area postrema, the dorsal motor nucleus of the vagus, and the nucleus ambiguus. The majority of the vagal fibers lead into the NTS. Most of the information that is received by the NTS is spread to various areas of the brain such as the hypothalamus, the central nucleus of the amygdala, and other nuclei in the brainstem.

The efferent fibers of the vagus nerve lead to the heart, lungs, stomach, intestines, liver, pancreas, and kidneys [20, 33].

Despite the high amount of parasympathetic fibers of the vagus nerve, the auricle branch is the only peripheral branch of the vagus nerve. Thus, He et al. [20] theorized that auricular vagal stimulation can alter the autonomic as well as the central nervous system via the concha to the NTS and further on to the corresponding structures of the brain. They suggested the existence of an "auriculovagal afferent pathway."

7. Detection of the Auricular Acupuncture Points

French and Chinese physicians suggested already in the 1970s that the auricle can not only be used for therapy but also for the diagnosis of dysfunctions in the body [22, 34–36].

The survey of Oleson et al. [22] investigated the theory by examining patients with musculoskeletal pain. In 75.2% of the cases there was an accordance between the conventional medical diagnosis and the auricular diagnosis. It is believed that the reaction of the ear is caused again through neurological pathways. In normal conditions, the ear is "electrically silent," but under pathological conditions the ear turns "electrically active" [16].

The inspection and palpation of the auricle are primary steps for the detection of "reactive points." Abnormalities like swelling, blushing, desquamation, or tenderness can be signs of pathological conditions in the body.

Mechanical and electrical point finders (Figure 6) are also used for a more precise detection of acupuncture points. Mechanical point finders depend very much on the sensitivity of the patients which can often lead to distorted results, whereas electrical point finders supply more objective results by measuring the electrical skin resistance. Recently, point finders with laser have been applied for the identification of "reactive acupuncture points" as well [15, 22].

Besides inspection, palpation, and point detectors, the vascular autonomic signal (VAS) is also used for the finding of auricular points.

8. Vascular Autonomic Signal (VAS)

Paul Nogier discovered this signal in 1966 assuming that there was a connection between the ear and the heart. Later, he realized that it was a response of the generalized autonomic nervous system.

The VAS is a vasculocutaneous reflex that can be felt like a stationary wave of the pulse on the arterial wall (compare Figure 7). This pulse is the result of cardiac output and its rebound as the blood piles up against the arterioles and capillaries. It is a response and adjustment of the nervous system. Any stimulation of the skin by light, laser, heat, or touch can provoke the VAS.

In Europe, the VAS became another method used for finding "reactive" auricular points. Hereby, practitioners use the radial artery pulse for its detection. VAS also allows the identification of treatable points which are not painful but still show a dysfunction of the body.

Figure 6: Point finder [10].

Figure 7: Detection of VAS. Modified from [10].

While in the past practitioners used their thumb to palpate the VAS, nowadays it can also be detected with bidirectional Doppler ultrasonography [11].

9. Methods of Stimulating the Ear Points

9.1. Acupressure. Auricular acupressure is a noninvasive method that stimulates the acupuncture points by applying pressure using fingers, knuckles, or dull objects like magnet beads or vaccaria seeds.

9.2. Needle Acupuncture. There are different methods of stimulating the ear points. The most common method is acupuncture. Hereby needles are used for the stimulation of the auricle. In former days people used fish bones, sharp stones, or bamboo clips as acupuncture material. Later, these materials were replaced with gold or silver. Today, the needles are made of high tensile stainless steel and coated in gold or silver. Gold needles are used for reducing (supplying) and silver needles are used for reinforcing (drainage). On the back of the ear they are applied the other way round. The needles stay in place for about 20–45 minutes.

Sometimes after an acupuncture treatment semipermanent needles are attached to certain acupuncture points to enhance the effect of the treatment. These needles are solely made of high tensile stainless steel and stay in the ear for an average time of one week [10, 37].

9.3. Electro Acupuncture. Electroacupuncture is a new modern technique of controlled electrical stimulation on the ear. Hereby, small electrical currents are applied to needles which have been inserted at specific acupuncture points.

The stimulation can either be on low frequency (2 Hz) or high frequency (100 Hz) depending on the pathological issue.

A new auricular electroacupuncture device called P-Stim was invented in the early 1990s by the Austrian physician Dr. Szeles and has been subject of many studies mainly on the treatment of chronic and acute pain since then [38, 39].

9.4. Laser Acupuncture. Laser acupuncture is a noninvasive method that stimulates auricular acupuncture points by applying laser light. It will be further discussed in the following chapters.

10. Adverse Reactions of Auricular Acupuncture

Adverse reactions happen very rarely in auricular acupuncture. So far, there have been reports neither on life-threatening reactions nor on irreversible or serious adverse events after acupuncture. Minor events can be local pain, local bleeding, or infections after needle acupuncture. There have been nine documented cases of perichondritis and one case of chondritis since 1980. In the worst case these diseases can lead to chondronecrosis or even deformation of the auricle. Out of these ten cases, eight patients received permanent needle acupuncture. Proper disinfection and regular inspection of permanent needles can help to avoid such side effects [40].

It has been suggested that patients with diabetes mellitus, immunosuppressed patients, or weakened patients suffering from chronic diseases should not receive permanent needle treatment [41].

11. Important Points in Auricular Acupuncture

As mentioned earlier, there is a huge amount of auricular points used in the Chinese as well as in the Western system. In the following, the most commonly used and researched points in auricular acupuncture will be introduced.

12. Shenmen

Shenmen, meaning "spirit gate," is located at the apex of the triangular fossa (Figure 8). This point is derived from the Chinese system but is also used in the Western system. Generally, it is used in combination with other auricular points. It is also the most commonly used point in auricular acupuncture mainly for the treatment of pain, stress, anxiety, and depression as well as alcohol or drug abuse, cessation of smoking, and weight loss [1, 19, 20, 25, 30, 42–62].

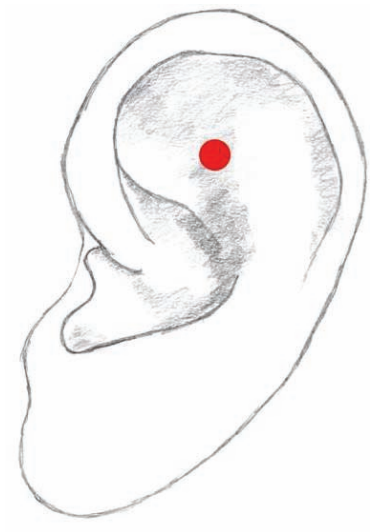


Figure 8: Shenmen [7, 63].

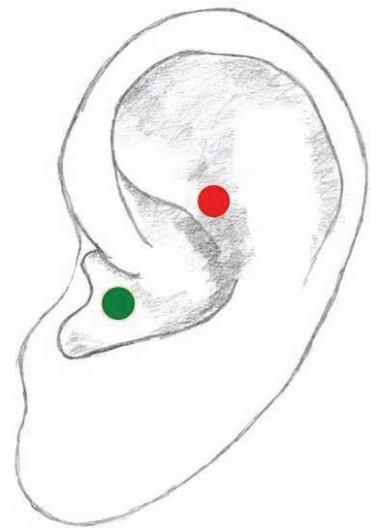


Figure 9: Heart point of the Western system (red), Heart point of the Chinese system (green) [7, 63].

13. Heart Point

There are two different locations of the Heart point in auricular acupuncture. According to the Western system, the Heart point is located in the antihelix whereas in the Chinese system it is situated in the hemiconcha inferior (Figure 9).

In most of the studies, the Chinese Heart point and its considered effect on the cardiovascular system and vagal activity have been investigated [25–27, 31, 64–66].

In their survey on advanced auricular acupuncture, Frank and Soliman [67] explained the shifting of the location of the Heart point and the Chinese Shenmen according to the three phases which depend on the stage of illness.

Phase 1, representing the original map of the inverted fetus, stands for normal physiology or acute pathology. In phase 2, the inverted fetus is represented in an upright

position on the map and stands for degenerative conditions. Phase 3 shows the fetus in a transverse position and stands for subacute and chronic conditions.

With this advanced approach and the Western point of view of the representation of the anatomy and the organs on the auricle, Frank and Soliman [67] showed that Shenmen correlates with the Western auricular points of the spleen in phase 1, the thalamus in phase 2, and the liver in phase 3. They also showed that through the shift the location of the Chinese and the Western Heart point coincide in phase 2.

14. Thalamus Point

The Thalamus point is situated on the lower edge of the antitragus (Figure 10). This point is mainly used in combination with Shenmen and the Lung point in pain therapy [1, 53].

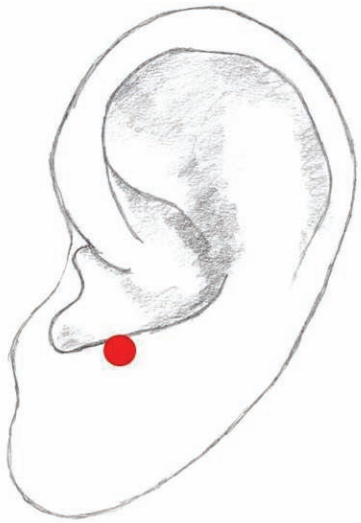


Figure 10: Thalamus point [7, 63].

15. Point Zero

Point Zero is located on a notch in the crus of the helix (Figure 11). It is one of the most important points of the auricle and was first described by Paul Nogier. He named this point "Point Zero" to express its function as a balancing point responsible for the homeostatic in the body in analogy to the "center of the body" according to the tenets of TCM [10, 19].

This point is used in the treatment of pain relief in combination with other auricular acupuncture points including Shenmen [53].

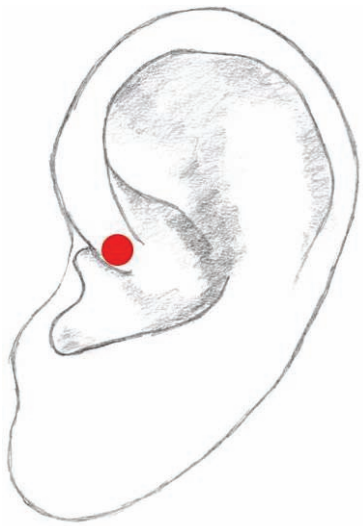


Figure 11: Point Zero [7, 63].

16. Lung Points

There are 2 Lung points (Figure 12). Both are situated in the hemiconcha inferior, representing the right or the left side of the lung. They are also two of the most commonly used auricular acupuncture points. In most studies only one point is stimulated. The Lung points are mainly used for the treatment of alcohol or drug dependency and pain relief especially after surgery. Studies show that auricular acupuncture might have an impact on the autonomic nervous system [1, 25, 28, 31, 43–45, 48, 51, 55–57].

17. More Details on the Auricular Acupuncture Points

Studies which investigated the effects of auricular acupuncture on pathological conditions often used a combination of auricular acupuncture points and other auricular or even body acupuncture points.

Studies on pain therapy after surgeries, for example, used Shenmen and one of the Lung points as basic points in combination with the auricular acupuncture point corresponding to the treated area, for example, the point for the knee joint after a knee surgery [55, 57].

Studies on drug and alcohol dependencies used a combination of auricular points suggested by the National Acupuncture Detoxification Association (NADA) including Shenmen, Sympathetic point, Kidney point, Liver point, and Lung point [43, 44, 48].

Studies investigating the effects of auricular acupuncture on the autonomic nervous system tested either the Heart point or the Lung point individually or in comparison to body acupuncture points. Both points have their locations in the concha, the area which is innervated mainly by the vagus nerve [25–28, 64, 65, 68]. There are also studies investigating the effect of Shenmen on anxiety compared to other auricular acupuncture points or body acupuncture points [58, 61].

Many studies provide poor information about the names or the exact locations of the treated auricular acupuncture points; mostly they only refer to the anatomical areas that had been treated. This lack of information is one of the problems in comparing the studies and makes it difficult to prove the effectiveness of auricular acupuncture [24, 47, 49, 69–73].

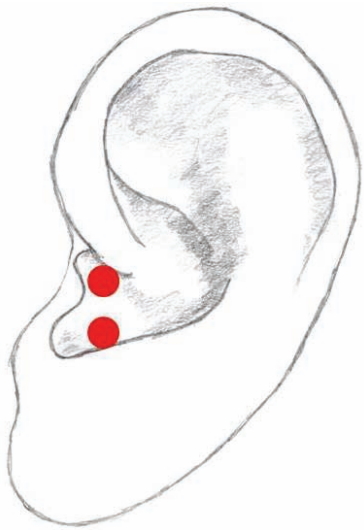


Figure 12: Lung points [7, 63].

18. Recent Studies on Auricular Acupuncture

This section covers recent studies of auricular acupuncture which were conducted either by acupressure, needling, or electro acupuncture. Studies on auricular laser acupuncture will be treated later on separately.

In former days, auricular acupuncture was mainly used for the treatment of sciatica and low back pain. After Paul Nogier's discovery of the microsystem on the ear, numerous clinical and experimental studies on the effects of auricular acupuncture have been carried out mainly in Europe, Asia, and America. Studies show that besides the already known success in treating low back pain many other diseases can be cured by using auricular acupuncture.

A review of Chen [46] showed that auricular acupuncture offers a vast field of applications. Fifty six kinds of illnesses including diseases of the respiratory tract, the circulatory system, the digestive system, the urinary system and the nervous system, diseases in the fields of otolaryngology, ophthalmology, dermatology, paediatrics, and gynaecology as well as acute abdomen, obesity, drinking, and smoking cessation were discussed in these studies. This review included retrospective as well as randomized controlled studies. Auricular acupuncture was effective in most cases especially in the treatment of hypertension, acute pain of the digestive system, dermatological diseases—like psoriasis, urticarial, or acne—and obesity. Due to the small amounts of treated people and randomized controlled (RCT) studies, the quality of this review is poor but still it shows that auricular acupuncture has the potential of being effective in many fields of medicine.

18.1. Pain. There are many studies on pain management with auricular acupuncture. It has shown its effectiveness on acute as well as chronic pain. Low back pain, headache, and pain caused by osteoarthritis were treated with auricular acupuncture. It was even used for postoperative pain control [1, 39].

Studies have shown that auricular acupuncture can be effective especially in the treatment of postoperative pain which would further result in a decrease of analgesic requirement. More studies should be conducted for more reliable and conclusive scientific evidence [55–57, 72, 74].

18.2. Obesity. There is evidence that auricular acupuncture can be used to achieve weight loss. Hsu et al. [49] ran a randomized controlled study with 60 obese women. These women were treated with auricular acupuncture over a period of 3 months. Although there was no change in body weight, body mass index, and waist circumference, there was a significant difference in the levels of obesity-related hormones. Particularly the hormone ghrelin showed an increase and the hormone leptin showed a decrease after 3 months of treatment. Previous studies have shown that obese people have a lower level of ghrelin than people with normal weight and that an increase of this level can be associated with weight loss.

18.3. Anxiety. Studies on anxious patients before operations or dental treatments and on older patients with postoperative anxiety have given evidence of the effectiveness of auricular acupuncture in these fields. The mechanism is still inconclusive, but scientists believe that auricular acupuncture might have an impact on the release of neurotransmitters such as serotonin which helps in the regulation of anxiety [42, 58, 61, 75, 76].

However, there is still no evidence on a successful treatment of patients with generalized anxiety disorder or anxiety neurosis [76].

18.4. Cardiovascular System. Recent studies on rats showed that auricular acupuncture regulates the cardiovascular function by activating baroreceptor sensitive neurons in the nucleus tractus solitarius (NTS). The arterial pressure and the heart rate were significantly reduced by auricular acupuncture [26, 77].

Another Chinese publication reported a decrease in systolic as well as diastolic pressure in patients with hypertension after electric pulse stimulation. The total effective rate had been 90.7% [68].

18.5. Insomnia. There is evidence that auricular acupuncture can also be effective in cases of insomnia. A combination of auricular acupressure with routine care was more efficient than solely auricular acupressure. However, due to methodological limitations and inconclusive results further evidence and research have yet to be considered [47, 52, 78].

18.6. Dermatological Conditions. Only a few studies have been implemented on psoriasis vulgaris. So far, there is one survey that shows a therapeutic effect of auricular acupuncture in combination with a decoction of TCM [79].

Auricular acupuncture has also been used for the treatment of verruca plana or flat warts. This randomized work showed a statistically better outcome in the treatment group than in the control group [80].

However, in both cases further investigations need to be considered to have more scientific evidence on the treatment of psoriasis vulgaris as well as verruca plana.

18.7. Opiate Addiction and Alcohol Abuse. The euphoric feeling of cocaine is assumed to be triggered by blocking the reuptake of the neurotransmitters noradrenalin, serotonin, and mainly dopamine. Hereby cocaine acts as an indirect dopamine agonist [48].

Auricular acupuncture is believed to have its effect by stimulating the vagus nerve at one of the Lung points on the auricle. This leads to a stimulation of the hypothalamus, which leads to the release of serotonin and further to an activation of methionine encephalin which inhibits the release of γ-aminobutyric acid (GABA). The inhibition of GABA causes an increase in dopamine. Hypothetically this mechanism reduces cravings and hereby supports the patient on his path to self-recovery [48].

Besides a Lung point the NADA determined further auricular points which presumably help not only drug addicts but also patients with alcohol problems during their protracted withdrawal. Research showed that patients described amongst others feelings of wellbeing and relaxation, peacefulness and harmony, reductions of anxiety and drug consumption. However, the craving for drugs and alcohol remained and the effects were only temporary [43].

Further studies also showed a positive effect on combined therapies. For example, auricular acupuncture combined with carbamazepine treatment showed good results for alcohol withdrawals and combined with psychotherapy it was effective for drug patients [81, 82].

Here, too, more randomized controlled studies with higher number of patients need to be performed in order to receive more scientifically significant evidence on the effect of auricular acupuncture.

18.8. Epileptic Seizures. In the last 20 years epilepsy, and especially drug-resistant epilepsy, has been successfully treated with vagus nerve stimulation (VNS) [83].

He et al. [29] hypothesize that auricular acupuncture can also be a possible treatment for epileptic seizures just as the VNS therapy. Clinical trials showed that auricular electroacupuncture led to a decrease of frequency and severity of seizures.

It is still unclear how the antiseizure effect works, but it is assumed that auricular acupuncture as well as VNS triggers more than one neuromodulatory mechanism which leads to an effective treatment [33].

He et al. [29] suggest two mechanisms for the success of auricular acupuncture. Firstly, the activation of the NTS leading to a suppression of the affected parts in the brain and secondly on the assumption of Granata et al. [84] that epilepsy might be caused by immune-mediated processes, the activation of the cholinergic anti-inflammatory pathway [84].

Recent studies on trigeminal nerve stimulation (TNS) have also been successful in the treatment of epileptic seizures [85–87].

18.9. Smoking Cessation. In the last decades auricular as well as body acupuncture had been a very common method to help people to lead a life without tobacco. White et al. [88] published a large review of smoking cessation both in auricular as well as in body acupuncture. Although this study showed that the effects of acupuncture were not better than other methods of smoking cessation, the authors pointed out that there is evidence of the effect of acupuncture and further bias-free studies need to be conducted in order to prove these effects. A new method of auricular electro acupuncture, called "Smokex-Pro," has been previously developed. A recently conducted study showed that after the treatment 47.9% of the patients stayed abstinent for at least 2 years [89].

18.10. Summary of Recent Studies. These aforementioned studies have shown that auricular acupuncture can be used in many fields of medicine and there is evidence of its effect. However, the lack of information, the small sample size, and the difference in study parameters give most of the studies a poor quality and make them difficult to compare. For more significant evidence, further investigations need to be conducted.

19. Laser in Auricular Acupuncture

19.1. Definition and History. The word "Laser" is an acronym for "Light Amplification by Stimulated Emission of Radiation." The properties of laser such as its extreme monochromaticity, its polarisation, and its high coherence make the laser so unique and versatile [90].

19.2. History of the Laser. In 1917, Albert Einstein laid the foundation of the laser technology based on the principle of stimulated emission. Also based on this principle Charles H. Townes invented in 1954 the first MASER—short for microwave amplification by the stimulated emission of radiation—for which he received (with Alexander M. Prokhorov and Nikolai G. Basov) the Nobel Prize in 1964. In 1958, Townes and Arthur L. Schawlow published their study "Infrared and optical maser" which was used as a basis for the construction of the first laser in 1960 by the physicist Theodor Maiman. A development of various kinds of lasers ensued [91].

19.3. Laser in Medicine. In the early 1960s a Ruby laser was introduced into medicine for the photocoagulation of the retina. Due to severe side effects, the treatment was discontinued. In the late 1960s, an Argon laser was developed for the use of detached retina treatment.

Today, laser therapy is used in many fields of medicine apart from ophthalmology also in dermatology, otolaryngology, dental medicine, general surgery, and vascular surgery. Depending on the aim of the treatment lasers can be of high intensity or low intensity.

High-intensity (level) lasers have the ability to cut, destroy, or cauterize tissues due to their thermal effect whereas the effect of low-intensity (level) lasers (LLL),

also called "soft laser" or "cold laser," is believed to be caused by the interaction of electromagnetic radiation with the tissue. It has been shown to be effective in wound healing, musculoskeletal pain, and rheumatoid arthritis as well as in laser acupuncture [90, 91].

19.4. History of Laser Acupuncture. Between 1970 and 1972 reports on successful therapies of hypertension and asthma with laser body acupuncture in the USSR were published [92].

The Canadian Friedrich Plog suggested the use of laser in body acupuncture already in 1973. In 1979, the Chinese surgeon Zhou started using laser acupuncture instead of needle acupuncture and acupressure for anaesthesia successfully. First studies on laser auricular acupuncture have been reported in 1984 conducted by Seitz and Kleinkort [93]. Different laser instruments have been developed over the years.

In 2001, the first "laser needles" (Figure 13) were invented at the University of Paderborn (Germany). The first scientific investigations were performed at the Medical University of Graz. For the first time it was possible to stimulate different acupuncture points at the same time [94–97].

19.5. Types of Laser in Auricular Acupuncture. Low-level lasers (LLL) are the most commonly used lasers in body acupuncture as well as auricular acupuncture. They are defined by wavelengths between 300 nm and 10,600 nm, power densities between 10^{-2} W/cm^2 and 10^0 W/cm^2, and energy densities between 10^{-2} J/cm^2 and 10^2 W/cm^2. The wavelength of a laser is defined by its laser medium which can be gaseous, fluid, solid, or semiconducting [98].

In laser acupuncture the most frequently used lasers are gas lasers, mainly the Helium Neon (HeNe) lasers. In recent years, semiconductor lasers such as Aluminium Gallium Arsenide (AlGaAs) lasers or Gallium Arsenide (GaAs) lasers have become more popular in laser acupuncture.

Most recently, ultralow level lasers (ULLL) have also been used in auricular acupuncture.

19.5.1. Helium Neon Laser. The Helium Neon (HeNe) laser, a low-level laser, was one of the first gas lasers. The medium of the laser is a mixture of Helium and Neon. In laser acupuncture, HeNe lasers have a wavelength of 632.8 nm [99].

19.5.2. Argon Laser. The Argon laser is also a low-level and gas laser. In laser acupuncture, the wavelength of an Argon laser is usually 514 nm [73].

19.5.3. Aluminium Gallium Arsenide Laser. The Aluminium Gallium Arsenide (AlGaAs) laser is a semiconducting low-level laser. In laser acupuncture, the standardized wavelength is 780 nm [99].

19.5.4. Gallium Arsenide Laser. The Gallium Arsenide (GaAr) laser is also one of the most commonly used semiconducting low-level lasers. Its standardized wavelength is 940 nm in laser acupuncture [99].

Figure 13: Laser needle for acupuncture.

19.5.5. Ultralow-Level Laser (ULLL). Recently, there have been also studies of ultralow-level lasers (ULLL) in auricular as well as body acupuncture. The output power of an ULLL is significantly smaller than of an LLL; consequently, the energy density and the power density are also a lot smaller [65, 100, 101].

19.6. "Laser Needles". In 2001, the first "laser needles" were invented at the University of Paderborn in Germany, with the trade name Laserneedle (Figure 14). Since then, many new developments of these laser needles evolved. This semiconducting laser instrument is equipped with up to 10 laser needles and has the ability to work on different wavelengths, mainly red (685 nm), infrared (788 nm), and violet (405 nm) [95].

19.7. Classification and Safety of Low-Level Lasers. According to the European Norm (EN 60825-1), low-level lasers are classified as "3R" lasers equivalent to the old classification "3b," meaning that radiation can be a risk of serious damage to the eye. To avoid this risk, the patient and acupuncturist should wear special glasses during the treatment. Laser as well as the treatment room must be marked specifically [10, 102].

20. Technical Parameters

20.1. Wavelength. The wavelength is one of the most important technical parameters in laser acupuncture. Its unit is expressed in nanometers (nm). A laser can have a wavelength spectrum between 240 nm and 3,000 nm. In laser acupuncture the lasers have wavelengths between 405 nm and 904 nm (Table 2). Lasers with wavelengths over 785 nm are infrared lasers in other words invisible light lasers. The wavelength is not only responsible for the coloring of the laser light but also how deep the laser light penetrates through the skin. For example, the red laser light with a wavelength of 685 nm has a penetration depth of about 4 cm [95, 103, 104].

20.2. Output Power. The output power is the power level of the laser light. Its unit is given in milliwatts (mW). In laser acupuncture, the applied dose depends on the output power of the laser. The higher the output power the higher the power density influences the depth of penetration. The designation of average output power is also very important especially when using a pulsed laser [95].

Figure 14: Laser needles. Modified from [97].

Table 2: Wavelengths. Modified from [15].

Colour	Wavelength
Extreme violet	400 nm
Middle violet	420 nm
Blue-violet	440 nm
Middle blue (icy blue)	470 nm
Blue-green	500 nm
Green	530 nm
Green-yellow	560 nm
Sunlight	570 nm
Yellow	580 nm
Orange-yellow	590 nm
Orange	600 nm
Red-orange	610 nm
Light red	650 nm
Dark red	780 nm

20.3. Power Density. The power density is expressed in terms of watt per cm^2 (W/cm^2) or milliwatt per cm^2 (mW/cm^2). It states the intensity of the laser beam and is in indirect proportion to the diameter of the laser beam [95].

Schikora [105] calculated that the power density had to be more than 1.3 W/cm^2 for laser acupuncture to achieve the same effect as needle acupuncture.

Calculation of the power density (mW/cm^2) is as follows:

$$\text{Power Density } \left(\text{mW/cm}^2\right) = \frac{\text{Output Power (mW)}}{\text{Beam Area } \left(\text{cm}^2\right)}. \quad (1)$$

20.4. Energy Density. The unit of the energy density is watt-seconds per cm^2 (Ws/cm^2), which equals Joules per cm^2 (J/cm^2). The energy density is the treatment dose and states the amount of energy supplied per cm^2 for the time of irradiation [95].

Calculation of the energy density (Ws/cm^2)

$$\text{Energy Density } \left(\text{Ws/cm}^2\right) = \frac{\text{Output Power (mW)} \times \text{Time (s)}}{\text{Beam Area } \left(\text{cm}^2\right)}. \quad (2)$$

20.5. Beam Diameter or Beam Area. Another important parameter is the beam area or the diameter of the beam (cm^2). With one of these two parameters the energy density can be calculated if the output power and the time are stated.

Calculation of the Beam area (cm^2)

$$\text{Beam Area } \left(\text{cm}^2\right) = \text{Diameter (cm)} \times 0.7854. \quad (3)$$

20.6. Dose Range. According to the study of Litscher and Opitz [95], the dose range can be "from 0.001 J/cm^2 (=Ws/cm^2) to 10 J/cm^2 (=Ws/cm^2) and more."

20.7. Continuous or Pulsed Laser. A laser can have pulsed or continuous waves. There is the assumption that pulsed laser light can "interfere with other pulsing biological phenomena" which would make a continuous waved laser a better option for laser acupuncture treatment. As mentioned previously, it is important to calculate the average output power when using pulsed lasers [95].

20.8. Time of Radiation. The time of radiation is also an important parameter in laser acupuncture. Most of the time it is in seconds (s). It is an important parameter for the calculation of energy density. The radiation time can last from 1 s to up to 100 s [37].

21. Important Study Parameters

According to Birch [106] studies on acupuncture respectively on auricular acupuncture should fulfil additional criteria to the already existing international standards of medical journals in order to achieve sufficient evidence.

Most important parameters that should be considered in studies of auricular laser acupuncture are listed in the following.

21.1. Research Question. The question of research needs to be clearly defined. On the basis of this research question, a proper study model shall be designed in order to achieve a meaningful result [106].

21.2. Randomized Controlled Studies. The use of randomized controlled studies has often been discussed in the literature. Still they are considered to be necessary for the efficiency of a study and stay as a gold standard [48, 106, 107].

21.3. Sample Size. The sample size in a study is also a very important parameter for the evidence of the effectiveness of auricular acupuncture. A smaller amount can be used in pilot studies however, in studies with more reference groups

the amount of people has to be larger in order to give sufficient evidence. In most of the studies of auricular acupuncture, the number of treated patients is often too low [106].

21.4. Criteria. The inclusion and exclusion criteria need to be accurately defined and strictly executed [106].

21.5. Acupuncturist. Acupuncturists need to have very good qualifications which have to be stated in detail in the study. If there is more than one acupuncturist involved in the study then they should receive special training in accordance with the study. Particularly in auricular acupuncture with needles the points have to be needled correctly otherwise the results have a higher chance of being distorted. However, the risk of falsified results decreases by using a laser [106].

21.6. Nomenclature and Location. Due to the different nomenclatures in auricular acupuncture, the name and the exact location of the points need to be stated clearly in studies.

21.7. Description of the Acupuncture Proceeding. The description of the proceeding has to include detailed information on the treated points, the system (e.g., Chinese or Western), the material used, the number and frequency of treatments, and the methods such as manual needling, electrical stimulation or laser stimulation. Studies on electroacupuncture and laser acupuncture need to provide proper information on technical parameters [106].

21.8. Period of Treatment. Another important parameter for the quality of a study is the time period of the treatment and the follow-up. Birch [106] suggested a time period of at least 3 months, preferably one year. The length of periods can vary depending on the pathological conditions; longer durations are also suggested for studies on drugs and alcohol abuse [108].

21.9. Number of Treatments. The amount of treatments and the time between treatments are very important parameters for reproducible studies in laser auricular acupuncture [109].

21.10. Reference Groups. Birch [106] recommended that the acupuncture treatment should always be compared to 3 different groups. The acupuncture treatment should be compared to sham acupuncture group, a group receiving standard medical therapy as well as a group which does not receive any treatment. Most of the studies compare auricular acupuncture to sham treatment groups or standard medical treatment groups.

21.11. Sham Acupuncture versus Placebo Acupuncture. Birch [106] believes in most cases sham acupuncture with needles is used under wrong assumptions. Sham treatment is often mistakenly equated to placebo treatment. The chosen sham acupuncture points are not necessarily used for treatment of the medical condition that is investigated; however, they can still have an impact on the body when they are needled.

In the study of Irnich et al. [110], sham laser acupuncture was conducted by deactivating the laser irradiation but not the red light or acoustic sound. In this case, sham laser acupuncture cannot be counted as a placebo treatment. It can only be treated as a placebo treatment if the laser stays entirely switched off. This must be clearly stated in the studies.

21.12. Additional Medication. Studies where auricular acupuncture is used as an adjunctive treatment and studies of certain pathological conditions necessitate additional use of medical treatment, for example, withdrawal symptoms, anxiety, pain, anaesthesia, or hypertension. Here, the medication needs to be documented accurately [106].

22. Human Skin

22.1. Optical Properties of the Skin. The optical properties of the human skin play an important role in laser acupuncture. The multilayered, inhomogeneous, and anisotropic structure of the skin determines the absorption and scattering of the radiation.

Melanin and haemoglobin are the main substances responsible for the absorption of the laser light. Melanin, responsible for the pigmentation of the skin, is mainly situated in the dermis and haemoglobin mainly in the epidermis. Further substances such as bilirubin, carotene, lipids, cell nuclei, and filamentous proteins may also contribute to the absorption of the laser light.

The scattering of the radiation is mainly caused by the filamentous proteins Keratin, which is found in the epidermis and responsible for the thickness of the skin, and collagen in the dermis. Melanosomes in the epidermis, cell nuclei, cell walls, and other structures can also have an influence on the scattering of the radiation.

Due to the dynamic of these substances their influence on the penetration of the laser light varies among human beings. Just to name a couple of examples, the thickness of the skin starts decreasing at the age of 45; the difference in the pigmentation of the Caucasian and the black population is caused by the different concentration of melanin [103, 111, 112].

22.2. Penetration of the Laser Light. The optical properties and the wavelength are the two main factors for the penetration of the laser light. It is believed that in body acupuncture the nociceptive structures lie 2-3 cm underneath the skin's surface. To achieve this depth, the wavelength must be between 605 nm and 850 nm which can be reached by red and infrared lasers [103, 105].

Recent studies with violet lasers have shown an effect on the blood flow velocity in the basilar artery by stimulating body acupuncture points. The penetration depth of the violet laser with a wavelength of 405 nm is about 1 mm [94].

Esnouf et al. [113] conducted a study evaluating the penetration depth of an Aluminium Gallium Arsenide laser with a wavelength of 850 nm. It showed that "most of the laser radiation was absorbed within the first 1 mm of the skin." With this new finding it can be assumed that a violet laser

can have the same effect as red or infrared lasers. Due to its low penetration depth the violet laser might even be the laser of first choice in auricular acupuncture since the penetration depth on the auricle does not necessarily require the same depth as in body laser acupuncture.

22.3. "Deqi" Sensation. In TCM the "Deqi" sensation is a signal to confirm the correct positioning of the needle in body acupuncture. "Deqi" can be seen as a benchmark of efficient acupuncture. It has been described as a feeling of numbness, prickling, flowing, or heaviness around the acupuncture point. Acupuncturists can make the "Deqi" sensation more perceptible by rotating or moving the needle back and forth during the therapy. In auricular acupuncture patients have described the "Deqi" feeling as a warm sense on the ear during treatment [45, 114].

Recent studies on body acupuncture suggest that "Deqi" sensation can also be achieved through laser acupuncture. In the studies the patients identified the "Deqi" sensation as a heavy sense or an electrical current or a feeling of an ant bite [94, 115].

23. Methods of Verification

In the last years, different noninvasive bioengineering methods have been used for verifying laser acupuncture.

Methods for the measurement of the *peripheral effect* are

(i) Laser Doppler Flowmetry (LDF) and

(ii) Laser Doppler Imaging (LDI).

The *cerebral effects* can be measured with

(i) multidirectional Transcranial Ultrasound Doppler Sonography (TCD),

(ii) cerebral Near-Infrared Spectroscopy (NIRS), and

(iii) functional Magnetic Resonance Imaging (fMRI).

23.1. Laser Doppler Flowmetry (LDF). Laser Doppler flowmetry is a method to measure changes in the microcirculation. Flux is the main parameter and is defined as the product of average velocity and concentration of red blood cells in superficial vessels. This technique uses the Doppler effect—also called Doppler shift—which is based on the shifting of the light caused by moving blood cells [104, 116].

23.2. Laser Doppler Imaging (LDI). This method is also based on the principle of the Doppler effect. Hereby, the reflected light acquires data that are transformed into a color-coded picture displaying the distribution of tissue perfusion [104].

23.3. Multidirectional Transcranial Ultrasound Doppler Sonography (TCD). The TCD is a relatively new recording technique in acupuncture which is able to measure the blood flow velocity in the middle cerebral artery, the anterior cerebral artery, the posterior cerebral artery, the supratrochlear artery, and the ophthalmic artery. The TCM Research Center in Graz developed a helmet for transcranial ultrasound investigations where the probe holder construction was situated at the "windows to the brain," meaning transnuchal, transtemporal, and transorbital (Figure 15) [104].

23.4. Functional Magnetic Resonance Imaging (fMRI). With this technique, it is possible to measure metabolic and circulatory changes of the brain. Hereby the fMRI uses the magnetic properties of oxygenated and deoxygenated haemoglobin to identify changes in their concentration in the blood [104].

23.5. Near Infrared Spectroscopy (NIRS). NIRS is a noninvasive spectroscopic technique that measures the functional activities in the brain by using an optical window in the near-infrared light spectrum. The spectral range must be between 630 nm and 1300 nm, so the light can get through the cranium to investigate the metabolism in the cerebral cortex [104].

23.6. Bispectral Index (BIS) and Electroencephalogram (EEG). The EEG is a method to measure electrical brain activities. In auricular acupuncture, EEG is mainly used to examine the activities of the cerebral cortex.

The BIS is an important numerical descriptor of the EEG which is mainly used in anaesthesia [30, 104].

24. Advantages

Taking into account that acupuncturists handle the laser equipment with care and responsibility, the advantages opposite the disadvantages in laser auricular acupuncture are outbalanced.

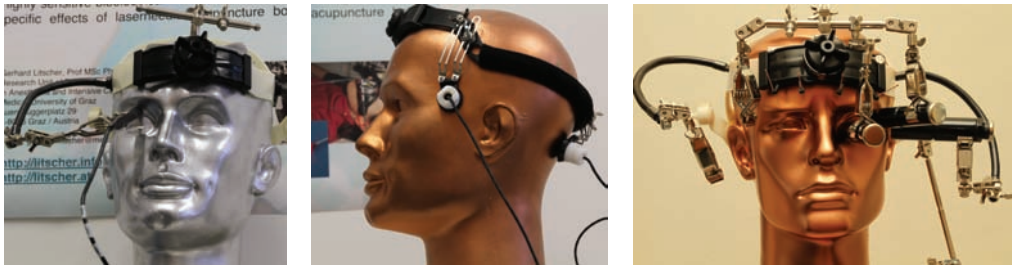
24.1. General Advantages. Auricular acupuncture is often used as an adjunctive treatment, for example, during withdrawal therapies or for pain relief. It has shown that patients treated additionally with auricular acupuncture required less medication which further leads to a reduction of side effects and secondary diseases and consequently results in a decrease of costs [54–57, 72, 74].

Auricular acupuncture therapy does not require an inpatient treatment. Patients can be treated as outpatients which would further lead to a decrease of inpatients following a reduction of costs.

24.2. Comparison to Auricular Acupuncture with Needles. The laser treatment being a noninvasive method is one of the biggest benefits. Patients with fear of needles as well as children of all ages can be treated. The low risks of local bleeding and infections such as perichondritis and chondritis disappear entirely [99].

In studies of auricular laser acupuncture a placebo treatment can be simulated easier than in studies of acupuncture with needles.

Auricular acupuncture with laser has also the advantage that it takes less time than with needles [37].



(a) (b) (c)

Figure 15: "Windows of the brain": transorbital ((a), (c)), transnuchal ((b) right), transtemporal ((b) left, (c) right). Modified from [104].

24.3. Comparison to Body Acupuncture with Laser. Auricular acupuncture has two main advantages versus body acupuncture in needling as well as in laser treatment; on one side auricular acupuncture is easier to apply because the patients do not need to get undressed and on the other side the stimulation is not as sophisticated as in body acupuncture [59].

25. Disadvantages

Risk of serious damage to the eye can be reduced drastically or even erased by cautious use of the laser instrument and also the wearing of safety glasses during the treatment.

26. Recent Studies and Their Quality

Not many studies have been published up to now. This is no surprise considering that auricular acupuncture has only found its way into medicine in the last 60 years and laser therapy only in the last 40 years. By using the keywords "auricular" and "acupuncture" and "laser" on PubMed as few as 28 publications were found. The first paper was published in the year 1982. Out of these 28 publications, 17 studies were published in English, 6 in Russian, 3 in Chinese, and 2 in Italian.

Only 14 publications investigated specifically the effects of auricular acupuncture with laser. In the following 10 of these studies will be discussed in detail; the remaining 4 studies were either in Russian or Chinese and were not taken for further evaluation.

With the exception of 2 studies, different subjects were investigated which made it difficult to compare the results of the publications with each other in order to find sufficient evidence of auricular acupuncture with laser.

26.1. Experimental Pain. King et al. [51] investigated on 80 healthy subjects whether laser auricular acupuncture can reduce pain or its threshold. Eighty healthy subjects with regard to the exclusion and inclusion criteria were chosen to take part. The subjects were randomly divided into 2 groups, an experimental group which received laser treatment on Shenmen, Lung point, Wrist point, and Dermis point and a control group which received sham acupuncture on the same points but without the laser being activated. Each point was stimulated for 30 s. For the stimulation of the acupuncture points, a continuous waved HeNe laser with a wavelength of 632.8 nm, an average power of 1 mW and a peak power of 1 mW was used. For the simulation of the pain an electric electrode was fixed on the left wrist of the subjects. There was no further information on the technical parameters such as power density, energy density, or the diameter of the laser beam. In 71% (29 subjects) of the experimental group there was an increase in the threshold of the pain whereas in the control group the increase was only 33% (13 subjects).

The outcome shows evidence that laser auricular acupuncture can have an effect on pain. Further studies need to be conducted for more evidence; however, the missing technical parameters make a reproducibility of this study difficult. Studies on patients with real pain instead of experimental pain should also be investigated.

26.2. Insomnia. In the Chinese study of Yao [117], 46 patients were treated for insomnia under the aspects of TCM. The treated auricular points were Shenmen, Endocrine point, Subcortex point, Brain point, Heart point, Kidney point, Spleen point, Stomach point, Liver point, and Kidney point. The laser instrument was a PU-1 semiconductor laser with a wavelength of 830 nm, an output power of 3 mW. The diameter of the laser beam was 2 mm. The irradiation time lasted for 1 minute. The treatment was once a day, 12 times with pauses of 5-to 7-day in-between therapy. The results showed that 69.5% of the patients (32 patients) were able to sleep for more than 7 hours, 28.3% of the patients (13 patients) were able to sleep between 5 and 6 hours. Auricular acupuncture had no effect on only one patient (2.2%). The total effectiveness was 97.8%.

This outcome shows that auricular acupuncture can be effective in the treatment of insomnia. However, a description of the exact location of the points was missing and there was no information whether the patients received laser treatment on all of the mentioned points or only on certain points. The technical parameters did not include if it was a pulsed or continuous waved laser. No reference groups were selected. No inclusion or exclusion criteria were defined and the amount of people treated was too low to give sufficient evidence on this subject.

Further studies need to be conducted for more evidence. Due to the lack of information, a reproducibility of this study is very difficult.

26.3. Acne Vulgaris. In a Chinese study of Sun [118], 68 patients were treated for acne vulgaris. These patients were randomly divided into a treatment group of 36 people who received laser auricular acupuncture and into a control group of 32 people who received body acupuncture with needles. For auricular acupuncture the points Lung, Spleen, Large Intestine, Sanjiao, Endocrine, Adrenal Gland, and Cheek were stimulated with an HeNe laser. Only 3 or 4 of these points were used for each treatment; however, there was no further information how often or in which combination these points were used and no description of the exact location of the auricular points. The HeNe laser had power density of 25 mW/cm^2. The points were treated with this laser from a distance of 30–50 cm for 3–5 minutes per session. Altogether, the patients received 10 treatments. In the control group the patients were treated 10 times on different body acupuncture points with needles.

After the treatment 28 patients of the treatment group had no lesions left, in 6 cases 70% of the lesions were gone and only 2 patients had a cure rate of 30–70% of the lesions, whereas in the control group only 15 patients had a full cure of the lesions, in 14 cases 70% of the lesions were gone and only 30–70% of the lesions disappeared on 3 patients [118].

The results show evidence of the impact of auricular laser acupuncture. However, the exact location and combination of the auricular points, further technical parameters, for example, beam diameter or energy density, and a definition of exclusion and inclusion criteria were missing. Further studies with higher sample size and a third nontreatment reference group should be conducted for further proof of the effect of auricular laser acupuncture. The lack of information makes it again difficult to reproduce this study.

26.4. Alcohol Addiction and Withdrawal. In recent years auricular acupuncture has been subject to many studies on alcohol addiction or alcohol withdrawals. So far two studies on different subjects in this field have been published on auricular acupuncture with laser.

Zalewska-Kaszubska and Obzejta [73] investigated the possibility of auricular acupuncture as an adjunctive treatment of alcohol abuse and of its influence of the β-endorphin level in the plasma which is believed to have an influence on alcohol withdrawal. Fifty three patients were treated with a combination of auricular laser acupuncture and body laser acupuncture. The patients were chosen by exclusion and inclusion criteria. Out of these 53 patients only 15 patients finished the study. The patients were not divided into reference groups.

The auricular points were stated in numbers 82, 83, 87, 51, and 55. According to Rubach [37], point number 82 is Point Zero, number 83 is the Anxiety point, 87 is the Stomach point, 51 is the Vegetative point I, and 55 is Shenmen. These points were stimulated with a semiconducting Argon laser with a wavelength of 540 nm, an output power of 100 mW, and a beam area of 0.05 cm^2. The irradiation time lasted for 10 s per point.

A spot on the neck vessel projection was stimulated by an HeNe laser with a wavelength of 632.8 nm, an output power of 25 mW, and a beam area of 0.2 cm^2. The irradiation time was 5 min. There was no further information about the exact location of this spot.

The whole treatment consisted of 4 sessions. Each session lasted 20 days. The auricle was stimulated every 2nd day and the neck vessel was continuously stimulated over a 20-day period. Inbetween the sessions there was a break of one week. For the valuation of the β-endorphin level, the patients gave blood before the first laser treatment and one day after each laser session. They also had to undertake the Beck Depression Inventory-Fast Screen (BDI-FS) after each session for evaluating their state of mind.

The results showed an increase in the β-endorphin level and according to the BDI-FS a positive mood in the patients. The authors believe that an increase in the β-endorphin levels is linked to an increase in the mood and consequently a reduction of alcohol withdrawal [73].

This study gave sufficient information on the technical parameters of the lasers but insufficient information on the location of the stimulated auricular as well as body points. Although the results showed a positive outcome, the low number of patients which completed the study and the missing of reference groups call for further studies with better quality and information.

Trümpler et al. [119] investigated in their randomized controlled study the duration of alcohol withdrawal by comparing laser acupuncture with needle acupuncture and sham laser acupuncture on the auricle. Forty eight inpatients were chosen under certain criteria and were randomly divided into three reference groups. The points were chosen individually with the help of an electronic point finder. The most frequently stimulated points were—according to the nomenclature of Oleson—the Diaphragm point, the Cheerfulness point, the Insomnia point, the Sympathetic point, the Spleen point, the Laterality point, a Lung point, and Shenmen. A semiconducting laser with a wavelength of 830 nm (name: Modulus; product of schwa-medico, Inc., Ehringshausen, Germany) was used for the auricular laser acupuncture. No further technical parameters such as output power, diameter of the beam, or beam area were given. Each auricular point was stimulated for 60 s. In the laser sham acupuncture group the laser was inactivated. The needle acupuncture was performed with stainless steel needles to a depth of 1–3 mm for about 40 min.

The treatment was applied daily until the end of withdrawal symptoms. Besides the acupuncture treatment, patients received Clomethiazole or in case of intoleration benzodiazepines on an individual basis. Other medications were kept unchanged for the time of the study.

The outcome of this study did not show any difference of the withdrawal duration inbetween the reference groups. The needle acupuncture treatment seemed to have more efficiency than laser acupuncture treatment. However, there was no difference between sham and laser acupuncture. There was no information whether the sham acupuncture was applied with an entirely switched off laser or the laser irradiation was just deactivated. The authors see the cause for this insufficient result in the small number of patients, the different degrees of severity of diseases induced by long-term

alcohol abuse, the different pharmacological management of withdrawal, and unequal provision of care [119].

For further improvement of this study's quality a non-treatment group should have been added as another reference group. Also, the combination of the individual auricular points should have been listed more precisely; maintaining to the standard use of the NADA might lead to a more meaningful result and better comparison for further studies.

26.5. Chronic Allergic Dermatoses. Hou et al. [50] investigated the effects of auricular laser acupuncture on 35 patients suffering from eczemas, on 46 patients suffering from urticaria, on 41 suffering from fascial cosmetic dermatitis, and on 25 patients suffering from atopic dermatitis. The auricular acupuncture points Shenmen, Urticaria, and Subcortex were stimulated with a semiconducting AlGaAs laser each for 3 minutes. There was no further technical information of the laser. The whole treatment consisted of 2 sessions. Each session had a 5-minute treatment with intervals of 3-4 days inbetween. Medical treatment of antihistamine and corticosteroid was discontinued and a serum IgE level was determined before and after treatment. Only in the cases of eczema and urticarial, the IgE levels decreased after the treatment. The symptoms of all four dermatoses, however, improved significantly.

Even though the results show evidence in the treatment of chronic dermatoses with auricular acupuncture, the deficiency of information, and the small amount of patients give this study a poor quality.

For further evidence, a randomized controlled study with a larger number of patients, exact information of technical parameters, and definition of criteria as well as a longer period of treatment including a follow-up should be conducted.

26.6. Laser Acupuncture for Adolescent Smokers. In this double-blind randomized study of Yiming et al. [62], auricular laser acupuncture was investigated for cessation of smoking on adolescent smokers. Three hundred thirty patients between the age of 12 and 18 years were divided into a laser acupuncture group and a sham acupuncture group. 128 adolescents of the laser acupuncture group completed the study and were treated on the auricular acupuncture points Ershénmén (probably Shenmen), Kó (probably Mouth), Fèi (probably Lung), and Wàibi. One hundred fourty adolescents of the sham acupuncture group completed the study and were treated on the same auricular points but without any radiation.

An HeNe laser with a wavelength of 632.8 nm, an output power of 2.5 to 3 mW, and a diameter of 1 mm was used for the laser stimulation. The auricular points were stimulated from a distance of about 1 mm for 60 s. The distance was chosen so that no pain or sensation could be felt. The treatment consisted of 12 sessions. Three sessions were given per week.

The results showed no significant effect of auricular laser acupuncture compared to sham acupuncture. In both groups around 21% of the patients stopped smoking after the first treatment. A three-month follow-up showed a complete cessation of 25% of 101 patients in the laser acupuncture group and 26% of 107 patients in the sham acupuncture group [62].

The quality of this study was low. For a reproduction of this study, the nomenclature of the points and its localization should have been described according to the nomenclature of the WHO [14]. The details of the technical parameters were imprecise. It is also questionable whether with a distance of about 1 mm the laser acupuncture can have the same effect than by placing the laser needle directly on the skin.

26.7. Animal Experiment: Microcirculation. Komori et al. [70] investigated in 40 rabbits the changes in microcirculation after auricular stimulation. The rabbits were randomly allocated into 4 reference groups with 10 rabbits each. The first group received needle acupuncture treatment, the second received near-infrared lamp irradiation, the third group received laser acupuncture stimulation, and the fourth group acted as control group and received no treatment. Stainless needles were used for the needle acupuncture treatment and a near-infrared lamp with a wavelength of 1540 nm and an energy density of 40 mW/mm^2 were used for the near-infrared treatment. The irradiation time was 1 s. A semiconducting AlGaAs laser with a wavelength of 830 nm, an output power of 60 mW, an energy density of 39 mW/mm^2,and a diameter of 1.4 mm were used for the laser acupuncture. The rabbit ear chamber method was used for the verification of the microcirculation. This method facilitates the observation of a single blood vessel via intravital microscopy as well as effects of different interventions on peripheral hemodynamics.

The stimulated area on the rabbits' ears corresponds to the concha of the human auricle. The results showed a significant increase in the arteriolar diameter, the blood flow velocity and the blood flow rate after needle acupuncture, near-infrared lamp irradiation as well as laser acupuncture. Several studies have been conducted examining the relation of laser as well as needle acupuncture to the blood flow. Substance P—a neurotransmitter and calcitonin-related peptide—and Nitric oxide—responsible for vasodilatation—seemed to play an important role in this mechanism [70].

This study was of good quality and it proved that auricular acupuncture has an effect on the microcirculation. It can be considered to have an effect on diseases caused by poor peripheral blood flow. Further studies should be performed on this subject.

26.8. Ultralow-Level Laser: Postural Instability. Bergamaschi et al. [100] investigated on 34 elderly patients the effects of auricular as well as body laser acupuncture with an ultralow-lever laser. The patients were divided into 3 reference groups. nine patients received auricular laser acupuncture, 9 patients received body laser acupuncture, and 16 patients received sham auricular acupuncture, hereby the laser stayed switched off. A semiconducting laser (product: BioliteLP020) with an output power of 0.03 mW was used. The irradiation was administered in 20 half-second flashes. The single point energy dose was about 0.3 mJ. The bioactive body acupuncture points were selected with a point finder. Instead of stimulating auricular acupuncture points auriculotherapy

zones according to the Auriculoterapia Posturale secondo Scoppa method were stimulated. For the verification of the results, 4 balance tests were performed before treatment, right after treatment within 15 minutes of the first test, within 1 hour after the first test, and 3 days after the first test. The results showed an average improvement of 15% after the treatment and a long-term improvement of 5–10% 3 days after the treatment in body as well as auricular laser acupuncture.

The results show slight evidence on the effects of body and auricular laser acupuncture. For further evidence, more studies on this subject need to be conducted with a higher amount of treated patients, a definition of exclusion and inclusion criteria plus the medication of the patients should be taken into account.

26.9. Ultralow-Level Laser: Violet Laser Stimulation on Rats. Gao et al. [65] conducted a study testing the effects of a violet laser on heart rate, heart rate variability, and mean arterial blood pressure on anaesthetised rats by stimulating two body acupuncture points (Baihui (GV20) and Zusanli (ST36)) and the Heart point situated on the inferior concha of the auricle. The points were stimulated separately with a continuous waved laser (product: Conrad Electronic, SE) with a wavelength of 405 nm and an output power of 1 mW. The irradiation time lasted 2 minutes. The blood pressure was measured via a polyethylene catheter on the left common carotid artery connected to a blood pressure transducer and amplifier. The mean heart rate and the heart rate variability were monitored with an electrocardiogram (ECG) and measured before, during, and after the stimulation.

The results showed changes in the heart rate on all acupuncture points especially after the stimulation of Baihui. The total heart rate variability changed insignificantly on all acupuncture points. The mean arterial blood pressure decreased slightly after stimulating the Baihui acupoint [65].

This study showed that a violet laser can have an impact on physiological neurovegetative parameters. However, there was no evidence that a violet laser had more effect on auricular acupuncture than on body acupuncture. Quite the contrary, the stimulation on Baihui showed more effect than on the Heart point. Further studies need to be carried out for further evidence.

27. Conclusion

Recent studies have shown that auricular laser acupuncture can have an effect on the body.

However, a comparison of the results is not given because of the small amount of studies, different subjects of research, and different study approaches. Studies in the past have often shown low quality due to insufficient and different levels of information.

In order to achieve a higher grade of comparability the different technical and study parameters discussed previously have to be stated accurately. Furthermore, study designs of the same subject need to be reconciled with each other. To achieve meaningful and comparable results, further studies following these proposals need to be conducted.

Recent conclusive studies of auricular acupressure, auricular electroacupuncture and auricular acupuncture, with needles should also be consulted for further studies on auricular laser acupuncture for example studies investigating the mechanism of auricular acupuncture. There is strong evidence that the influence on the autonomic nervous system through the stimulation of the ABVN can be one of its mechanisms [20, 26, 77].

By taking all these basic conditions into consideration, future studies on auricular laser acupuncture will be more significant and will show stronger evidence of its effect.

Conflict of Interests

The authors declare that they have no conflict of interests.

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References

[1] G. N. Asher, D. E. Jonas, R. R. Coeytaux et al., "Auriculotherapy for pain management: a systematic review and meta-analysis of randomized controlled trials," *Journal of Alternative and Complementary Medicine*, vol. 16, no. 10, pp. 1097–1108, 2010.

[2] P. Whittaker, "Laser acupuncture: past, present, and future," *Lasers in Medical Science*, vol. 19, no. 2, pp. 69–80, 2004.

[3] F. Bahr, K. Bushe-Centmayer, L. Dorfer et al., *The Big Book of Classical Acupuncture*, Elsevier, Munich, German, 2007.

[4] T. Schnura, *Diagnosis and Point Selection According to TCM*, Elsevier, Munich, German, 2009.

[5] J. Jia, Y. Yu, J.-H. Deng et al., "A review of Omics research in acupuncture: the relevance and future prospects for understanding the nature of meridians and acupoints," *Journal of Ethnopharmacology*, vol. 140, no. 3, pp. 594–603, 2012.

[6] L. Dorfer, M. Moser, F. Bahr et al., "A medical report from the stone age?" *The Lancet*, vol. 354, no. 9183, pp. 1023–1025, 1999.

[7] T. Oleson, *Auriculotherapy Manual: Chinese and Western Systems of Ear Acupuncture*, Elsevier, London, UK, 2003.

[8] G. Chen, "Advances in researches on auriculo-acupoints," *Journal of Traditional Chinese Medicine*, vol. 11, no. 3, pp. 216–223, 1991.

[9] L. Gori and F. Firenzuoli, "Ear acupuncture in European traditional medicine," *Evidence-Based Complementary and Alternative Medicine*, vol. 4, no. 1, pp. 13–16, 2007.

[10] F. Bahr and B. Strittmatter, *The Big Book of Ear Acupuncture*, Hippokrates, Stuttgart, Germany, 2010.

[11] J. Chalmers, "Modern auricular therapy: a brief history and the discovery of the vascular autonomic signal," *Journal of Chinese Medicine*, vol. 84, pp. 5–8, 2007.

[12] B. L. Frank, *Auricular Medicine and Auricular Therapy—A Practical Approach*, Author House, Bloomington, Ind, USA, 2007.

[13] B. L. Frank and N. Soliman, "Obesity treatment through auricular therapy and auricular medicine," *Medical Acupuncture*, vol. 14, no. 1, pp. 33–35, 2002.

[14] World Health Organization, *Report of the Working Group on Auricular Acupuncture Nomenclature*, Lyon, France, 1990.

[15] R. Helling and M. Feldmeier, *Auriculomedicine According to Nogier*, Hippokrates, Stuttgart, German, 1999.

[16] N. Soliman and B. L. Frank, "Auricular acupuncture and auricular medicine," *Physical medicine and rehabilitation clinics of North America*, vol. 10, no. 3, pp. 547–554, 1999.

[17] E. T. Peuker and T. J. Filler, "The nerve supply of the human auricle," *Clinical Anatomy*, vol. 15, no. 1, pp. 35–37, 2002.

[18] A. Waldeyer, *Human Anatomy*, Walter de Gruyter, Berlin, German, 2003.

[19] T. Oleson, "Auriculotherapy stimulation for neuro-rehabilitation," *NeuroRehabilitation*, vol. 17, no. 1, pp. 49–62, 2002.

[20] W. He, X. Wang, H. Shi et al., "Auricular acupuncture and vagal regulation," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 786839, 6 pages, 2012.

[21] N. Ueno, H. Sudo, Y. Hattori, K. Yuge, T. Miyaki, and H. Ito, "Innervation of the external ear in humans and the musk shrew," *Journal of Otolaryngology of Japan*, vol. 96, no. 2, pp. 212–218, 1993 (Japanese).

[22] T. D. Oleson, R. J. Kroening, and D. E. Bresler, "An experimental evaluation of auricular diagnosis: the somatotopic mapping of musculoskeletal pain at ear acupuncture points," *Pain*, vol. 8, no. 2, pp. 217–229, 1980.

[23] H. Heine, *Anatomical Correlates of Acupuncture Points*, vol. 7 of *Spektrum der Wissenschaft*, German, 1993.

[24] W. Y. Chung, H. Q. Zhang, and S. P. Zhang, "Peripheral muscarinic receptors mediate the anti-inflammatory effects of auricular acupuncture," *Chinese Medicine*, vol. 6, no. 1, article 3, 2011.

[25] X.-Y. Gao, S.-P. Zhang, B. Zhu, and H.-Q. Zhang, "Investigation of specificity of auricular acupuncture points in regulation of autonomic function in anesthetized rats," *Autonomic Neuroscience: Basic and Clinical*, vol. 138, no. 1-2, pp. 50–56, 2008.

[26] X. Y. Gao, Y. H. Li, K. Liu et al., "Acupuncture-like stimulation at auricular point Heart evokes cardiovascular inhibition via activating the cardiac-related neurons in the nucleus tractus solitarius," *Brain Research*, vol. 1397, pp. 19–27, 2011.

[27] X.-Y. Gao, L. Wang, I. Gaischek, Y. Michenthaler, B. Zhu, and G. Litscher, "Brain-modulated effects of auricular acupressure on the regulation of autonomic function in healthy volunteers," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 714391, 8 pages, 2012.

[28] E. Haker, H. Egekvist, and P. Bjerring, "Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects," *Journal of the Autonomic Nervous System*, vol. 79, no. 1, pp. 52–59, 2000.

[29] W. He, P.-J. Rong, L. Li, H. Ben, B. Zhu, and G. Litscher, "Auricular acupuncture may suppress epileptic seizures via activating the parasympathetic nervous system: a hypothesis based on innovative methods," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 615476, 5 pages, 2012.

[30] C.-C. Hsu, C.-S. Weng, M.-F. Sun, L.-Y. Shyu, W.-C. Hu, and Y.-H. Chang, "Evaluation of scalp and auricular acupuncture on EEG, HRV, and PRV," *American Journal of Chinese Medicine*, vol. 35, no. 2, pp. 219–230, 2007.

[31] R. La Marca, M. Nedeljkovic, L. Yuan, A. Maercker, and U. Ehlert, "Effects of auricular electrical stimulation on vagal activity in healthy men: evidence from a three-armed randomized trial," *Clinical Science*, vol. 118, no. 8, pp. 537–546, 2010.

[32] L. K. McCorry, "Physiology of the autonomic nervous system," *American Journal of Pharmaceutical Education*, vol. 71, no. 4, article 78, 2007.

[33] T. R. Henry, "Therapeutic mechanisms of vagus nerve stimulation," *Neurology*, vol. 59, no. 6, pp. S3–S14, 2002.

[34] H. L. Huang, *Ear Acupuncture*, Rodale Press, Emmons, Pa, USA, 1974.

[35] P. F. M. Nogier, *Treatise of Auriculotherapy*, Maisonneuve, Moulins-les-Metz, France, 1972.

[36] M. Wexu, *The Ear Gateway To Balancing the Body: A Modern Guide to Ear Acupuncture*, ASI Publishers, New York, NY, USA, 1975.

[37] A. Rubach, *Principles of Ear Acupuncture—Microsystem of the Auricle*, Hippokrates, Stuttgart, Germany, 2001.

[38] Eagle Advancement Institute 2011, West Bloomfield, http://www.eagleadvancementinstitute.com/pstim/faq.asp.

[39] S. M. Sator-Katzenschalger and A. Michalek-Sauberger, "P-Stim auricular electroacupuncture stimulation device for pain relief," *Expert Review of Medical Devices*, vol. 4, no. 1, pp. 23–32, 2007.

[40] H. Strzyz and G. Ernst, "Adverse reactions to acupuncture," *Schmerz*, vol. 11, no. 1, pp. 13–19, 1997.

[41] H. S. Jones, "Clinical records. Auricular complications of acupuncture," *Journal of Laryngology and Otology*, vol. 99, no. 11, pp. 1143–1145, 1985.

[42] Y.-C. P. Arai, A. Ito, S. Hibino, S. Niwa, and W. Ueda, "Auricular acupunctures are effective for the prevention of postoperative agitation in old patients," *Evidence-based Complementary and Alternative Medicine*, vol. 7, no. 3, pp. 383–386, 2010.

[43] L. Bergdahl, A. H. Berman, and K. Haglund, "Patients' experience of auricular acupuncture during protracted withdrawal," *Journal of Psychiatric and Mental Health Nursing*, 2012.

[44] M. L. Bullock, T. J. Kiresuk, R. E. Sherman et al., "A large randomized placebo controlled study of auricular acupuncture for alcohol dependence," *Journal of Substance Abuse Treatment*, vol. 22, no. 2, pp. 71–77, 2002.

[45] J. L. Capodice, Z. Jin, D. L. Bemis et al., "A pilot study on acupuncture for lower urinary tract symptoms related to chronic prostatitis/chronic pelvic pain," *Chinese Medicine*, vol. 2, article 1, 2007.

[46] H. Chen, "Recent studies on auriculoacupuncture and its mechanism," *Journal of Traditional Chinese Medicine*, vol. 13, no. 2, pp. 129–143, 1993.

[47] Y. C. Hai, Y. Shi, S. N. Chi, M. C. Sai, K. K. L. Yung, and L. Z. Qing, "Auricular acupuncture treatment for insomnia: a systematic review," *Journal of Alternative and Complementary Medicine*, vol. 13, no. 6, pp. 669–676, 2007.

[48] A. D'Alberto, "Auricular acupuncture in the treatment of cocaine/crack abuse: a review of the efficacy, the use of the national acupuncture detoxification association protocol, and the selection of sham points," *Journal of Alternative and Complementary Medicine*, vol. 10, no. 6, pp. 985–1000, 2004.

[49] C.-H. Hsu, C.-J. Wang, K.-C. Hwang, T.-Y. Lee, P. Chou, and H.-H. Chang, "The effect of auricular acupuncture in obese women: a randomized controlled trial," *Journal of Women's Health*, vol. 18, no. 6, pp. 813–818, 2009.

[50] Y.-H. Hou, F. Xu, and S.-X. Wu, "Effect of auricular acupuncture with low power laser on four chronic allergic dermatoses and serum IgE level," *Chinese Medical Sciences Journal*, vol. 20, no. 4, p. 281, 2005.

[51] C. E. King, J. A. Clelland, C. J. Knowles, and J. R. Jackson, "Effect of helium-neon laser auriculotherapy on experimental pain threshold," *Physical Therapy*, vol. 70, no. 1, pp. 24–30, 1990.

[52] M. S. Lee, B.-C. Shin, L. K. P. Suen, T.-Y. Park, and E. Ernst, "Auricular acupuncture for insomnia: a systematic review," *International Journal of Clinical Practice*, vol. 62, no. 11, pp. 1744–1752, 2008.

[53] R. C. Niemtzow, "Imaginary acupuncture," *Medical Acupuncture*, vol. 21, no. 4, pp. 225–228, 2009.

[54] S. M. Sator-Katzenschlager, G. Scharbert, S. A. Kozek-Langenecker et al., "The short- and long-term benefit in chronic low back pain through adjuvant electrical versus manual auricular Acupuncture," *Anesthesia and Analgesia*, vol. 98, no. 5, pp. 1359–1364, 2004.

[55] T. I. Usichenko, M. Hermsen, T. Witstruck et al., "Auricular acupuncture for pain relief after ambulatory knee arthroscopy—a pilot study," *Evidence-Based Complementary and Alternative Medicine*, vol. 2, no. 2, pp. 185–189, 2005.

[56] T. I. Usichenko, M. Dinse, M. Hermsen, T. Witstruck, D. Pavlovic, and C. Lehmann, "Auricular acupuncture for pain relief after total hip arthroplasty—a randomized controlled study," *Pain*, vol. 114, no. 3, pp. 320–327, 2005.

[57] T. I. Usichenko, S. Kuchling, T. Witstruck et al., "Auricular acupuncture for pain relief after ambulatory knee surgery: a randomized trial," *Canadian Medical Association Journal*, vol. 176, no. 2, pp. 179–183, 2007.

[58] S.-M. Wang and Z. N. Kain, "Auricular acupuncture: a potential treatment for anxiety," *Anesthesia and Analgesia*, vol. 92, no. 2, pp. 548–553, 2001.

[59] S.-M. Wang, P. DeZinno, E. C. Lin et al., "Auricular acupuncture as a treatment for pregnant women who have low back and posterior pelvic pain: a pilot study," *American Journal of Obstetrics and Gynecology*, vol. 201, no. 3, pp. 271.e1–271.e9, 2009.

[60] J. F. Wang, H. X. Bao, Y. H. Cai, J. H. Zhang, and P. J. Tong, "Case-control study on application of auricular acupuncture for the treatment of analgesia during perioperative period in total hip arthroplasty," *Zhongguo Gu Shang*, vol. 25, no. 3, pp. 220–223, 2012 (Chinese).

[61] S. Wu, J. Liang, X. Zhu, X. Liu, and D. Miao, "Comparing the treatment effectiveness of body acupuncture and auricular acupuncture in preoperative anxiety treatment," *Journal of Research in Medical Sciences*, vol. 16, no. 1, pp. 39–42, 2011.

[62] C. Yiming, Z. Changxin, W. S. Ung, Z. Lei, and L. S. Kcan, "Laser acupuncture for adolescent smokers—a randomized double-blind controlled trial," *American Journal of Chinese Medicine*, vol. 28, no. 3-4, pp. 443–449, 2000.

[63] B. Strittmatter, *Pocket Atlas for Ear Acupuncture*, Hippokrates, Stuttgart, German, 2001.

[64] X.-Y. Gao, K. Liu, B. Zhu, and G. Litscher, "Sino-European transcontinental basic and clinical high-tech acupuncture studies-part 1: auricular acupuncture increases heart rate variability in anesthetized rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 817378, 2012.

[65] X.-Y. Gao, K. Liu, B. Zhu, and G. Litscher, "Sino-European transcontinental basic and clinical high-tech acupuncture studies–part 3: violet laser stimulation in anesthetized rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 402590, 2012.

[66] H. Q. Huang and S. Z. Liang, "Improvement of blood pressure and left cardiac function in patients with hypertension by auricular acupuncture," *Zhong Xi Yi Jie He Za Zhi*, vol. 11, no. 11, pp. 654–643, 1991 (Chinese).

[67] B. L. Frank and N. Soliman, "Shen Men: a critical assessment through advanced auricular therapy," *Medical Acupuncture*, vol. 10, no. 2, 1999.

[68] X. Y. Gao and B. Zhu, "Analysis on blood-pressure-decreasing effect of electric pulse stimulation of auricular acupoint Jiangyagou in the patient of hypertension," *Zhongguo Zhen Jiu*, vol. 25, no. 7, pp. 474–476, 2005 (Chinese).

[69] C.-S. Jia, X.-S. Ma, X.-F. Li et al., "Clinical study on auricular acupoint penetration needling along the skin for treatment of a variety of pain syndrome and dysfunction," *Journal of Traditional Chinese Medicine*, vol. 31, no. 3, pp. 169–172, 2011.

[70] M. Komori, K. Takada, Y. Tomizawa et al., "Microcirculatory responses to acupuncture stimulation and phototherapy," *Anesthesia and Analgesia*, vol. 108, no. 2, pp. 635–640, 2009.

[71] T. Shiraishi, M. Onoe, T.-A. Kojima et al., "Effects of bilateral auricular acupuncture stimulation on body weight in healthy volunteers and mildly obese patients," *Experimental Biology and Medicine*, vol. 228, no. 10, pp. 1201–1207, 2003.

[72] T. I. Usichenko, C. Lehmann, and E. Ernst, "Auricular acupuncture for postoperative pain control: a systematic review of randomised clinical trials," *Anaesthesia*, vol. 63, no. 12, pp. 1343–1348, 2008.

[73] J. Zalewska-Kaszubska and D. Obzejta, "Use of low-energy laser as adjunct treatment of alcohol addiction," *Lasers in Medical Science*, vol. 19, no. 2, pp. 100–104, 2004.

[74] T. I. Usichenko, M. Dinse, V. P. Lysenyuk, M. Wendt, D. Pavlovic, and C. Lehmann, "Auricular acupuncture reduces intraoperative fentanyl requirement during hip arthroplasty—a randomized double-blinded study," *Acupuncture and Electro-Therapeutics Research*, vol. 31, no. 3-4, pp. 213–221, 2006.

[75] A. Michalek-Sauberer, E. Gusenleitner, A. Gleiss, G. Tepper, and E. Deusch, "Auricular acupuncture effectively reduces state anxiety before dental treatment—a randomised controlled trial," *Clinical Oral Investigations*, vol. 16, no. 4, pp. 1517–1522, 2012.

[76] K. Pilkington, G. Kirkwood, H. Rampes, M. Cummings, and J. Richardson, "Acupuncture for anxiety and anxiety disorders—a systematic literature review," *Acupuncture in Medicine*, vol. 25, no. 1-2, pp. 1–10, 2007.

[77] R. P. Liu, J. L. Fang, P. J. Rong, Y. Zhao et al., "Effects of electroacupuncture at auricular concha region on the depressive status of unpredictable chronic mild stress rat models," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 789674, 7 pages, 2013.

[78] W. F. Yeung, K. F. Chung, M. M. Poon et al., "Acupressure, reflexology and auricular acupressure for insomnia: a systematic review of randomized controlled trials," *Sleep Medicine*, vol. 13, no. 8, pp. 971–984, 2012.

[79] C. J. Lu, Y. Xiang, X. L. Xie, M. L. Xuan, and Z. H. He, "A randomized controlled single-blind clinical trial on 84 outpatients with psoriasis vulgaris by auricular therapy combined with optimized Yinxieling Formula," *Chinese Journal of Integrative Medicine*, vol. 18, no. 3, pp. 186–191, 2012.

[80] S. Ning, F. Li, L. Qian et al., "The successful treatment of flat warts with auricular acupuncture," *International Journal of Dermatology*, vol. 51, no. 2, pp. 211–215, 2012.

[81] M. Karst, T. Passie, S. Friedrich, B. Wiese, and U. Schneider, "Acupuncture in the treatment of alcohol withdrawal symptoms: a randomized, placebo-controlled inpatient study," *Addiction Biology*, vol. 7, no. 4, pp. 415–419, 2002.

[82] A. Margolin, S. K. Avants, and R. Arnold, "Acupuncture and spirituality-focused group therapy for the treatment of HIV-positive drug users: a preliminary study," *Journal of Psychoactive Drugs*, vol. 37, no. 4, pp. 385–390, 2005.

[83] R. E. Elliott, A. Morsi, O. Tanweer et al., "Efficacy of vagus nerve stimulation over time: review of 65 consecutive patients with treatment-resistant epilepsy treated with VNS >10years," *Epilepsy and Behavior*, vol. 20, no. 3, pp. 478–483, 2011.

[84] T. Granata, H. Cross, W. Theodore, and G. Avanzini, "Immune-mediated epilepsies," *Epilepsia*, vol. 52, no. 3, pp. 5–11, 2011.

[85] E. E. Fanselow, "Central mechanism of cranial nerve stimulation for epilepsy," *Surgical Neurology International*, vol. 3, no. 4, pp. 247–254, 2012.

[86] C. M. Degiorgio, J. Soss, I. A. Cook et al., "Randomized controlled trial of trigeminal nerve stimulation for drug-resistant epilepsy," *Neurology*, vol. 80, no. 9, pp. 786–791, 2013.

[87] S. Karceski, "Trigeminal nerve stimulator: a new treatment for seizures?" *Neurology*, vol. 80, no. 9, pp. 95–97, 2013.

[88] A. R. White, H. Rampes, and E. Ernst, "Acupuncture for smoking cessation," *Cochrane Database of Systematic Reviews*, vol. 2, Article ID CD000009, 2002.

[89] B. Breivogel, B. Vuthaj, B. Krumm, J. Hummel, D. Cornell, and A. Diehl, "Photoelectric stimulation of defined ear points (smokex-Pro method) as an aid for smoking cessation: a prospective observational 2-year study with 156 smokers in a primary care setting," *European Addiction Research*, vol. 17, no. 6, pp. 292–301, 2011.

[90] J. R. Basford, "Laser therapy: scientific basis and clinical role," *Orthopedics*, vol. 16, no. 5, pp. 541–547, 1993.

[91] K. Graudenz and C. Raulin, "From Einstein's quantum theory to modern laser therapy. The history of lasers in dermatology and aesthetic medicine," *Hautarzt*, vol. 54, no. 7, pp. 575–582, 2003.

[92] N. F. Gamaleya, "Laser biomedical research in the USSR," in *Laser Applications in Medicine and Biology*, M. L. Wohlbarsht, Ed., vol. 3, pp. 1–172, Plenum, New York, NY, USA, 1977.

[93] L. Seitz and J. A. Kleinkort, "Low-power laser: its application in physical therapy," in *Thermal Agents in Rehabilitation*, S. Michlovitz, Ed., pp. 217–238, F A Davis, Philadelphia, Pa, USA, 1986.

[94] G. Litscher, T. Huang, L. Wang, and W. Zhang, "Violet laser acupuncture-part 1: effects on brain circulation," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 3, no. 4, pp. 255–259, 2010.

[95] G. Litscher and G. Opitz, "Technical parameters for laser acupuncture to elicit peripheral and central effects: state-of-the-art and short guidelines based on results from the Medical University of Graz, the German Academy of Acupuncture, and the scientific literature," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 697096, 5 pages, 2012.

[96] Y. C. Zhou, "An advanced clinical trial with laser acupuncture anesthesia for minor operations in the oro-maxillofacial region," *Lasers in Surgery and Medicine*, vol. 4, no. 3, pp. 297–303, 1984.

[97] G. Litscher and D. Schikora, *Laserneedle-Acupuncture. Science and Practice*, Pabst Science, Lengerich, Germany, 2005.

[98] W. Posten, D. A. Wrone, J. S. Dover, K. A. Arndt, S. Silapunt, and M. Alam, "Low-level laser therapy for wound healing: mechanism and efficacy," *Dermatologic Surgery*, vol. 31, no. 3, pp. 334–340, 2005.

[99] G. D. Baxter, C. Bleakley, and S. McDonough, "Clinical effectiveness of laser acupuncture: a systematic review," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 1, no. 2, pp. 65–82, 2008.

[100] M. Bergamaschi, G. Ferrari, M. Gallamini, and F. Scoppa, "Laser acupuncture and auriculotherapy in postural instability—a preliminary report," *JAMS Journal of Acupuncture and Meridian Studies*, vol. 4, no. 1, pp. 69–74, 2011.

[101] L. Lorenzini, A. Giuliani, L. Giardino, and L. Calzà, "Laser acupuncture for acute inflammatory, visceral and neuropathic pain relief: an experimental study in the laboratory rat," *Research in Veterinary Science*, vol. 88, no. 1, pp. 159–165, 2010.

[102] Berufsgenossenschaft der Feinmechanik und Elektrotechnik (BFGE) 2001, Anwendung neuer Laser entsprechend der Stellungnahme des Fachausschuss Elektrotechnik zur Berücksichtigung der Norm DIN EN 60825-1 (VDE 0837 Teil 1), http://www.doku.net/artikel/neuelaserk.htm.

[103] R. R. Anderson and J. A. Parrish, "The optics of human skin," *Journal of Investigative Dermatology*, vol. 77, no. 1, pp. 13–19, 1981.

[104] G. Litscher, "Integrative laser medicine and high-tech acupuncture at the Medical University of Graz, Austria, Europe," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 103109, 21 pages, 2012.

[105] D. Schikora, "Laserneedles in acupuncture," in *Laserneedle Acupuncture. Science and Practice*, G. Litscher and D. Schikora, Eds., pp. 1–17, Pabst Science, Lengerich, Germany, 2005.

[106] S. Birch, "Clinical research on acupuncture: part 2. Controlled clinical trials, an overview of their methods," *Journal of Alternative and Complementary Medicine*, vol. 10, no. 3, pp. 481–498, 2004.

[107] M. Aickin, "Beyond randomization," *Journal of Alternative and Complementary Medicine*, vol. 8, no. 6, pp. 765–772, 2002.

[108] D. Cowan, "Methodological issues in evaluating auricular acupuncture therapy for problems arising from the use of drugs and alcohol," *Acupuncture in Medicine*, vol. 29, no. 3, pp. 227–229, 2011.

[109] P. A. Jenkins and J. D. Carroll, "How to report low-level laser therapy (LLLT)/photomedicine dose and beam parameters in clinical and laboratory studies," *Photomedicine and Laser Surgery*, vol. 29, no. 12, pp. 785–787, 2011.

[110] D. Irnich, N. Salih, M. Offenbächer, and J. Fleckenstein, "Is sham laser a valid control for acupuncture trials?" *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 485945, 8 pages, 2011.

[111] J. L. Leveque, P. Corcuff, J. De Rigal, and P. Agache, "In vivo studies of the evolution of physical properties of the human skin with age," *International Journal of Dermatology*, vol. 23, no. 5, pp. 322–329, 1984.

[112] T. Lister, P. A. Wright, and P. H. Chappell, "Optical properties of human skin," *Journal of Biomedical Optics*, vol. 17, no. 9, pp. 1–14, 2012.

[113] A. Esnouf, P. A. Wright, J. C. Moore, and S. Ahmed, "Depth of penetration of an 850nm wavelength low level laser in human skin," *Acupuncture and Electro-Therapeutics Research*, vol. 32, no. 1-2, pp. 81–86, 2007.

[114] K. Landgren, *Ear Acupuncture—A Practical Guide*, Elsevier, Churchill Livingstone, Pa, USA, 2008.

[115] G. Litscher, "Yes, there is Deqi sensation in laser acupuncture," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 198254, 2013.

[116] G. Litscher, L. Wang, and E. Huber, "Effects of laserneedle stimulation on microcirculation and skin temperature," in *Laserneedle Acupuncture*, G. Litscher and D. Schikora, Eds., pp. 64–72, Pabst Science, Lengerich, Germany, 2005.

[117] S. Yao, "46 cases of insomnia treated by semiconductor laser irradiation on auricular points," *Journal of Traditional Chinese Medicine*, vol. 19, no. 4, pp. 298–299, 1999.

[118] L. Sun, "He-Ne laser auricular irradiation plus body acupuncture for treatment of acne vulgaris in 36 cases," *Journal of Traditional Chinese Medicine*, vol. 26, no. 3, pp. 193–194, 2006.

[119] F. Trümpler, S. Oez, P. Stähli, H. D. Brenner, and P. Jüni, "Acupuncture for alcohol withdrawal: a randomized controlled trial," *Alcohol and Alcoholism*, vol. 38, no. 4, pp. 369–375, 2003.

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Research Article

Near-Infrared Spectroscopy for Objectifying Cerebral Effects of Laser Acupuncture in Term and Preterm Neonates

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Laser acupuncture (LA) becomes more and more relevant in neonates and infants. With near-infrared spectroscopy (NIRS), a continuous and noninvasive measurement of tissue oxygenation is possible. Aim was to investigate, whether the application of LA was associated with any changes in regional cerebral oxygen saturation ($rcSO_2$) in term and preterm neonates. The study included 20 neonates (12 males, 8 females). The Large Intestine 4 acupuncture point (LI 4, *Hegu*) was stimulated by a microlaser needle (10 mW, 685 nm laser needle EG GmbH, Germany) for 5 minutes, bilaterally. All neonates underwent polygraphic recording during undisturbed daytime sleep, including heart rate (HR), peripheral oxygen saturation (SpO_2), and measurement of nasal flow. Using NIRS, $rcSO_2$ was measured continuously. Cerebral fractional tissue oxygen extraction (cFTOE) was calculated. We did not observe any significant changes in SpO_2 and HR values during the whole observation period. However, there was a significant decrease in $rcSO_2$ ($P = 0.003$) within postintervention period, accompanied by a significant increase in cFTOE ($P = 0.010$) in postintervention period.

1. Introduction

There is an increasing interest in complementary and alternative medicine (CAM) in particular in herbal medicine, homeopathy, and traditional Chinese medicine (TCM) to treat the pediatric population [1–3]. TCM has been practiced in China for over 2000 years as the main form of medical treatment before the introduction of western medicine approximately 100 years ago. TCM includes Tuina (i.e., Chinese massage at acupoints), moxibustion, and all types of acupuncture (acupressure, needle acupuncture, electric acupuncture, and laser acupuncture (LA)) [4]. Positive effects of acupuncture on reduction of pain and agitation in children have been reported [5]. However it is unknown whether repeated needle stimulation may alter sensory processing and responses to subsequent painful stimuli [6] or demonstrates an increased infection risk in premature babies [7]. LA application is painless and can avoid infections, which could be an important alternative to manual acupuncture in infants [8–14]. However, the applied doses, duration of stimulation, peripheral, and central effects of LA are an ongoing discussion [15, 16]. Evidence derived from functional magnetic resonance imaging demonstrated that stimulation of different acupuncture points, for example, LI 4 (*Hegu*) or

Liv 3 (*Taichong*) induced specific patterns of brain activity in adults [17] and children [18]. This brain pattern activation is based on the indirect representation of neuronal activity and metabolic changes, particularly the relative changes in concentration of deoxygenated haemoglobin (HHb). Near-infrared spectroscopy (NIRS) has been used to measure cerebral tissue oxygenation and changes in oxygen delivery and oxygen consumption within a tissue compartment [19]. In 1978, Chen and Erdmann [20] firstly described the effects of acupuncture on oxygenation, followed by several studies demonstrating the effect of acupuncture using NIRS in adults [21] and concluded that changes in peripheral and cerebral activities can be quantified and are reproducible using NIRS. The limited evidence of LA studies in children shows similar results compared to adults [22, 23]. However, the central effect of LA in newborn infants has not been evaluated. The aim of the study was to measure changes of regional cerebral tissue oxygenation in term and preterm neonates undergoing LA.

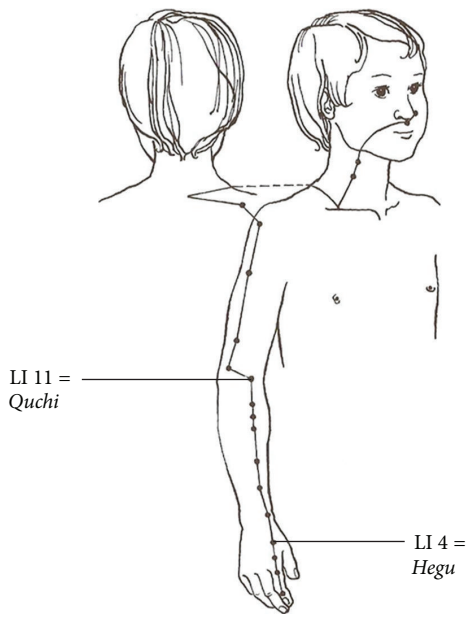

Figure 1: LI 4 (*Hegu*) is located in the Large Intestine Meridian in the middle of the 2nd metacarpal bone on the radial side. The Large Intestine Meridian begins on the index finger and travels along the arm over the shoulder to end outside of the nose. LI 4 (*Hegu*) is considered to be one of the most effective acupuncture points for general pain control, especially of the head. Modified by the author H. Tenk: Internship of Pediatric Chinese Acupuncture, 3 ed, W. Maudrich, 1994.

2. Materials and Methods

2.1. Participants. Preterm and term newborns admitted to the Neonatal Intensive Care Unit of the University Hospital of Graz scheduled for sleep studies were included. Infants were excluded with known genetic anomalies or major malformations, pathology in cranial ultrasound or abnormal neurological examination, elevated bilirubin levels, suspected intrauterine infection, and suspected sepsis or septic shock. Infants >28 weeks gestation were also excluded if they had any need for respiratory support or oxygen during study phase. Infants <28 weeks gestation were only included if they required <30% oxygen or had only needed continuous positive airway pressure. The university ethics committee approved the study, and parental written informed consent was obtained.

Measurements were performed after 2 hours of undisturbed daytime sleep in supine position using a Babytherm 8000 incubator (Dräger GmbH Lübeck, Germany). During the measurements no medication was administered; the ambient temperature and humidity were kept constant. The infant was comforted with a pacifier if needed, and the eyes were protected with an eye shield (Biliband, Natus Medical Inc., San Carlos, CA, USA) as previously described [24].

Heart rate, oxygen saturation, and breathing movements were recorded. Cerebral oxygenation was measured using NIRO 300 (Hamamatsu, Japan). The optodes were placed on the left side of the forehead with an interoptode distance of 4 cm and a sampling rate of 2/s. Light shielding was performed with a slim cap.

The NIRO 300 continuously measures changes in the oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb) concentration, and regional oxygen saturation (rSO_2 (the NIRO 3oo displays the regional oxygen saturation as "tissue oxygen index (TOI)"). Measurement of rSO_2 was performed using the Spatially Resolved Spectroscopy (SRS) method, in which the tissue absorption coefficient is determined from the regionally dependent weakening of light. A detector especially developed for the SRS method registers the emitted near-infrared light from the light source into tissue and allowed rSO_2 calculation using the SRS algorithm. All parameters were stored within a multichannel system.

As acupuncture point Large Intestine 4 (LI 4, *Hegu*) was selected. LI 4 (*Hegu*) is located in the Large Intestine Meridian in the middle of the 2nd metacarpal bone on the radial side (Figure 1). There are a total of 20 points on the Large Intestine Meridian. The pathway begins at the index finger along the arm over the shoulder and ends on the face just lateral of the nose. For LA, a laser needle (laser needle GmbH, Glienicke/Nordbahn, Germany) was used, which has been previously described by Litscher et al. [25–27].

The laser needle used for acupuncture provides continuous laser light with a wavelength of 685 nm and an output power of 10 mW per laser needle. An output power of 10 mW (diameter 500 μm) and a radiation time of 5 min resulted in an power density of about 1,5 J/cm^2 per acupuncture point [27].

Before LA was performed, the skin at the acupuncture point was disinfected, and the laser needles were fixed to the skin with a special adhesive tape, bilaterally at LI 4 (*Hegu*). Once the baby fell asleep, the fixed laser needle was activated for 5 minutes and left undisturbed on the child's LI 4 (*Hegu*), bilaterally (for at least 10 minutes).

Figure 2 shows the infant with the applied and activated laser needle.

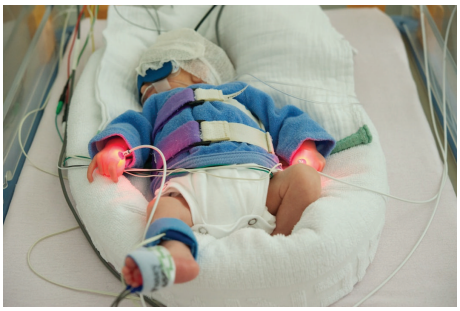

Figure 2: Demonstrates the infant with the applied laser needle and the near-infrared spectroscopy monitoring secured through the slim cap.

Table 1: Values are median and range for continuous data and absolute counts for categorical data.

Investigated neonates	$N = 20$
Male/female	12/8
Preterm/term	17/3
Birthweight	2120 g (690 g–3600 g)
AGA/SGA	19/1
GA (in completed weeks)	34 + 0 (27–40)
Day of life at the time of investigation	22 (11–68)
GA (in completed weeks) at the time of investigation	37 + 2 (34–42)
Weight at the time of investigation	2353 g (1882–3685 g)
HCT in %	43.4%

GA: gestational age; AGA: appropriate for date; SGA: small for date; HCT: hematocrit.

To include data in the analysis, there had to be, a "stable period" lasting 3 min before activating the laser needle without body movements, without apnoea or periodic breathing, without variations in heart rate exceeding 15%, and without variations in oxygen saturation measurements. For further analysis, NIRS parameters were recorded before, during, and after laser needle acupuncture with a sampling rate 2/sec.

The prospective protocol consisted of two baseline periods, each lasting 5 minutes, one before (preintervention period), one after intervention (postintervention period). To depict dynamic changes during intervention, the intervention period was divided into 10 periods, each lasting 30 seconds. Mean values of peripheral oxygen saturation (SpO_2), heart rate (HR), regional cerebral oxygen saturation ($rcSO_2$) were calculated for the two baseline periods, as well as for the 10 intervention periods. Cerebral fractional tissue oxygen extraction (cFTOE) was calculated for each period ((SpO_2–$rcSO_2$)/SpO_2) [28]. Data are presented as mean and 95% confidence interval. In this analysis, we investigated the changes in SpO_2, HR, $rcSO_2$, and cFTOE within the intervention period compared to preintervention and postintervention periods using a linear mixed model with a fixed effect for time and a first order autoregressive covariance structure. A P value of < 0.05 was considered as a statistical significance. The statistical analyses were performed using IBM SPSS Statistics (release 19.0.0. 2010, Chicago, IL, USA, SPSS Inc., an IBM company).

3. Results and Discussion

3.1. Results. The study group encompassed 20 neonates with a gestational age ranging from $26^{6/7}$ to $40^{6/7}$ weeks and a birth weight of 690 to 3680 g. Mean (range) measurements were performed on day 22 (11–68) after birth. Table 1 shows the demographic data of the study population.

We did not observe any significant changes in SpO_2 and HR values during the whole observation period. However, there was a significant decrease in $rcSO_2$ ($P = 0.003$) within postintervention period, accompanied by a significant increase in cFTOE ($P = 0.010$) in postintervention period (Figures 3 and 4).

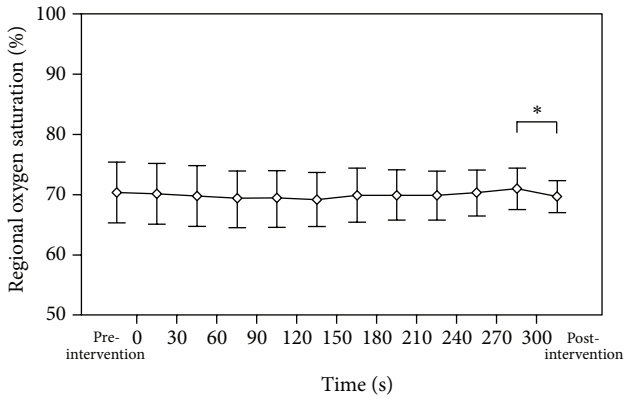

Figure 3: There was a significant decrease in regional cerebral oxygen saturation ($rcSO_2$) during postintervention period ($P = 0.003$).

3.2. Discussion. There is an increasing interest in complementary medical treatment of infants and newborn; however, the evidence is scare. A recent meta-analysis demonstrated that [29] acupuncture could be a safe nonpharmacologic treatment option for pain reduction in term and preterm infants. The current study investigated cerebral oxygenation and physiological parameter during LA. The results of the study can be summarised as follows: (i) significant decrease in regional cerebral oxygen saturation ($rcSO_2$) in postintervention period, (ii) significant increase in cerebral fractional tissue oxygen extraction (cFTOE), and (iii) no changes at all in peripheral oxygen saturation or heart rate during the whole observation period. This is the first study to demonstrate changes in cerebral tissue oxygenation in association with LA in term and preterm infants.

Oxygen delivery is the product of blood flow and oxygen content. In neonates, the cardiac output is dependent on HR. In our study, there were no significant differences in HR, which leads us to assume that there were no differences between both groups as regards to blood flow. The oxygen content of arterial blood supplying the brain equates SpO_2, which did not show any significant changes either. Therefore,

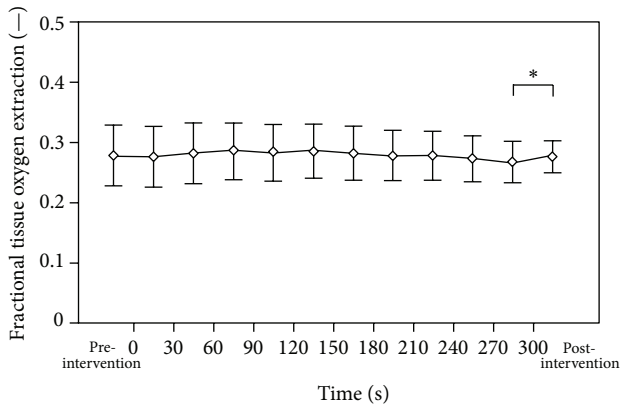

Figure 4: There was a significant increase in the cerebral fractional tissue oxygen extraction (cFTOE) during postintervention period ($P = 0.010$).

we hypothesize that the observed significant changes in $rcSO_2$ and cFTOE were due to changes in regional cerebral tissue perfusion and oxygen extraction. Our findings suggest that there was a decrease in local arterial blood supply after discontinuation of LA, expressed in a decrease of $rcSO_2$. This was accompanied by an increase in regional tissue oxygen extraction, expressed in an increase of cFTOE. Furthermore, we hypothesize that the increase of local cerebral tissue perfusion may have been a slow process, and that may be the reason why there were no significant changes measureable during LA. There are some similarities to reported results of LA in adults [27, 30]. Litscher and Wang reported an increase of O_2Hb and TOI after needle and laser acupuncture in adult patients [31]. Furthermore, Litscher described a possible correlation between manual needle acupuncture, laser acupuncture, electrical punctual stimulation, and changes in regional cerebral oxygenation in more than 100 volunteers [32].

LI 4 *(Hegu)*, which is considered to be one of the most effective acupuncture points for general pain control, was used in the current study. Manual acupuncture applied to LI 4 (*Hegu*) activates the sympathetic and parasympathetic nervous systems in healthy individuals [33]. Stimulating LI 4 (*Hegu*) bilaterally resulted in a more immediate effect than unilateral stimulation [34]. LI 4 (*Hegu*) has been used to treat infantile colic [35–38] and has been described for analgesia [39]. Furthermore, LI 4 (*Hegu*) induces specific patterns of brain activity in adults and children during manual acupuncture, electroacupuncture, and LA [40, 41]. These investigations are based on the indirect representation of neuronal activity and the resulting metabolic changes, particularly the relative changes in concentration of HHb. NIRS is an established approach to noninvasive measure peripheral and cerebral tissue oxygenation [42]. Near-infrared light penetrates deep into the tissue allowing to monitor tissue oxygenation. The oxygen-dependent absorption of light by haemoglobin enables the calculation of relative changes in the oxygenated and deoxygenated haemoglobin [43]. The advantages of NIRS are (i) noninvasive, (ii) low risk, (iii) continuity, and (iv) particularly suitable for the neonatal population due to their thin scalp and skull. The application of the method is easy, and it is used in several studies to measure cerebral [44–46] and peripheral oxygenation in term and preterm newborn [47, 48]. Our results demonstrate that NIRS can be used to measure changes of cerebral tissue oxygenation in term and preterm neonates undergoing LA.

Currently there are only a few studies that investigated the effect of acupuncture in neonates [49]. In children, acupuncture has been demonstrated to have positive effects on pain [39, 50, 51]. In comparison, there is a lack of data in newborn infants about safety of acupuncture and the response to acupuncture. Current evidence suggests that acupuncture is a safe modality for pediatric patients. However, fewer needles should be used when treating infants compared to adults [52, 53]. Case reports and case series have been described for neonates, and early infancy has been carried out, for example, as a therapy for infantile colic [35–38, 54], pain treatment, and newborn abstinence syndrome [55, 56]. A major limitation of acupuncture in newborns is their skin vulnerability with a potential to damage the skin resulting in a potential entry wound for infectious diseases. It is also unknown whether repeated needle stimulation may alter sensory processing and responses to subsequent painful stimuli, in the same manner like heel sticks, necessary to take blood samples [6, 39, 57]. LA is a painless procedure, and therefore, it becomes a more and more relevant alternative to manual acupuncture in infants [7–14]. But the central and peripheral effects and the applied doses in neonates and infants undergoing laser acupuncture are a matter of fact in ongoing discussions. Recently, acupuncture was considered to be included in the pain management algorithm for children as an effective nonpharmacological approach [58, 59].

The current study has some additional limitation not previously mentioned. The acupuncture effect of laser stimulation depends on the power density at the acupuncture point.

For a laser output power of 10 mW, the resulting power density at the acupuncture point is in the order of 5 W/cm^2. An output power of 10 mW and a time of radiation of 5 min result in an energy dosage of about 1,5 J/cm^2. Maybe with a higher energy dosage (higher laser output power and/or a longer radiation time) the results could be more significant, especially during LA [27].

But based on our recent published data about the changes of the skin temperature in preterm infants undergoing laser acupuncture [15], it seems rational and safe to use the same laser needle with the same output power (10 mW) and the same time of stimulation (five minutes). Another limitation of our study was the small number of infants included in the study. Future research should focus on alternative or adjunctive nonpharmacological therapy to understand the utility, safety, and effectiveness of acupuncture in newborns and infants and investigate central effects of LA in neonates, by changing the time of stimulation and/or the energy doses.

4. Conclusions

There was a significant decrease in $rcSO_2$ during postintervention period. This was accompanied by a significant

increase in cFTOE. This was in contrast to SpO_2 and HR, where no changes could be observed. Therefore, we hypothesize that observed changes were due to changes in regional cerebral perfusion and oxygen supply. This is the first study to demonstrate changes of cerebral tissue oxygenation caused by laser acupuncture in term and preterm neonates.

Abbreviations

CAM:	Complementary and alternative medicine
TCM:	Traditional Chinese medicine
LA:	Laser acupuncture
NIRS:	Near-infrared spectroscopy
O_2Hb:	Oxyhemoglobin
HHb:	Deoxyhemoglobin
TOI:	Tissue oxygenation index
HR:	Heart rate
SpO_2:	Peripheral oxygen saturation
rSO_2:	Regional oxygen saturation
$rcSO_2$:	Regional cerebral oxygen saturation
cFTOE:	Cerebral fractional tissue oxygenation
SRS:	Spatial resolved spectroscopy
LI 4 (*Hegu*):	Large Intestine 4.

Disclosure

The authors do not have any commercial associations that might create a conflict of interests in connection with this paper. They thank the parents for allowing them to study their infants, and the nurses involved in the treatment of the neonates. They also thank Evelyne Ziehenberger for her help in the realization of the study.

References

[1] E. Ernst, "Prevalence of complementary/alternative medicine for children: a systematic review," *European Journal of Pediatrics*, vol. 158, no. 1, pp. 7–11, 1999.

[2] S. Vohra, S. Surette, D. Mittra, L. D. Rosen, P. Gardiner, and K. J. Kemper, "Pediatric integrative medicine: paediatrics? Newest subspecialty?" *BMC Pediatrics*, vol. 12, no. 1, article 123, 2012.

[3] J. Snyder and P. Brown, "Complementary and alternative medicine in children: an analysis of the recent literature," *Current Opinion in Pediatrics*, vol. 24, no. 4, pp. 539–546, 2012.

[4] M. Loo, *Pediatric Acupuncture*, Churchill Livingstone, New York, NY, USA, 2002.

[5] K. Hunt and E. Ernst, "The evidence-base for complementary medicine in children: a critical overview of systematic reviews," *Archives of Disease in Childhood*, vol. 96, no. 8, pp. 769–776, 2011.

[6] A. Taddio, V. Shah, C. Gilbert-MacLeod, and J. Katz, "Conditioning and hyperalgesia in newborns exposed to repeated heel lances," *The Journal of the American Medical Association*, vol. 288, no. 7, pp. 857–861, 2002.

[7] W. Raith, G. Litscher, W. Müller, and B. Urlesberger, "Laseracupuncture—a possible alternative treatment for agitation and pain in neonates?" *Pediatric Anesthesia*, vol. 23, no. 2, pp. 205–206, 2013.

[8] J. Zhang, X. Li, J. Xu, and E. Ernst, "Laser acupuncture for the treatment of asthma in children: a systematic review of randomized controlled trials," *Journal of Asthma*, vol. 49, no. 7, pp. 773–777, 2012.

[9] M. I. Karaman, O. Koca, E. V. Küçük, M. Öztürk, M. Güneş, and C. Kaya, "Laser acupuncture therapy for primary monosymptomatic nocturnal enuresis," *Journal of Urology*, vol. 185, no. 5, pp. 1852–1856, 2011.

[10] E. Radvanska, K. Kamperis, A. Kleif, L. Kovács, and S. Rittig, "Effect of laser acupuncture for monosymptomatic nocturnal enuresis on bladder reservoir function and nocturnal urine output," *Journal of Urology*, vol. 185, no. 5, pp. 1857–1861, 2011.

[11] S. Gottschling, S. Meyer, I. Gribova et al., "Laser acupuncture in children with headache: a double-blind, randomized, bicenter, placebo-controlled trial," *Pain*, vol. 137, no. 2, pp. 405–412, 2008.

[12] K. Stockert, B. Schneider, G. Porenta, R. Rath, H. Nissel, and I. Eichler, "Laser acupuncture and probiotics in school age children with asthma: a randomized, placebo-controlled pilot study of therapy guided by principles of traditional Chinese medicine," *Pediatric Allergy and Immunology*, vol. 18, no. 2, pp. 160–166, 2007.

[13] W. Gruber, E. Eber, D. Malle-Scheid et al., "Laser acupuncture in children and adolescents with exercise induced asthma," *Thorax*, vol. 57, no. 3, pp. 222–225, 2002.

[14] A. Stellon, "The use of laser acupuncture for the treatment of neurogenic pruritus in a child—a case history," *Acupuncture in Medicine*, vol. 23, no. 1, pp. 31–33, 2005.

[15] W. Raith, G. Litscher, I. Sapetschnig et al., "Thermographical measuring of the skin temperature using laser needle acupuncture in preterm neonates," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 614210, 5 pages, 2012.

[16] R. T. Chow, "Dose dilemmas in low level laser therapy—the effects of different paradigms and historical perspectives," *Laser Therapy Journal*, no. 13, pp. 102–108, 2012.

[17] W. Huang, D. Pach, V. Napadow et al., "Characterizing acupuncture stimuli using brain imaging with FMRI—a systematic review and meta-analysis of the literature," *PLoS ONE*, vol. 7, no. 4, Article ID e32960, 2012.

[18] Y. Wu, Z. Jin, K. Li et al., "Functional magnetic resonance imaging activation of the brain in children: real acupoint versus sham acupoint," *Journal of Child Neurology*, vol. 25, no. 7, pp. 849–855, 2010.

[19] F. F. Jöbis, "Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters," *Science*, vol. 198, no. 4323, pp. 1264–1267, 1977.

[20] G. S. Chen and W. Erdmann, "Effects of acupuncture on tissue oxygenation of the rat brain," *Southern Medical Journal*, vol. 71, no. 4, pp. 392–398, 1978.

[21] G. Litscher, L. Wang, and E. Huber, "Changes in cerebral near infrared spectroscopy parameters during manual acupuncture needle stimulation," *Biomedizinische Technik*, vol. 47, no. 4, pp. 76–79, 2002 (German).

[22] G. Litscher, "NIR spectroscopy explores the mysteries of acupuncture," *Spectroscopy Europe*, vol. 17, no. 1, pp. 8–14, 2005.

[23] G. Litscher, "NIR spectroscopy explores the mysteries of acupuncture," *Spectroscopy Asia*, vol. 1, no. 1, pp. 8–14, 2005.

[24] W. Raith, G. M. Schmölzer, B. Resch, M. Seewann, W. Müller, and B. Urlesberger, "Laser acupuncture as a possible treatment for an agitated infant—a preterm after 28 weeks of gestation," *German Journal of Acupuncture and Related Techniques*, vol. 51, no. 3, pp. 33–36, 2008.

[25] G. Litscher and D. Schikora, "Cerebral vascular effects of noninvasive laserneedles measured by transorbital and transtemporal Doppler sonography," *Lasers in Medical Science*, vol. 17, no. 4, pp. 289–295, 2002.

[26] G. Litscher, "Cerebral and peripheral effects of laserneedle-stimulation," *Neurological Research*, vol. 25, no. 7, pp. 722–728, 2003.

[27] G. Litscher and D. Schikora, Eds., *Laserneedle-Acupuncture: Science and Practice*, Pabst Science, Berlin, Germany, 2005.

[28] G. Naulaers, B. Meyns, M. Miserez et al., "Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation: a validation study in piglets," *Neonatology*, vol. 92, no. 2, pp. 120–126, 2007.

[29] W. Raith, B. Urlesberger, and G. M. Schmölzer, "Efficacy and safety of acupuncture in preterm and term infants," *Evidence-Based Complementary and Alternative Medicine*, 2013.

[30] G. Litscher and D. Schikora, "Near-infrared spectroscopy for objectifying cerebral effects of needle and laserneedle acupuncture," *Spectroscopy*, vol. 16, no. 3-4, pp. 335–342, 2002.

[31] G. Litscher and L. Wang, "Cerebral near infrared spectroscopy and acupuncture—results of a pilot study," *Biomedizinische Technik*, vol. 45, no. 7-8, pp. 215–218, 2000.

[32] G. Litscher, "Bioengineering assessment of acupuncture—part 5: cerebral near-infrared spectroscopy," *Critical Reviews in Biomedical Engineering*, vol. 34, no. 6, pp. 439–457, 2006.

[33] E. Haker, H. Egekvist, and P. Bjerring, "Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects," *Journal of the Autonomic Nervous System*, vol. 79, no. 1, pp. 52–59, 2000.

[34] T. B. Lin and T. C. Fu, "Effect of electroacupuncture on blood pressure and adrenal nerve activity in anesthetized rats," *Neuroscience Letters*, vol. 285, no. 1, pp. 37–40, 2000.

[35] K. Landgren, N. Kvorning, and I. Hallström, "Acupuncture reduces crying in infants with infantile colic: a randomised, controlled, blind clinical study," *Acupuncture in Medicine*, vol. 28, no. 4, pp. 174–179, 2010.

[36] K. Landgren, N. Kvorning, and I. Hallström, "Feeding, stooling and sleeping patterns in infants with colic—a randomized controlled trial of minimal acupuncture," *BMC Complementary and Alternative Medicine*, vol. 11, article 93, 2011.

[37] M. Reinthal, S. Andersson, M. Gustafsson et al., "Effects of minimal acupuncture in children with infantile colic—a prospective, quasi-randomised single blind controlled trial," *Acupuncture in Medicine*, vol. 26, no. 3, pp. 171–182, 2008.

[38] M. Reinthal, I. Lund, D. Ullman, and T. Lundeberg, "Gastrointestinal symptoms of infantile colic and their change after light needling of acupuncture: a case series study of 913 infants," *Chinese Medicine*, vol. 6, article 28, 2011.

[39] A. Ecevit, D. A. Ince, A. Tarcan, M. T. Cabioglu, and A. Kurt, "Acupuncture in preterm babies during minor painful procedures," *Journal of Traditional Chinese Medicine*, vol. 31, no. 4, pp. 308–310, 2011.

[40] W. Wang, L. Liu, X. Zhi et al., "Study on the regulatory effect of electro-acupuncture on Hegu point (LI4) in cerebral response with functional magnetic resonance imaging," *Chinese Journal of Integrative Medicine*, vol. 13, no. 1, pp. 10–16, 2007.

[41] C. M. Siedentopf, F. Koppelstaetter, I. A. Haala et al., "Laser acupuncture induced specific cerebral cortical and subcortical activations in humans," *Lasers in Medical Science*, vol. 20, no. 2, pp. 68–73, 2005.

[42] J. Menke, U. Voss, G. Möller, and G. Jorch, "Reproducibility of cerebral near infrared spectroscopy in neonates," *Biology of the Neonate*, vol. 83, no. 1, pp. 6–11, 2003.

[43] M. Wolf and G. Greisen, "Advances in near-infrared spectroscopy to study the brain of the preterm and term neonate," *Clinics in Perinatology*, vol. 36, no. 4, pp. 807–834, 2009.

[44] B. Urlesberger, G. Pichler, E. Gradnitzer, F. Reiterer, G. Zobel, and W. Müller, "Changes in cerebral blood volume and cerebral oxygenation during periodic breathing in term infants," *Neuropediatrics*, vol. 31, no. 2, pp. 75–81, 2000.

[45] G. Pichler, M. C. van Boetzelar, W. Müller, and B. Urlesberger, "Effect of tilting on cerebral hemodynamics in preterm and term infants," *Biology of the Neonate*, vol. 80, no. 3, pp. 179–185, 2001.

[46] N. Tax, G. Pichler, K. Grossauer et al., "Tilting the head changes cerebral haemodynamics in neonates," *Neonatology*, vol. 100, no. 3, pp. 253–259, 2011.

[47] G. Pichler, K. Grossauer, P. Klaritsch et al., "Peripheral oxygenation in term neonates," *Archives of Disease in Childhood: Fetal and Neonatal Edition*, vol. 92, no. 1, pp. F51–F52, 2007.

[48] G. Pichler, J. Heinzinger, P. Klaritsch, H. Zotter, W. Müller, and B. Urlesberger, "Impact of smoking during pregnancy on peripheral tissue oxygenation in term neonates," *Neonatology*, vol. 93, no. 2, pp. 132–137, 2008.

[49] M. T. Thiel and K. Stockert, "Acupuncture and neonatology," *Journal of Chinese Medicine*, no. 97, pp. 50–57, 2011.

[50] K. J. Kemper, R. Sarah, E. Silver-Highfield, E. Xiarhos, L. Barnes, and C. Berde, "On pins and needles? Pediatric pain patients' experience with acupuncture," *Pediatrics*, vol. 105, no. 4, pp. 941–947, 2000.

[51] H. Jun, Z. Cuiping, and C. Shunhai, "Electric acupuncture treatment of peripheral nerve injury," *Journal of Traditional Chinese Medicine*, vol. 15, pp. 114–117, 1995.

[52] V. Jindal, A. Ge, and P. J. Mansky, "Safety and efficacy of acupuncture in children: a review of the evidence," *Journal of Pediatric Hematology/Oncology*, vol. 30, no. 6, pp. 431–442, 2008.

[53] D. Adams, F. Cheng, H. Jou, S. Aung, Y. Yasui, and S. Vohra, "The safety of pediatric acupuncture: a systematic review," *Pediatrics*, vol. 128, no. 6, pp. 1575–1587, 2011.

[54] H. Skjeie, T. Skonnord, A. Fetveit, and M. Brekke, "A pilot study of ST36 acupuncture for infantile colic," *Acupuncture in Medicine*, vol. 29, no. 2, pp. 103–107, 2011.

[55] B. Golianu, E. Krane, J. Seybold, C. Almgren, and K. J. S. Anand, "Non-pharmacological techniques for pain management in neonates," *Seminars in Perinatology*, vol. 31, no. 5, pp. 318–322, 2007.

[56] W. Raith, G. Pichler, H. Zotter, W. Mueller, and B. Urlesberger, "Detection of psychic ear acupuncture points in a newborn infant with neonatal abstinence syndrome," *Journal of Alternative and Complementary Medicine*, vol. 16, no. 4, pp. 345–346, 2010.

[57] K. R. . Gentry, K. L. McGinn, A. Kundu, and A. M. Lynn, "Acupuncture therapy for infants: a preliminary report on reasons for consultation, feasibility, and tolerability," *Pediatric Anesthesia*, vol. 22, no. 7, pp. 690–695, 2012.

[58] V. Y. Sohn, D. Zenger, and S. R. Steele, "Pain management in the pediatric surgical patient," *Surgical Clinics of North America*, vol. 92, no. 3, pp. 471–485, 2012.

[59] J. C. I. Tsao, S. Evans, M. Meldrum, T. Altman, and L. K. Zeltzer, "A review of CAM for procedural pain in infancy—part II: other interventions," *Evidence-Based Complementary and Alternative Medicine*, vol. 5, no. 4, pp. 399–407, 2008.