As a stimulating point in acupuncture, acupoint has unique microcirculatory features, and its dynamics vary greatly depending on health status. Acupoint sensitization is defined as the transformation of an acupoint from a “silenced status” (healthy) to an “activated status” (disease). Our previous study demonstrated that acupoint sensitization is associated with an increase in the level of local blood perfusion. However, the structural changes in microcirculation during acupoint sensitization have yet to be elucidated because the high-resolution microcirculation imaging of acupoints has been difficult to obtain. In this study, the structural changes in microcirculation at the Zusanli (ST36), Yanglingquan (GB34) and nonacupoint sites on days 0, 7 and 21 were dynamically observed during acupoint sensitization in an experimental knee osteoarthritis mouse model by using optical-resolution photoacoustic microscopy. The results showed that no significant differences in microvessel density, the distribution of vessel diameters or vascular tortuosity were observed at the GB34, ST36 or nonacupoint sites among days 0, 7 and 21. We proposed that acupoint sensitization may not be associated with the structural changes in microcirculation and that the microcirculatory changes during acupoint sensitization are more likely to be functional. The functional characteristics of the sensitized acupoints warrant further investigation.

**KEYWORDS**
acupoint sensitization, microcirculation, microcirculatory structure, optical-resolution photoacoustic microscopy, photoacoustic imaging
INTRODUCTION

Since acupuncture was first introduced to the Western world in the 1970s, it has been generally recognized as an effective therapy and has become one of the most common complementary and alternative therapies worldwide. As the stimulating point and effector, acupoints have been widely considered the core focus of acupuncture research. Previous studies have shown that acupoints are not fixed; instead, their positions and functions can change dynamically depending on the physiological and pathological conditions of the body [1, 2]. During the process of dynamic changes, acupoint sensitization is defined as the transformation of an acupoint from a “silenced status” (healthy) to an “activated status” (disease) [3, 4]. Acupoint sensitization significantly affects the receptive field size and the sensitivity of an acupoint and thus enhances its therapeutic function [5], which includes receiving stimuli and regulating body function. In addition, as a reflection of disease activity on the body surface, a sensitized acupoint can hold high clinical value [6–8]. Therefore, the structural changes and functional characteristics of microcirculation during acupoint sensitization need to be clarified to improve diagnostic and treatment capabilities, as well as better understand of acupoint connotation.

Microcirculation plays a pivotal role in metabolic supply and metabolism, regulating blood flow and maintaining homeostasis. Numerous studies have demonstrated that acupoints exert unique microcirculatory characteristics [9, 10]. Local microcirculation has been identified as an important target of acupuncture and is closely associated with the acupuncture effects [11]. Prior research in this area has primarily focused on the effects of acupuncture on blood perfusion in local acupoints and its underlying mechanisms. However, the structural and functional characteristics of microcirculation during the sensitization have been neglected to some extent. Instead, previous studies on acupoint sensitization have primarily focused on neural mechanisms [3–5]. A lack of imaging studies on acupoint sensitization has limited by the objective observation on sensitized acupoints and implications for clinical practice. Through laser speckle imaging, our previous study demonstrated for the first time that acupoint sensitization is associated with an increased level of local blood perfusion [12]. However, a high-resolution microcirculation imaging is more difficult to obtain, and the structural changes in microcirculation during acupoint sensitization remain largely elusive.

As a new frontier of microcirculatory imaging, photoacoustic imaging (PAI) possesses some advantageous characteristics; it is noninvasive, has real-time processing and results in high-resolution images [13, 14]. PAI is implemented by an effective combination of optical imaging and ultrasonic imaging. In PAI, the tissue sample is often irradiated by a short-pulsed laser beam. Locally absorbed light is converted into heat, and an acoustic wave (photoacoustic wave) is subsequently generated by thermoelastic expansion. Deposition of optical energy in the tissues has been mapped based on the differences in photoacoustic waves across different biological tissues [15]. PAI not only exhibits the characteristics of optical imaging such as high contrast and sensitivity but also possesses the advantages of ultrasound imaging, such as higher spatial resolution at depths [16, 17]. Optical-resolution photoacoustic microscopy (OR-PAM) is a major form of PAI that can detect the structural and functional changes in the microcirculation within a certain range of depth at the capillary level [18, 19]. Moreover, quantitative analysis of OR-PAM can be accurately determined through the extraction of vascular information such as vessel diameters, microvessel density (MVD) and vascular tortuosity [20, 21]. As a powerful tool for studying the microcirculation in vivo, OR-PAM has been used in preclinical studies of a wide range of therapeutic areas, including ophthalmology [22], oncology [23], neurology [24] and physiology [25].

In the present study, we aimed to characterize the structural changes in microcirculation at several acupoints such as Yanglingquan (GB34) and Zusanli (ST36) by using PAI. OR-PAM was used for in vivo imaging and measuring the process of acupoint sensitization in a mouse model of moniodoacetate (MIA)-induced knee osteoarthritis (OA). This study aimed to not only reveal the connotation of acupoints and clarify the microcirculatory characteristics of sensitized acupoints but also expand the application of PAI in the biomedical field. Our findings can be added to the debate on the microcirculatory characteristics of sensitized acupoints and support the negative structural changes in microcirculation during acupoint sensitization.

MATERIALS AND METHODS

2.1 Experimental animals

Male BALB/c mice were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd (Animal Lot: SCXK[Jing]2016–0006). BALB/c mice weighed 30.0 ± 2.0 g and were 8 weeks old. The animals were housed in a fenced facility in the Experimental Animal Center of Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences at a controlled temperature (24 ± 2°C) under a 12-h dark/light cycle, with sterile drinking water and a standard pellet diet available ad libitum. The experimental protocol was approved by the Medical and Laboratory Animal Ethics Committee at Beijing University of Chinese Medicine.

2.2 Animal groups and interventions

Twelve BALB/c mice were divided into three groups randomly (n = 4 per group): the normal control (N) group, the normal saline (NS) group and the OA group.
As previously described by Pitcher [26], 0.5 mg MIA (Sigma, St. Louis, Missouri) was dissolved in 10 μL of sterile normal saline. On day 0 after PAI, the mice in the OA group were injected with MIA solution into the right knee joint through the infrapatellar ligament. The mice in the NS group were injected with 10 μL of normal saline. No intervention was carried out in the N group.

### 2.3 Photoacoustic imaging

In this study, a custom-built OR-PAM system was used to acquire all the experimental data. The major components of OR-PAM system included the following: (a) a 532-nm nanosecond-pulsed laser (GKNQL-532; Beijing Guoke Laser Co., Beijing, China) with a repetition rate of 2 kHz for photoacoustic signal excitation, (b) a single-element ultrasound transducer (V214-BC-RM; Olympus-NDT, Massachusetts, USA) with 50-MHz center frequency to detected the resultant photoacoustic waves, such as photoacoustic signals, (c) a 2-channel data acquisition (DAQ) card (CS1422; Gage Applied Technologies Inc., Lockport, New York) to digitize the photoacoustic signals at a sampling rate of 200 MS/s and (d) a precision 3-axis motorized linear stage (VT-80; Physik Instrumente, Karlsruhe, Germany) to acquire the volumetric data from mechanical raster scanning. A more detailed description of the OR-PAM system can be found in our previous publications [27, 28]. The lateral resolution of OR-PAM was measured to be ~4 μm by imaging a sharp metallic blade edge. For in vivo experiments, the laser pulse energy shed on the sample surface was measured to be ~200 nJ, corresponding to an optical fluence of ~16 mJ/cm² on the skin surface when the laser is focused ~250 μm below the skin surface. The fluence of each laser pulse on the tissue was well below 20 mJ/cm², in accordance with the American National Standard Institute (ANSI) safety limit.

On days 0, 7 and 21, PAI was performed in each group of mice. The room was controlled at a temperature of 25 ± 1°C, a relative humidity of 55% ± 10%, and a wind speed of <1 m/s. During OR-PAM scanning, the mice were anesthetized with isoflurane (3% for induction, 1%-2% for maintenance) mixed with oxygen (1 L/minute), delivered through an anesthesia machine (Matrix VMR, MIDMARK, Dayton, USA). Respiration rate was monitored using an SA Instruments Model 1025 monitoring and gating system (Stony Brook, New York) and maintained throughout the experimental period at 50% to 70% breaths per min by adjusting isoflurane levels. Body temperature was maintained at approximately 37°C using a temperature controller (69002; RWD Life Science, Shenzhen, China). In the experiments, the mice were positioned after anesthesia to ensure that the lower limbs could not move freely. The hair of the lower limbs was removed 24 h before anesthesia. A 4.5 × 6.5 mm region in the lateral right lower limb of mice was scanned. The ranges of the GB34, ST36 and nonacupoint sites were defined on the images according to the location of the acupoints in mice [29, 30].

### 2.4 Quantitative analysis of microvascular morphological parameters

A dedicated microvascular quantification algorithm, as described previously [20, 28], was used to quantitatively analyze the changes in microcirculatory structure at the GB34, ST36 and nonacupoint sites. The distribution of vessel diameters, MVD and vascular tortuosity were calculated and compared between groups.

Vessel diameter distribution was summed up by analyzing the vertical distance between the centerline and both sides of the blood vessel walls. MVD was defined as the ratio between the number of pixels with a signal intensity of 1 and the total number of pixels, which could be calculated from the two-dimensional binary images of the GB34, ST36 and nonacupoint sites. Vascular tortuosity was measured by three different parameters, namely, distance metric (DM), inflection count metric (ICM) and the sum of angles metric (SOAM). DM was defined as the ratio between the actual path length of a vessel segment in each subdomain and the linear distance between the two ends of the vessel. ICM was defined as the ratio between DM and the number of vessel inflection points. SOAM was defined as the sum of the curvature at all voxels along the centerline of a vessel normalized by the vessel's actual path length. The postprocessing of all acquired images was carried out using MATLAB (R2014a; MathWorks, Natick, Massachusetts) software on a PC with an Intel(R) Core(TM) i7 CPU @3.9 GHz and a 64 GB RAM.

### 2.5 Safranin-O with fast green staining

On day 21 after PAI, the mice in each group were killed by an intraperitoneal injection of pentobarbital (150 mg/kg body weight). The knee joints were extracted and fixed in 10% neutral formalin. Safranin-O with fast green staining is the most commonly used method for cartilage staining [31, 32], clearly revealing the hierarchical structure of cartilage and reflecting the morphologies of cartilage and subchondral cancellous bone [33]. These staining methods were used to assess the damage of articular cartilage. After 10 days in 5% formic acid, the knee joints were sectioned with a 5-μm slicer. The sections were dewaxed, dehydrated and stained in 1% fast green for 1.5 minutes. Next, the sections were stained by 0.5% safranin-O for 1.5 minutes, differentiated by ethanol, and washed with tap water. After vitrification with dimethylbenzene and neutral balata fixation, the sections were observed under a light microscope (CX31, Olympus Corporation, Japan).

Quantitative histological analysis of OA cartilage was performed according to the method previously described by Pritzker [34]. First, the histopathology grade assessment of OA cartilage was conducted based on the key pathological
features of six grades. Then, OA staging was determined according to the horizontal extent of the involved cartilage surface, divided into five stages. Finally, OA scores were calculated using the following equation: OA scores = grade × stage, where OA score represents a combined assessment, based on the severity (grade) and extent (stage) of OA in the articular cartilage.

2.6 | Statistical analysis
The statistical analysis was performed using SPSS software, version 17.0 (SPSS, Inc., Chicago, Illinois), and the data were expressed as the means ± SD. Two-way repeated measures ANOVA and Kruskal-Wallis ANOVA were used to analyze the differences between groups for microcirculatory structure and OA scores, respectively. Statistical significance was set to $P < 0.05$.

3 | RESULTS
3.1 | Safranin-O with fast green staining of articular cartilage and OA scores
The results of the safranin-O with fast green staining of the articular cartilage sections as well as OA quantitative measurements (OA scores) are shown in Figure 1. The articular surface and cartilage morphology of the N and NS groups were intact without evidence of damage. The cartilage pathological changes in the OA group included erosion and matrix loss, delamination of superficial layer and cartilage erosion. The range of damage varied from 10% to 25%, to the extent of 50%. The OA scores in the OA group were significantly higher than those in the N and NS groups ($P < 0.01$). However, no significant differences in OA scores were observed between the N and NS groups.

3.2 | PAI of GB34, ST36 and nonacupoint sites on days 0, 7 and 21
The results of PAI are presented in Figures 2–6. Microcirculation in the $4.5 \times 6.5$ mm region of the lateral right lower limb of mice was clearly observed. The microvessel diameter ranged from 5 to 90 μm, primarily between 20 and 60 μm at the GB34, ST36 and nonacupoint sites. The microcirculatory structures of the GB34, ST36 and nonacupoint sites were clearly distinguished at different time periods. No significant differences in the microvascular changes of the GB34, ST36 and nonacupoint sites were found on days 0, 7 and 21 among the N, NS and OA groups ($P < 0.01$). Similarly, no apparent alterations in vascular structure or angiogenic activity were observed among the three groups.

3.3 | Quantitative analysis of microcirculatory structure at GB34, ST36 and nonacupoint sites on days 0, 7 and 21
The results of the quantitative analysis are presented in Figures 7–8. Quantitative analysis of
microcirculatory structure revealed no significant differences in diameter distribution, MVD, DM, ICM or SOAM at the GB34, ST36 and nonacupoint sites among the N, NS and OA groups on days 0, 7 and 21. Similarly, no remarkable changes were observed in diameter distribution, MVD, DM, ICM or SOAM of the GB34, ST36 and nonacupoint sites among days 0, 7 and 21 in the same group.

FIGURE 2  A, Illustrated diagram of the GB34, ST36 and nonacupoint sites in the mouse. The GB34 site is at the depression below the capitulum fibulae posterolateral to the knee joint. The ST36 site is located in the anterior tibial muscle, and 5 mm lateral and distal to the anterior tubercle of the tibia. The nonacupoint site was located 4 mm outside of the ST36 site. B, Maximum amplitude projection (MAP) image of the lateral right lower limb, where the GB34, ST36 and nonacupoint sites appear as bright spots. Scale bar is 500 μm. C, The GB34, ST36 and nonacupoint sites are marked by black spots on the surface of right lower limb. The GB34, ST36 and nonacupoint sites are represented by orange, blue and green arrows, respectively.

FIGURE 3  A, The maximum amplitude projection (MAP) images (4.5 × 6.5 mm region) of the lateral right lower limb. B, Close-up image (green dashed square) of microvessels with diameters of 5, 15, 25, 40, 50 and 60 μm denoted by white, orange, green, blue, purple and gray arrows, respectively. Scale bar is 500 μm.
Acupuncture has been used as an effective alternative therapy for knee OA throughout the world. A growing body of evidence has shown that acupuncture can alleviate knee OA-related pain, improve knee function [35, 36] and often act synergistically with other medications [37]. According to traditional Chinese medicine, acupoints exert a dual role as disease severity indicators and regulators of body functions [4]. Thus, the acupoints used in clinical practice often represent the reflection points of a specific disease (ie, sensitized acupoints). Extensive clinical research on the acupuncture of knee OA has increased the regularity of acupoint selection for OA treatment and provided an important insight into the selection of acupoints. Therefore, in this study, knee OA was chosen as the carrier for acupoint sensitization. In
particular, the treatment of knee OA with acupuncture is primarily through several acupoints (eg, GB34 and ST36) surrounding the knee [38]. These two acupoints were selected as the target acupoints in this study to better clarify the structural changes of microcirculation during acupoint sensitization. In addition, a nonacupoint site control was selected by considering the influence of elevated inflammatory mediators in the blood after exposure to disease.

The results of safranin-O with fast green staining demonstrated that the articular cartilages in the OA group suffered significantly more damage than those in the N and NS groups, as indicated by their OA scores. No significant differences in the OA scores were observed between the NS

FIGURE 5 The maximum amplitude projection (MAP) and corresponding computed vascular centerlines overlaid with segmented volumetric optical-resolution photoacoustic microscopy (OR-PAM) images of the GB34, ST36 and nonacupoint sites in the NS group. Scale bar is 200 μm
and N groups, suggesting that the disturbances from injection can be excluded. Taken together, our data suggest that a reliable OA animal model can be successfully constructed for the study of acupoint sensitization.

This study, for the first time, applied OR-PAM to acupuncture research and achieved in vivo microcirculatory imaging of acupoints at \( \approx 10 \ \mu m \) of lateral resolution.

Multistage monitoring of the GB34, ST36 and nonacupoint sites revealed that the imaging effect was more desirable at day 0 than at days 7 and 21, attributes to the thickened skin and tissue edema caused by repeated imaging and MIA injection, respectively. Although the imaging effects were slightly different, the microcirculatory structures of the three target sites could be clearly distinguished at the indicated day 0.

**FIGURE 6** The maximum amplitude projection (MAP) and corresponding computed vascular centerlines overlaid with segmented volumetric optical-resolution photoacoustic microscopy (OR-PAM) images of the GB34, ST36 and nonacupoint sites in the OA group. Scale bar is 200 \( \mu m \).
time points. The variations in photoacoustic amplitudes on the indicated time points are presumably due to the physiological changes in mice. The influence of OR-PAM system performance drift on in vivo results was excluded by measuring photoacoustic signals of the same black tape on different days for calibration purpose. During in vivo imaging, the mice were handled with the same anesthesia and heating conditions on different days. Moreover, the distance between mice and the imaging head of OR-PAM was kept the same on different days to maintain a consistent imaging setup. Considering the variations of photoacoustic amplitudes on different days, the intensity of photoacoustic signals was not selected as a quantitative endpoint in this study. Instead, the diameter distribution, MVD, DM, ICM and SOAM were selected as the quantitative endpoints. These parameters can be extracted accurately if the SNR of an image is higher than a certain threshold (SNR > 1.7). In this study, the SNR values of all images selected for quantitative analysis were found to be higher than the above-mentioned threshold.

PAI results showed that there were no significant differences in MVD, tortuosity and diameter distribution at the GB34, ST36 and nonacupoint sites on days 0, 7 and 21. These results suggest that the microcirculatory structures of the GB34, ST36 and nonacupoint sites do not change during acupoint sensitization in knee OA. Our previous study indicated that the acupoint sensitization in knee OA is associated with an increased level of local blood perfusion at the GB34 and ST36 sites [12]. Based on these findings, we hypothesized that acupoint sensitization primarily contribute to the functional changes in microcirculation. Moreover, it was speculated that the acupoint sensitization on microcirculation is more likely to be functional and thus displays a functional characteristic. Given the rapid development of functional microcirculatory imaging based on photoacoustic microscopy [39, 40] and different metabolic characteristics of meridian points, especially in oxygen metabolism [41], further research is warranted to clarify the functional characteristics of sensitized acupoints.

The functional changes in the microcirculation during acupoint sensitization such as hemoglobin oxygen saturation, blood flow and rate of oxygen metabolism should be investigated.

This study not only makes a substantial breakthrough in the in vivo imaging of acupoints but also lays a strong scientific foundation for high-resolution imaging of acupoint sensitization. To the best of our knowledge, this study is the first time that acupoint sensitization in knee OA has been shown to have no association with the structural changes in microcirculation, suggesting that microcirculatory changes during acupoint sensitization are more likely to be functional. These findings may provide new insights and directions for future research. One challenge of this study was the longitudinal monitoring of vascular changes over multiple days. This strategy is difficult to implement with OR-PAM and has rarely been reported in other studies, which provides a useful reference and lays an important foundation for future studies.
Because acupoint sensitization is a dynamic process and its functional changes may be sensitive [12], the selection of observation time is crucial to revealing the exact microcirculatory changes during acupoint sensitization. In this study, days 0, 7 and 21 were selected based on the general rule of OA experiments [26] and the objective requirement for comparing before and after models established in the same group due to the potential individual differences during acupoint sensitization. Specifically, the first 7 days are considered as the acute stage of experimental model, at which pain-related behavioral changes emerge and progress rapidly [42, 43]. Furthermore, behavioral changes are relatively stable, and the occurrence bone destruction is observed at day 21. Whether the negative results are related to the missing of intermediate time points such as day 3 (early phase) and 14 (middle phase), as well the timing characteristics of acupoint sensitization in microcirculatory structure in knee OA warrant further investigation. Moreover, the organic changes in acupoints such as pachyderma, cutaneous pigmentation, hydroderma and other pathological changes of skin tend to appear in the later stage of severe cases. It is suggested that the structural changes in microcirculation lag behind functional changes and are closely associated with disease severity. Therefore, multistage monitoring and dynamic observation of the microcirculatory changes during acupoint sensitization should be applied in future research. In addition, more attention is required for the severity of disease, which may influence the implications of acupoint sensitization. Moreover, the acupoint sensitization occurs in an acupoint-dependent manner [12]. Although GB34 and ST36 are highly representative in knee OA acupoint sensitization and have been commonly applied to knee OA treatment, the changes in microcirculatory structure for other related acupoints warrant further investigation. Therefore, further expansion of the relevant acupoints, such as Dubi (ST35) and Yinlingquan (SP9), should be taken into consideration.

The use of OR-PAM still possesses some limitations, especially the imaging depth of 1 mm. Relatively shallow imaging depth failed to clarify the connotation of sensitized acupoints in deep tissues. Although no consensus has yet been reached on the depth of acupoints, it is well accepted that acupoints are multilayered and characterized by three-dimensional structures [44]. Therefore, whether the negative results of this study are related to the shallow imaging depth of OR-PAM should be confirmed by additional studies. Photoacoustic computed tomography has promised a greater detection depth [45], which can be introduced into studies of acupoint sensitization. In addition, the time needed to acquire an image with OR-PAM is dependent on the imaging range, scanning step and scanning speed. For a 3 × 3 mm area, if we set the step between adjacent A lines and B-scans as 5 μm, mechanical axis scanning speed was set at 5 mm/s and imaging time of approximately 10 minutes; thus, the imaging did not occur in real time. However, we

![FIGURE 8](image)
believed that new fast MEMS scanning, as reported in a recent publication [40], could significantly improve the DAQ time. Hence, the clinical application of a real-time system is feasible, when a suitable volume data size is acquired using fast MEMS scanning. In addition to this, wearable real-time monitoring equipment [46, 47] can be applied for an accurate and convenient observation of microcirculation due to the involvement of repeated in vivo imaging at the same location. On the other hand, the quantitative algorithm used in this study was unable to stratify vessel diameter data. As a consequence, the quantitative algorithm analysis of microcirculatory structure should be strengthened and improved.

5 CONCLUSION

This study, for the first time, achieved the in vivo PAI of acupoints at ~10 μm lateral resolution by using OR-PAM. In an experimental knee OA mouse model, structural changes in the microcirculation were dynamically observed during acupoint sensitization. This study preliminarily confirmed that acupoint sensitization in knee OA is not associated with structural changes in the ST36, GB34 and nonacupoints sites. Hence, we speculated that the microcirculatory changes during acupoint sensitization are more likely to be functional. Further research should focus on the functional changes in the microcirculation during acupoint sensitization such as hemoglobin oxygen saturation, blood flow and rate of oxygen metabolism.

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REFERENCES

[1] S. Chen, Y. Miao, X. Nan, Y. Wang, Q. Zhao, E. He, et al., Evid. Based Complement. Alternat. Med. 2015, 158012, 2015.
[2] P. J. Rong, J. J. Zhao, L. L. Yu, L. Li, H. Ben, S. Y. Li, et al., Evid. Based Complement. Alternat. Med. 2015, 516851, 2015.
[3] L. L. Yu, L. Li, P. J. Rong, B. Zhu, Q. Q. Qin, H. Ben, et al., Evid. Based Complement. Alternat. Med. 2014, 768634, 2014.
[4] L. Li, L. Yu, P. Rong, H. Ben, X. Li, B. Zhu, et al., Evid. Based Complement. Alternat. Med. 2013, 391283, 2013.
[5] P. J. Rong, S. Li, H. Ben, L. Li, L. L. Yu, C. X. Cui, et al., Evid. Based Complement. Alternat. Med. 2013, 742195, 2013.
[6] Y. Chae, H. Y. Kim, H. J. Lee, H. J. Park, D. H. Hahn, K. An, et al., J. Physiol. Sci. 2007, 57, 115.
[7] D. Xie, Z. Liu, X. Hou, B. Zhang, J. Xiong, M. Yi, et al., Acupunct. Med. 2013, 31, 422.
[8] C. C. Yan, S. Zhang, Q. Q. Li, L. W. Zhang, X. R. Wang, N. N. Fu, et al., BMJ Open 2017, 7, e014438.
[9] M. Bürklein, W. Banzer, J. Altern. Complement. Med. 2007, 13, 33.
[10] H. Hsu, M. H. Huang, P. T. Chao, M. Y. Yan, T. L. Hsu, W. K. Wang, et al., Physiol. Meas. 2007, 28, N77.
[11] S. Min, H. Lee, S. Y. Kim, J. Y. Park, Y. Chae, H. Lee, et al., J. Altern. Complement. Med. 2015, 21, 46.
[12] N. Ding, J. Jiang, X. Liu, Y. Xu, J. Hu, Z. Li, Evid. Based Complement. Alternat. Med. 2018, 2018, 7308767.
[13] J. Yao, L. V. Wang, Laser Photonics Rev. 2013, 7, 758.
[14] L. V. Wang, S. Hu, Science 2012, 335, 1458.
[15] J. Yao, L. V. Wang, Contrast Media Mol. Imaging 2010, 6, 332.
[16] L. V. Wang, J. Yao, Nat. Methods 2016, 13, 627.
[17] J. Yao, J. Xia, L. V. Wang, Ultrason. Imaging 2016, 38, 44.
[18] S. Hu, K. Maslov, L. V. Wang, Opt. Lett. 2011, 36, 1134.
[19] K. Maslov, H. F. Zhang, S. Hu, L. V. Wang, Opt. Lett. 2008, 33, 929.
[20] Z. Yang, J. Chen, J. Yao, R. Lin, J. Meng, C. Liu, et al., Opt. Express 2014, 22, 1500.
[21] Q. Li, L. Li, T. Yu, Q. Zhao, C. Zhou, X. Chai, J. Biophotonics 2017, 10, 780.
[22] S. Hu, B. Rao, K. Maslov, L. V. Wang, Opt. Lett. 2010, 35, 1.
[23] C. Yeh, J. Liang, Y. Zhou, S. Hu, R. E. Sohn, J. M. Arbelet, et al., J. Biomed. Opt. 2016, 21, 20501.
[24] V. Tsytsev, S. Hu, J. Yao, K. Maslov, D. L. Barbou, L. V. Wang, J. Biomed. Opt. 2011, 16, 076002.
[25] Y. Liu, X. Yang, H. Gong, B. Jiang, H. Wang, G. Xu, et al., J. Biomed. Opt. 2013, 18, 76007.
[26] T. Pichler, J. Sousa-Valente, M. Makkangai, J. Vis. Exp. 2016, 111, e53746.
[27] L. Li, R. Lin, H. Wang, J. Meng, H. Zheng, L. song, Opt. Express 2013, 21, 7316.
[28] H. Zhao, G. Wang, R. Lin, X. Geng, L. song, T. Li, et al., J. Biomed. Opt. 2018, 23, 1.
[29] J. Yang, D. Wu, Y. Tang, H. Jiang, J. Biophotonics 2017, 10, 217.
[30] H. Jeon, S. Ryu, D. Kim, S. Koo, K. T. Ha, S. Kim, et al., Evid. Based Complement. Alternat. Med. 2017, 3971675, 2017.
[31] N. Ding, J. Jiang, P. Qin, Q. Wang, J. Hu, Z. Li, PLoS One 2018, 13, e0194022.
[32] I. C. Tuncay, B. H. Ozdemir, H. Demirors, O. Karaeminogullari, N. R. Tandogan, J. Investig. Surg. 2005, 18, 115.
[33] H. Ji, J. Li, J. Shao, D. He, Y. Liu, W. Fei, et al., Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 2017, 123, 320.
[34] K. P. Pritzker, S. Gay, S. A. Jimenez, K. Ostergaard, J. P. Pelletier, P. A. Revell, et al., Osteoarthr. Cartil. 2006, 14, 13.
[35] M. S. Corbett, S. J. Rice, V. Madurasinghe, R. Slack, D. A. Fayter, M. Harden, et al., Osteoarthr. Cartil. 2013, 21, 1290.
[36] S. Ahlsin, S. Saleem, A. M. Bharti, R. K. Iles, M. Aslam, Pain 2009, 147, 60.
[37] C. I. Mavrommatis, E. Argyra, A. Vadalouka, D. G. Vasilakos, Pain 2012, 153, 1720.
[38] N. Purepong, A. Jitvimonrat, E. Sithipornvorakul, S. Eksakuakul, P. Janwantanakul, Acupunct. Med. 2012, 30, 187.
[39] B. Ming, M. J. Kennedy, A. J. Dixon, N. Sun, R. Cao, B. T. Soetikno, et al., Opt. Lett. 2015, 40, 901.
[40] J. Yao, L. Wang, J. M. Yang, K. I. Maslov, T. T. Wong, L. Li, et al., Nat. Methods 2015, 12, 407.
[41] S. X. Zheng, X. H. Pan, J. S. Xu, C. Y. Xiu, Y. Q. Dong, X. Zhu, BMC Complement. Altern. Med. 2014, 14, 323.
[42] A. C. Ogbonna, A. K. Clark, C. Gentry, C. Hobbs, M. Makkangai, Eur. J. Pain 2013, 17, 514.
[43] L. J. Moilanen, M. Hämäläinen, E. Nummenmaa, P. Ilmarinen, K. Vuolteenaho, R. M. Nieminen, et al., Osteoarthr. Cartil. 2011, 23, 17.
[44] W. Chapple, J. Acupunct. Meridian Stud. 2013, 6, 270.
[45] R. Li, E. Phillips, P. Wang, C. J. Goergen, J. X. Cheng, J. Biophotonics 2016, 9, 124.
[46] J. Tang, L. Xi, J. Zhou, H. Huang, T. Zhang, P. R. Carney, et al., J. Cereb. Blood Flow Metab. 2015, 35, 1224.
[47] J. Tang, J. E. Coleman, X. Dai, H. Jiang, Sci. Rep. 2016, 6, 25470.