Effect of different twirling and rotating acupuncture manipulation techniques on the blood flow perfusion at acupoints

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Abstract

OBJECTIVE: To analyze the effect of different twirling and rotating acupuncture manipulation techniques on the blood flow perfusion at acupoints to provide a reference for the study of acupoint specificity and the quantification and effectiveness of acupuncture methods.

METHODS: Twenty healthy male or female college students each received four different acupuncture manipulation techniques, including simple acupuncture, uniform reinforcing-reducing needling, twirling reinforcing needling, and twisting reducing needling. The self-control method was applied. Acupuncture was performed by an acupuncture manipulation simulator at Neiguan (PC 6) and Zusanli (ST 36). The process of twirling and rotating was divided into seven timepoints. The PeriCam Perfusion Speckle Imager (PSI) System was used to collect the blood flow perfusion data at each acupoint. The specificity of twirling and rotating acupuncture manipulation was analyzed based on changes in the curve, video, and numerical blood flow perfusion data at each timepoint.

RESULTS: There were two peaks in the blood flow perfusion curve of twirling and rotating acupuncture manipulation; one appeared after 3 min of needle retention, and the other appeared 3 min after needle removal. The blood flow perfusion parameters showed that the greatest differences between the four manipulation techniques occurred after 5 and 10 min of needle retention. The specificity of various manipulation techniques was most obvious at these two timepoints. There were significant differences between the four manipulation groups in the blood flow perfusion and the relative change rates of blood flow perfusion at each timepoint.

CONCLUSION: Laser speckle imaging enables the real-time, non-invasive, rapid, and accurate collection of blood flow perfusion data during acupuncture. This imaging technique enables the easy attainment of various parameters such as visual images, two-dimensional curves, and data tables. At various timepoints, the four groups significantly differed regarding changes in blood flow perfusion and relative change rates of blood flow perfusion, which facilitated the differentiation of the four acupuncture manipulation methods. Based on this, further analysis could be conducted to study spatial distribution characteristics such as the influence area and flare area. The frequency domain analysis of
acupuncture manipulation curves is important in the study of the dose-effect relationship and specificity of acupuncture manipulation.

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Keywords: Acupuncture; Sensitivity and specificity; Blood flow perfusion; Laser speckle imaging

INTRODUCTION

Acupuncture manipulation is the most important basic skill in the clinical practice of acupuncture and moxibustion, and the type of manipulation is closely related to the clinical efficacy of acupuncture. Therefore, the analysis of acupuncture manipulation is an important part of the quantitative study of acupuncture techniques. The general quantitative study of acupuncture techniques focuses on the measurement of mechanical, electrical, and biological parameters. Continuous advancement of technology has facilitated the combination of acupuncture manipulation study and imaging techniques; this combination has vast research potential, as images can be used to analyze acupuncture manipulation. The acupuncture process has temporal and spatial characteristics that influence the effectiveness of treatment.1 In the present study, laser speckle imaging was used to analyze the effect of twirling and rotating acupuncture manipulation on the blood flow perfusion of the skin at acupoints. Laser speckle contrast imaging evaluates the blood flow by analyzing the spatial statistical properties of time-integrated speckles. Laser speckle contrast imaging analysis is simple, convenient, and non-invasive; furthermore, its extraction has high spatial and temporal resolution, with no need for the injection of contrast agents or drugs.2,3 These properties enable laser speckle contrast imaging to quantify the influence of different acupuncture and moxibustion therapies on the microcirculation of the body.4 With millisecond-scale temporal resolution and micrometer-scale spatial resolution, laser speckle contrast imaging analysis achieves real-time imaging of blood flow, which lays the foundation for the analysis and quantitative research of the effect of twirling and rotating acupuncture manipulation on the microcirculation.

The amount of blood flowing into tissue and organs per unit of time is referred to as the blood flow perfusion unit (PU),5 and is often calculated in mL · min⁻¹ · 100 g⁻¹. Needling exerts a direct effect on the superficial cutaneous microcirculation at acupoints. In recent years, an increasing number of studies have investigated how acupuncture influences blood flow perfusion and microcirculation blood volume to further the research into the acupuncture effect and dose-effect relationship.6,7 In the present study, a blood perfusion imager called the PeriCam Perfusion Speckle Imager (PSI) System was used to monitor the changes in blood flow perfusion and analyze the features of twirling and rotating acupuncture manipulation in accordance with the monitoring results. The present study could provide technological methods for future research into the specificity and evaluation of acupuncture manipulation and the assessment of acupoint functions.

MATERIALS AND METHODS

Subjects and grouping

The present study included 20 healthy volunteer subjects comprising eight men and 12 women with an average age of (25.8 ± 1.7) years who were students majoring in acupuncture and moxibustion and tuina. Random number table method was used to determine the sequence of subjects receiving the four types of acupuncture manipulation: twirling reinforcing needling, twisting reducing needling, uniform reinforcing-reducing needling, and simple acupuncture. All subjects were in good health with normal body temperature, no history of exogenous diseases in the past week, and had not previously undergone acupuncture and moxibustion treatment. Furthermore, the skin at the assessed acupoints was free of petechiae, ecchymosis, skin neoplasms, and other conditions that might affect the blood flow perfusion monitoring. The exclusion criteria were cardiocerebrovascular disease, peripheral vascular disease, connective tissue disease, diabetes mellitus, skin diseases, or recent intake of drugs that affect vascular activity; women were also excluded if the experiment was being performed within 3 d before, during, or 3 d after menstruation. This study was approved by the ethics committee of Yueyang Hospital affiliated with Shanghai University of Traditional Chinese Medicine (reference No. 2016-108) and all subjects provided written informed consent to be included in the study.

Experimental environment and conditions

Skin blood flow perfusion parameters are easily affected by factors such as ambient temperature, light, and air flow. Thus, the experiments were carried out in a quiet and dry room with a humidity level of 30%-40%, ambient temperature of (20 ± 2) °C, and no obvious air flow in a location that was not near any air conditioning vents. The experimental locations were normally lit, without direct sunlight and strong or weak light.

Acupuncture point selection

Zusanli (ST 36) and Neiguan (PC 6) were chosen for three main reasons. Firstly, these two acupoints are convenient to needle and collect data from and they are located on limbs, which are the focus of acupuncture microcirculation research. Secondly, these two acupoints are recognized to have a noticeable therapeutic effect and have been investigated in many studies, which facilitates cross-comparison study and mutual demonstra-
tion. Thirdly, as the present study mainly focused on the specificity of acupuncture manipulation, it is easiest to identify the commonalities of manipulation functions when only two acupoints are used in a comparative observation. The acupoint positions were based on the standards in Experimental Acupuncture and Moxibustion. Neiguan (PC 6) is located two cun above the distal palmar wrist crease, between the palmaris longus tendon and the flexor carpi radialis tendon. Zusanli (ST 36) is located three cun below Dubi (ST 35), on the anterior tibialis and the line connecting Dubi (ST 35) and Jixi (ST 41).

**Acupuncture manipulation simulator**
An acupuncture manipulation simulator developed by the Institute of Traditional Chinese Medicine Engineering at Shanghai University of Traditional Chinese Medicine was used to ensure that the subjects each received the same amount of acupuncture stimulation. The acupuncture manipulation simulator was purchased from Shanghai TCM shanxin medical technology Co., Ltd., (ZSF-01, Shanghai, China). The simulator replaced manual acupuncture needle insertion and manipulation. The simulator achieves objective quantification by ensuring that the actual stimulation quantity is identical to the set value, avoiding the interference of subjective factors. The maximum twisting and rotating angle of the instrument is $360^\circ \times 4/s$ with a stroke range of $\pm 720^\circ$ and a minimum adjustment angle of $1.5^\circ$.

**Pericam perfusion speckle imager (PSI) system**
The PeriCam PSI System used to collect the blood flow perfusion data. PeriCam PSI System was purchased from Perimed AB Company (Stockholm, Sweden). The wavelength of the monitoring laser was 785 nm, the power was 70 mw, video frame capture rate was 94 frames/s, scan frequency of video sampling was 50 Hz, resolution of the camera monitoring blood flow was $1388 \times 1038$ pixels, and resolution of the optical camera was $752 \times 580$ pixels.

**Experimental procedures**
The acupoints of the subjects were disinfected and marked. The subjects were instructed to avoid eating cold or hot food and avoid exercising within 1 h prior to the experiment, and were required to lie down and adapt to the conditions of the room for at least 15 min prior to receiving acupuncture. When the subject was in the appropriate posture, the operator began needling and performing acupuncture manipulation after obtaining Qi. The four acupuncture manipulation groups were simple acupuncture (needle retention without any manipulation (G0)), uniform reinforcing-reducing needling (G1), twirling reinforcing needling (G2), and twisting reducing needling (G3). The blood flow perfusion of the two acupoints was recorded by the PeriCam PSI System. Each subject received all four types of manipulation; the interval between data collection was at least 2 d. Figure 1 shows the data collection procedures in the present study.

**Observation indicators and parameters**
Data, curves, and videos of the acupuncture process: after completing the needling process, the curves, videos, and numerical data of the blood flow perfusion during the entire experiment were obtained. Data were recorded for 30 min in total, starting from before the needling was done and finishing after the needles were removed. The curve recorded the real-time dynamic changes in blood flow perfusion at the monitored acupoints. Color coding technology was used to show the blood flow perfusion at the monitored acupoints on the image. High perfusion areas were shown in red, while low perfusion areas were shown in blue. Intensity images and optical images were also obtained. Blood flow perfusion data showed numerical changes in the perfusion of acupoints. Figure 2 shows the curve, video, and numerical data interface of the blood flow perfusion at Neiguan (PC 6).

**Blood flow perfusion at different timepoints**
Most previous studies focused on the average blood
flow perfusion volume. Analysis results have proved that acupuncture manipulation changes the skin blood flow perfusion volume, but it is hard to detect differences between various types of manipulation. As the cumulative effect of acupuncture stimulation and time parameters are closely related to the blood flow perfusion volume, researchers have begun to pay more attention to the needle retention time. Therefore, to better clarify the changes in blood flow perfusion, the acupuncture process in the present study was divided into seven timepoints in accordance with the sequence followed in clinical practice: T1 (before acupuncture), T2 (when Qi was obtained), T3 (during acupuncture manipulation), T4 (during 5 min of needle retention), T5 (during 10 min of needle retention), T6 (during 15 min of needle retention), and T7 (during 20 min of needle retention). The average blood flow perfusion volume at these seven timepoints was analyzed to identify any differences between various acupuncture manipulation methods at different times. Blood flow perfusion at different timepoints is shown in Figure 3.

Relative change rates in blood flow perfusion

The changes in the assessed blood flow perfusion parameters are affected by two factors. One factor is the instantaneous changes caused by acupuncture manipulation stimulation, and the other is the regular changes in skin blood flow perfusion of the limbs due to the heartbeat, which interferes with the study of peripheral blood flow perfusion. In the present study, the obtained PU value was used to calculate the "relative change rates in blood flow perfusion volume" (F, a dimensionless quantity). F was calculated using the following formula.

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F = \frac{PU_{current\ time\ period} - PU_{previous\ time\ period}}{PU_{previous\ time\ period}} \times 100\%
\]

F was used in the present study to highlight the influence of acupuncture manipulation and exclude the interference caused by metabolic effects of the body and regular heartbeat.

Statistical analysis

SPSS software (version 19, IBM Corp., Armonk, NY, USA) was used for analysis, and the statistical significance level was set at 0.05. SPSS software (version 19, IBM Corp., Armonk, NY, USA) was used for analysis, and the statistical significance level was set at 0.05.

Figure 2 Blood flow perfusion curve, video, and numerical data obtained during the experiment
A: curve data; B: numerical data; C1: video image of blood perfusion; C2: video image of intensity; C3: video image of photo.

Figure 3 Screenshots of blood flow perfusion videos recorded at Neiguan (PC 6) at different timepoints during stimulation
A: T1 moment; B: T2 moment; C: T3 moment; D: T4 moment; E: T5 moment; F: T6 moment; G: T7 moment. T1: before acupuncture; T2: after obtaining Qi; T3: immediately after twirling manipulation was done; T4: after 5 min of needle retention; T5: after 10 min of needle retention; T6: after 15 min of needle retention; T7: after 20 min of needle retention.
USA) was used for the statistical analysis. Data were described as mean ± standard deviation. Normally distributed data were analyzed by one-way analysis of variance. The least significant difference method was used to analyze data with homogeneity of variance, while Tamhane’s T2 was used for data without homogeneity of variance. Non-normally distributed data were analyzed by the Kruskal-Wallis test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Changes in two-dimensional curves and videos of blood flow perfusion

Blood flow perfusion curves and videos of Zusanli (ST 36) and Neiguan (PC 6) were recorded in the simple acupuncture (G0), uniform reinforcing-reducing needling (G1), twirling reinforcing needling (G2), and twisting reducing needling (G3) groups. Figure 4 shows the perfusion curves of blood flow, which clearly demonstrates the change in the blood flow perfusion volume at the two acupoints caused by the four needling techniques. Compared with G0 (simple acupuncture), more pulsatile changes in blood flow perfusion were seen during needle retention in G1 (uniform reinforcing-reducing needling), G2 (twirling reinforcing needling), and G3 (twisting reducing needling). The pulsatile changes in blood flow perfusion during needle retention were related to the quivering of the acupuncture needles, which was affected by different manipula-

Figure 4 Two-dimensional curves of blood flow perfusion during the experiment

A: the curve of simple acupuncture at Neiguan (PC 6); B: the curve of simple acupuncture at Zusanli (ST 36); C: the curve of uniform reinforcing-reducing needling at Neiguan (PC 6); D: the curve of uniform reinforcing-reducing needling at Zusanli (ST 36); E: the curve of twirling reinforcing needling at Neiguan (PC 6); F: the curve of twirling reinforcing needling at Zusanli (ST 36); G: the curve of twisting reducing needling at Neiguan (PC 6); H: the curve of twisting reducing needling at Zusanli (ST 36).
tion techniques. Compared with G0 (simple acupuncture), the quivering of needles was more obvious in the other three groups.

Figure 5 shows screenshots of the blood flow perfusion videos recorded at Zusanli (ST 36) during stimulation by twirling reinforcing needling; there were changes in the brightness of blood flow perfusion at the seven timepoints. Compared with G0 (simple acupuncture), more pulsatile changes in blood flow perfusion were seen during needle retention in G1 (uniform reinforcing-reducing needling), G2 (twirling reinforcing needling), and G3 (twisting reducing needling). The pulsatile changes in blood flow perfusion during needle retention were related to the quivering of the acupuncture needles, which was affected by different manipulation techniques. Compared with G0 (simple acupuncture), the quivering of needles was more obvious in the other three groups.

Comparison of the curves and images of blood flow perfusion at Neiguan (PC 6) and Zusanli (ST 36) showed that the average blood flow perfusion volume at Neiguan (PC 6) was higher than that at Zusanli (ST 36) at the same time within the same manipulation group. The brightness and range of blood flow perfusion images at Neiguan (PC 6) were significantly stronger and larger than at Zusanli (ST 36).

The flare areas of blood flow perfusion appeared immediately after needle insertion (Figures 3 and 5). The blood flow perfusion images, flare areas with the largest range and the strongest brightness generally appeared after the needles had been retained for about 3 min. Furthermore, a flare area with large range and strong brightness appeared on the blood flow perfusion image about 3 min after the needles were removed. Figure 6 shows the curve of uniform reinforcing-reducing needling at Zusanli (ST 36) as an example; the two red square frames respectively represent the appearance times of two flares.

Changes in blood flow perfusion volume at different timepoints

Figure 7A shows the changes over time in the blood flow perfusion at Neiguan (PC 6) in the four manipulation groups. There were significant differences between the four groups at T4 (P < 0.05). Analysis of independent samples for the timepoints with disparity demonstrated that the blood flow perfusion in G1 was markedly different from that in G2 and G3.

Figure 7B shows the changes over time in the blood flow perfusion at Zusanli (ST 36) in the four manipulation groups. There were significant differences between the four groups at T4 and T5 (P < 0.05). Analysis of independent samples showed that the blood flow perfusion significantly differed between G0, G2, and G3 at T4. There was also a marked difference in blood flow perfusion between G1 and G0 at...
T4. At T5, there were marked differences in the blood flow perfusion between G2 and G3, and between G1 and G0 and G3.

**Changes in the relative change rates of blood flow perfusion**

Figure 8A shows the changes over time in the F at Neiguan (PC 6) in the four manipulation groups. There were significant differences between the groups at T4 ($P < 0.05$). Analysis of independent samples for the timepoints with disparity showed significant differences in the F between G2 and the other three groups at T4, and significant differences between G0 and G3 at T4.

Figure 8B shows the changes over time in the F at Zusanli (ST 36) in the four manipulation groups. There were significant differences between the groups at T2, T4, and T5. Analysis of independent samples for the timepoints with disparity showed that the F significantly differed between G2 and the other three groups at T2 and T4.

There were significant differences between G0, G2, and G1 at T5, which shows that the disparity in the F started to appear between G0 and the other three groups at T5.

**DISCUSSION**

**Effects on blood flow perfusion curves and videos**

The values and curves of blood flow perfusion increased significantly immediately after needle manipulation in all four groups, especially at the stage of obtaining Qi and during needle retention (Figure 6); thus, the blood flow perfusion volume of the acupoints was significantly higher after needle manipulation than before needling. The spots on the blood flow perfusion image were initially small and dim, and then became big and bright. In addition, the changes in the curves over time were complex. No clear differences could be seen between the four manipulation curves. The blood flow perfusion curves of twirling and rotating acupuncture manipulation were characterized by two peaks, one that appeared after 3 min of needle retention, and one that appeared 3 min after the needles were removed.

According to engineering signal analysis, transient pulsatile changes belong to high-frequency signal changes. Frequency domain analysis of blood flow perfusion parameters could be performed to further study the specificity of different acupuncture manipu-

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**Figure 6** Curve of uniform reinforcing-reducing needling at Zusanli (ST 36)

**Figure 7** Changes in blood flow perfusion volume at different timepoints
A: Neiguan (PC 6); B: Zusanli (ST 36). T1: before acupuncture; T2: after obtaining Qi; T3: immediately after the twirling manipulation was done; T4: after 5 min of needle retention; T5: after 10 min of needle retention; T6: after 15 min of needle retention; T7: after 20 min of needle retention.

**Figure 8** Relative change rates of blood flow perfusion at different timepoints
A: Neiguan (PC 6); B: Zusanli (ST 36). T1: before acupuncture; T2: after obtaining Qi; T3: immediately after twirling manipulation was done; T4: after 5 min of needle retention; T5: after 10 min of needle retention; T6: after 15 min of needle retention; T7: after 20 min of needle retention.
Evaluating techniques in terms of pulsatile changes in blood flow perfusion.

**Effects on blood flow perfusion**

At T1 (before needling) and T2 (after obtaining Qi), the blood flow perfusion did not significantly differ between the four groups. This revealed that interference factors of each manipulation group were well controlled before the experiment, which ensured that there were no significant differences in blood flow perfusion parameters before the manipulation. Based on this, it was most meaningful to analyze the blood flow perfusion at different timepoints after needle insertion.

Compared with G0 (simple acupuncture), the stimulation was higher in the other three groups. However, there were no significant differences between G0 and the other three groups in the blood flow perfusion at T3 (immediately after the manipulation was done).

Line graphs showed that the PU value at T3 (immediately after the manipulation was done) was lower than that at T2 (after obtaining Qi) (Figures 7). This suggests that after the strong stimulation produced by needling and obtaining Qi, continuing to increase the stimulating volume by reinforcing or reducing manipulation leads to a decrease in blood flow perfusion rather than an increase. Acupuncture reportedly controls sympathetic excitation and causes arteriole contraction. Acupuncture may also increase the number of metabolites regulating vasomotor control, leading to a decrease in microcirculation.

There were significant differences between each manipulation group in the blood flow perfusion parameters at T4 (after 5 min of needle retention) and T5 (after 10 min of needle retention). This showed that the four different acupuncture manipulation techniques could be distinguished in accordance with refined parameters at various timepoints, and that T4 (after 5 min of needle retention) was important in the study of the specificity of acupuncture manipulation. Further analysis of the data from T4 could play an important role in determining the specificity of needle manipulation and studying the dose-effect relationship of acupuncture manipulation, such as the time of Qi attainment and the time of needle retention.

As shown in the line graphs of the different acupuncture manipulation techniques, the maximum PU value of each group appeared at a different timepoint, which caused significant differences between G2 (twirling reinforcing needling) and G3 (twisting reducing needling), and between G2 (twirling reinforcing needling) and G1 (uniform reinforcing-reducing needling) at Neiguan (PC 6) at T4 (after 5 min of needle retention). Due to the temporal and spatial characteristics of the needling process, the spatial parameters (such as the blood flow perfusion area) that were influenced by twirling and rotating acupuncture manipulation must have been inconsistent.

Abundant blood flow perfusion at acupoints is an important reason for the low electrical resistance of acupoints. Thus, it is important to analyze the effect of acupuncture manipulation on the spatial parameters of blood flow perfusion.

**Effects on the relative change rates of blood flow perfusion**

There were significant differences in the F between G2 (twirling reinforcing needling) and the other three groups at T4 (after 5 min of needle retention). This revealed that twirling reinforcing needling caused the greatest changes in blood flow perfusion. The F at T4 (after 5 min of needle retention) effectively differentiated the twirling reinforcing needling group from the other three groups. It is important to study the specificity of blood flow perfusion related to acupuncture manipulation.

At Zusanli (ST 36), the F in G0 (simple needling) was very different from that in G2 (twirling reinforcing needling) and G1 (uniform reinforcing-reducing needling) at T5 (after 10 min of needle retention). Figure 8 shows that twirling reinforcing needling caused the most obvious changes in the F value during needle retention (T4-T7), followed by simple needling. G1 (uniform reinforcing-reducing needling) had the most stable F values.

In summary, the present study dynamically observed the influence of twirling and rotating acupuncture manipulation on blood flow perfusion at two acupoints and analyzed the time characteristics of twigling and rotating acupuncture manipulation. As various needling techniques with their own characteristics have commonalities, different twirling and rotating acupuncture manipulation techniques can be differentiated through PU changes and the F at different timepoints. This may serve as a reference for the quantification of acupuncture methods and lay a foundation for the study of acupoint specificity and acupuncture manipulation effects.

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