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Cerebral Blood Flow Monitoring by Diffuse Speckle Contrast Analysis during MCAO Surgery in the Rat

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The rodent model has been used frequently to understand stroke pathophysiology, due to its low cost and the large spectrum of genetic strains available. Here, we present a diffuse speckle contrast analysis system (DSCA) with a 1×2 optical switch that was used to non-invasively assess cerebral blood flow (CBF) changes in the rat during intraluminal suturing for middle cerebral artery occlusion (MCAO) surgery. The blood flow index (BFI) in the left hemisphere was lower than that in the right hemisphere because the left middle cerebral artery was occluded. Furthermore, the performance of the DSCA system was compared with that of commercial laser Doppler flowmetry. The changes in the BFI measured by the two systems were correlated strongly. The DSCA system was less sensitive to motion artifacts and able to measure relatively deep tissue flow in the rat's brain. In conclusion, the DSCA system secured CBF monitoring during surgery in a rodent model without craniotomy.

Keywords : Cerebral blood flow, Blood flow index, Diffuse speckle contrast analysis, Middle cerebral artery occlusion
OCIS codes : (000.1430) Biology and medicine; (110.6150) Speckle imaging; (170.3890) Medical optics instrumentation

I. INTRODUCTION

Stroke is a major disease leading to death in adults worldwide [1, 2]. Cerebral ischemic stroke has a high incidence, accounting for approximately 80% of strokes [3]. Animal models have been used widely to understand stroke pathophysiology and therapeutic interventions [4-6]. The rat model has been used mostly as an animal model for stroke studies due to its low cost and the similarity of the cranial blood circulation to that of humans [7-9]. The middle cerebral artery (MCA) is often influenced and triggered by stroke in humans. Middle cerebral artery occlusion (MCAO) has been developed as a clinical method in several models, including the photothrombosis model [10-12], the endothelin-1-induced stroke model [13, 14], and the intraluminal suture MCAO model [15-17]. In the intraluminal suture MCAO model, a silicon-coated filament suture is placed intraluminally at the origin of the MCA for a few minutes following the reperfusion step. It enables the induction of ischemic stroke without craniotomy, which makes the procedure easier to perform with minimal invasiveness, as other models require removal of the skull.

Several high-resolution cerebral blood flow (CBF) imaging techniques, including micro-computed tomography [18], diffusion tension imaging [19], and micro-magnetic resonance imaging [20], have been developed for the rodent model. However, these techniques are limited for real-time blood flow monitoring. To acquire real-time hemodynamic information rapidly, blood flow measurement systems, such as laser Doppler flowmetry (LDF) [21-24], laser speckle contrast imaging (LSCI) [25, 26], and diffuse correlation spectroscopy (DCS) [27, 28], have been applied to the rodent brain. To date, these systems have focused on functional changes in

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CBF in response to chemical [21], physical [22-24], or electrical [26] stimulation.

To improve the reliability of the MCAO model, a blood flow monitoring system can be applied during MCAO surgery in the rat brain. Many operators have used LDF to detect regional CBF during intraluminal-suture MCAO surgery due to this modality's ease of use [29, 30]. LSCI has also been used frequently for two-dimensional imaging of the rodent brain in the intraluminal suture MCAO model [31, 32] and the photothrombosis model [12]. However, this method of CBF measurement normally requires a craniotomy procedure in addition to special apparatus and complex techniques, as the measurement depth of the system is relatively shallow (~1 mm). Without craniotomy, diffuse optical tomography [33] and custom-designed dual-wavelength DCS flow oximetry [28] have been applied for rodent CBF mapping and analysis during MCAO surgery, as they enable the measurement of flow in relatively deep tissue (several centimeters).

In this study, we established a local CBF measurement technique, i.e., diffuse speckle contrast analysis (DSCA), to monitor changes in CBF in the intraluminal-suture MCAO rat model. DSCA was used to estimate the relative blood flow index (BFI), which depends on the correlation of speckled patterns caused by scatter movement inside the tissue. The system consists of a near-infrared laser, a charge coupled device (CCD) camera, and optical fibers. The DSCA system is based on the DCS configuration and the signal processing of LSCI [34-37]. Although LSCI involves simple data analysis and is easy to implement, its measurement range is limited to the superficial layer (~1 mm). DCS can non-invasively measure relatively deep blood flow (several centimeters), but it requires highly sensitive photon detectors and complex data analysis. The presented DSCA has various advantages, such as simple data analysis and setup, deep tissue flow measurements similar to DCS, and relatively fast signal processing.

The DSCA technique has been validated previously by measuring blood flow on a dry phantom, *in vivo* on a human arm, and in chick embryos. The DSCA has not been applied to rat brain monitoring during brain surgery. Here, we describe a DSCA system using an optical switch, including comparison with commercial LDF, which enables the precise monitoring of CBF information during intra-luminal-suture MCAO surgery. Using data on regional and global-CBF changes, we upgraded the system effectiveness in the MCAO rat model.

II. MATERIALS AND METHODS

2.1. Animal Preparation

The Animal Experiment Ethics Committee of Daegu Gyeongbuk Institute of Science and Technology approved this experimental protocol (approval no. DGIST-IACUC-0023). Nine male Sprague-Dawley rats (weight, 300-320 g) were anesthetized with 25 mg/kg Zoletil and 10 mg/mL Rompun via intraperitoneal injection. All rats were maintained at 37°C with a heating pad during the MCAO surgery.

2.2. Diffuse Speckle Contrast Analysis

An experimental DSCA setup for CBF measurement is shown in Fig. 1(a). A 785-nm-wavelength laser diode (100 mW, DL-785-100S; Crystalaser, Reno, NV, USA) was connected to a 1 × 2 optical switch (SW1X2; Sercalo, Neuchâtel, Switzerland) with multi-mode optical fibers. Two single-mode optical fibers were fixed in a CCD camera (F-033B; Stingray, London, England, UK). The optical switch distinguished the cross-talk signal when the detection light was overlaid on the detectors between neighboring probes. The optical switch was operated by a transistor-transistor logic signal from a data acquisition device, which controlled the sequential illumination of the laser beam on the tissues. To avoid biological damage due to high-power irradiation, the optical power of the laser was adjusted to about 6.5 mW [34].

Speckle contrast (K) was defined as described in Eq. (1).

$$K = \frac{\sigma}{< I >}, \tag{1}$$

where σ is the standard deviation and $< I >$ is the mean intensity in the region of interest (ROI). The contrast is related to the normalized variance (V_N) and unnormalized electric field autocorrelation ($G_1(\mathrm{r},\tau)$) for the given exposure time (T), as shown in Eq. (2).

$$K^2(T) = V_N(T) = \frac{2k}{T}\int_0^T (1-\frac{\tau}{T})[\mathrm{g}_1(\mathrm{r},\tau)]^2 d\tau, \tag{2}$$

where τ is the time delay and k is a constant dependent on the ratio of detector size to speckle size. $G_1(\mathrm{r},\tau)$ has been defined in greater detail in previous studies [34, 35]. Here, r is the distance between the source and detector. To linearize between the BFI and physiological blood flow, the BFI was defined as: $1/K^2$.

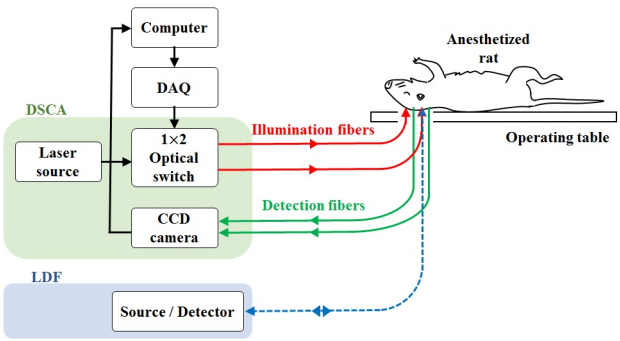

FIG. 1. Experiment configuration of the diffuse speckle contrast analysis (DSCA) system and laser Doppler flowmetry (LDF) during MCAO surgery.

2.3. MCAO Surgery Procedure

Transient MCAO was induced surgically in the rats according to the technique of Uluç et al. [17]. The superficial fascia was dissected under an operating microscope after the creation of a ventral midline incision. Careful sharp dissection was performed beneath the superficial fascia to expose a Y-shaped artery composed of the external, internal, and common carotid arteries (ECA, ICA, and CCA). Then, the ECA was tied off with a 6-0 silk suture as far distally as possible, and another silk suture around the ECA was placed loosely near the bifurcation of the Y-shaped artery. After the CCA and the ICA had been clipped near the bifurcation using microsurgical clips, an incision in the ECA was created between the two silk sutures. A 4-0 monofilament nylon suture with a silicon-coated tip (403956PK10; Doccol Corp., Sharon, MA, USA) was inserted into the ECA lumen toward the CCA. The nylon suture was turned from the CCA to the ICA lumen after removal of the microclip from the ICA. The suture was advanced 18-20 mm toward the MCA. Then, the microclip was removed from the CCA after a timer had been started to record occlusion time. In this experiment, the rats experienced 45 min occlusion time. After occlusion, the occluding suture was removed completely, and the ECA stump was tied off tightly. Then, the incision from the neck was closed with 3-0 silk sutures. Finally, the rats were placed in a recovery cage.

2.4. Experimental Protocols

The midline scalp was incised to expose the rat's skull before the installation of a probe supporter fabricated of polydimethylsiloxane. The probe supporter held all optical fibers perpendicular to the skull. The flexibility of the probe supporter allowed strong attachment along the curved skull surface, and the optical fibers were fixed vertically along the skull surface. The scalp around the probe was closed after attachment of the probe supporter. Then, the probe supporter and the neck were wrapped with a rubber band to prevent undesired motion during the surgery.

First, we compared CBF among three rats subjected to DSCA and LDF during MCAO. To date, the DSCA has been reported as a novel blood flow instrument, but it has not been compared with conventional instruments. Here, the CBF measurements by DSCA were compared with those obtained with commercial LDF (LDF-C1; Omegawave, Tokyo, Japan) to validate DSCA reliability. An LDF probe with one source and a detector pair was placed 4 mm lateral and 2 mm posterior to the bregma. The DSCA detector alone, without DSCA illumination, was located 4 mm lateral and 3 mm anterior to the bregma in Fig. 2. This probe detected the diffused light after being illuminated from the LDF laser with long coherence, a wavelength of 780 nm, and optical power of ~6 mW. The separation between the LDF probe and the DSCA detector was 5 mm, so the measuring depth of the DSCA was about 2.5 mm. Left hemispheric CBF of the rat was measured simultaneously

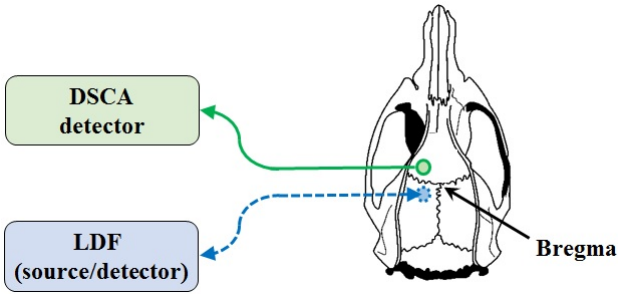

FIG. 2. Implementation of the laser Doppler flowmetry (LDF) and diffuse speckle contrast analysis (DSCA) probes to compare their performance.

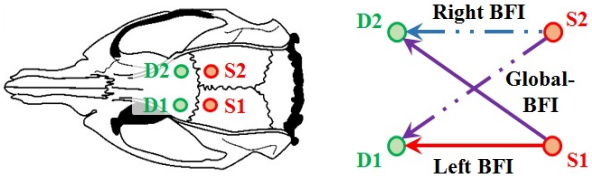

FIG. 3. Implementation of diffuse speckle contrast analysis (DSCA) probes to measure regional and global cerebral blood flow (CBF) (a) Placement of the DSCA probes on the rat skull for the measurement of regional CBF and global-CBF (S1 and S2, source 1 and source 2; D1 and D2, detector 1 and detector 2). (b) Optical paths for detecting the left, right, and global- blood flow indexes (BFIs). Note that the parallel directions are for the regional CBF measurements and diagonal directions are for the global-CBF measurements.

by LDF and DSCA during the MCAO surgery.

Next, DSCA-based CBF was measured in each of the two hemispheres (regional CBF) and across hemispheres (global-CBF) in six rats to enhance the reliability of left middle cerebral artery occlusion (L-MCAO) during the surgery. The laser sources were positioned 4 mm lateral and 2 mm posterior to the bregma in Fig. 3(a). The location of left laser source (S1) for left hemispheric CBF measurement is same as previous location of the LDF probe. The detection fibers were located 4 mm lateral and 3 mm anterior to the bregma. Regional CBF was measured in each hemisphere sequentially by manipulating the TTL ON/OFF signal of the optical switch in Fig. 3(b). The two detectors collected diffused light in parallel. Changes in regional CBF in the left and right hemispheres were measured by the left (S1-D1) and right (S2-D2) fiber pairs. The diagonal fiber pairs (S1-D2 and S2-D1) measured blood flow across the two hemispheres (global-CBF) of brain tissue. The S-D distance was about 9.43 ($\sqrt{8^2+5^2}$) mm, enabling a measurement depth of ~5 mm. The changes in regional CBF and global-CBF were monitored continuously during the MCAO surgery.

2.5. Signal Processing

All measurements were processed using LabVIEW software. A temporal domain analysis was introduced to increase the spatial resolution of the BFI analysis [35]. The exposure time of the CCD camera was set to 15.73 ms, so that the acquisition rate was 60 frames/s. In this study, the BFI was calculated using 20 frames, achieving a BFI acquisition speed of 3 Hz. The ROI window size was 11 × 11 pixels. The moving average of six window sizes was applied for smooth data processing. To calculate the percentage changes in the relative blood flow index (rBFI), the BFI was normalized to the baseline period (100%). Average rBFIs are expressed as means ± standard errors. Student's *t*-test was applied to analyze the changes in CBF with the criterion of $p < 0.05$.

III. RESULTS AND DISCUSSION

3.1. Comparison of CBF Data from LDF and DSCA

Figures 4(a) and 4(b) show CBF measurements acquired simultaneously by LDF and DSCA during MCAO surgery. The BFI responses to the CCA clip and MCAO were correlated strongly in both systems. The BFI decreased gradually when the left CCA (L-CCA) was clipped, and then decreased further after L-MCAO. The moving average scheme in the DSCA measurements, and the difference in sampling rate between LDF (~10 Hz) and DSCA (~3 Hz), led to a small time lag in the BFI measurements of DSCA. Although the DSCA sampling rate can be adjusted to 10 Hz using six frames, the spatial resolution decreased at this rate.

The LDF measurements were highly sensitive to motion artifacts, causing significant fluctuation in the BFI, particularly before L-CCA clipping in Fig. 4(a). LDF was susceptible to motion of the probe and the rat's head during the surgery. Thus, LDF required proper separation between the optical fiber tip and the tissue surface to increase detection sensitivity. Complicated procedures were required to implement LDF with the proper separation. This issue has been reported previously [28].

In Fig. 4(b), the DSCA measurement was less susceptible than the LDF measurement to motion artifacts because of its larger sensitive detection volume; the microvasculature flow was measured [39] simply by touching the measurement probe to the target surface. Compared with the fixed one-channel detection of LDF, the DSCA system accomplished multi-channel detection with relatively stable measurement.

In this experiment, LDF and DSCA shared the same light source; however, the systems had different measurement locations. LDF had a measuring depth of about 1 mm with respect to single scattering, and DSCA had a measuring depth of about 2.5 mm, considering multiple scattering; thus, the measurement depths were not the same [40]. We focused on observing blood flow in the superficial cortex

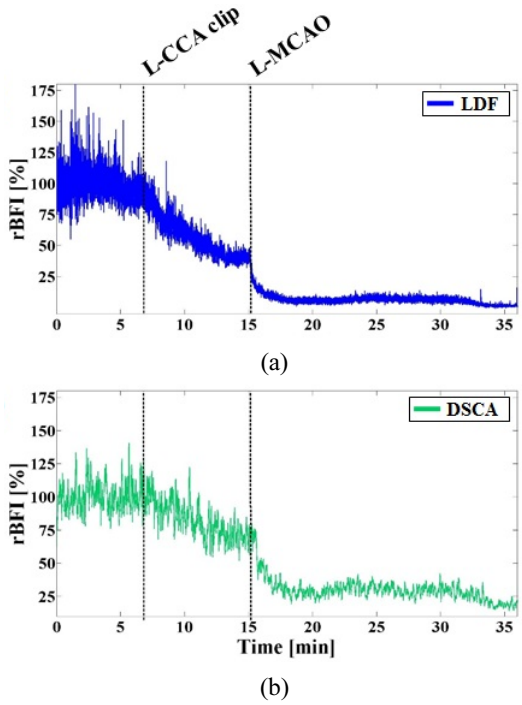

FIG. 4. Representative data of relative blood flow index (rBFI) changes during middle cerebral artery occlusion (MCAO) surgery. (a) Laser Doppler flowmetry (LDF) measurement. (b) Diffuse speckle contrast analysis (DSCA) measurement.

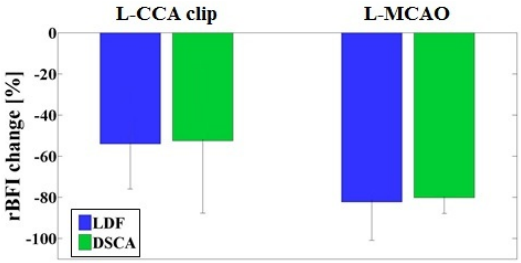

FIG. 5. The average of the relative blood flow index (rBFI) changes measured by laser Doppler flowmetry (LDF) and diffuse speckle contrast analysis (DSCA) in the left hemispheres of three rats during left common carotid artery (L-CCA) clipping and left middle cerebral artery occlusion (L-MCAO).

around the MCA, rather than measuring blood flow in the specific vessel. Interestingly, the measurement results showed fairly strong correlation, perhaps because the infarction of the blood vessels extended to the MCA, despite the slight difference in measurement position.

Figure 5 shows the mean decrease in CBF after CCA clipping and MCAO in three rats. The BFI reductions after L-CCA clipping and MCAO differed significantly ($p < 0.05$), indicating that MCAO produced much stronger occlusion than did L-CCA clipping. Both systems showed very similar mean BFI changes during the MCAO surgery, which reveals

the potential of the DSCA system to monitor blood flow, compared with a commercial LDF system.

3.2. Regional and Global-CBF Monitoring

Figure 6 shows the regional BFI changes in the left and right hemispheres and the global-BFI changes across hemispheres during transient MCAO surgery in the rat. When the L-CCA was clipped, the left BFI and global-BFI decreased gradually, in contrast to the constant regional BFI in the right hemisphere. When the left MCA was occluded, the left regional BFI decreased remarkably, whereas the global-BFI fluctuated more than with L-CCA clipping. The right regional BFI remained almost constant.

Figure 7 shows the mean reduction in BFI during L-CCA clipping and L-MCAO surgery in all rats ($n = 6$). The reductions in BFI in the left (-69.17% ± 9.92%), global (-45.83% ± 14.92%), and right (0.05% ± 5.84%) hemispheres after L-MCAO differed significantly ($p < 0.05$). As the similar DSCA result in Fig. 4, when L-MCAO was performed, mean BFI in left hemisphere decreased more than 50 % to baseline period. Simultaneous CBF measurements in left and right hemispheres during L-MCAO were able to increase the reliability of surgical success. In addition, we expect to study blood flow networks between the left and right brain, by accomplishing global-BFI measurement.

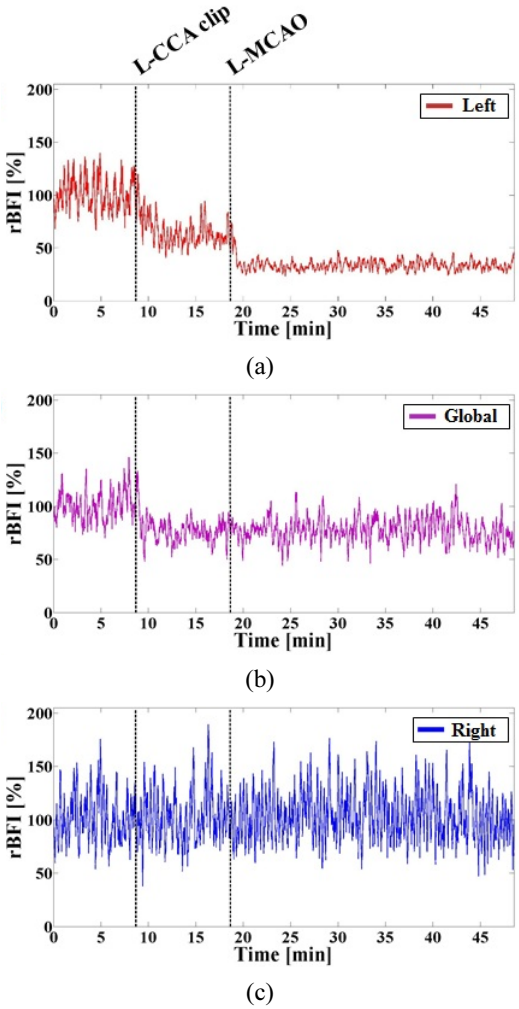

FIG. 6. Representative diffuse speckle contrast analysis (DSCA) data of the regional and global- blood flow index (BFI) changes during transient left middle cerebral artery occlusion (MCAO). (a) The relative blood flow index (rBFI) in the left hemisphere. (b) The rBFI across hemispheres. (c) The rBFI in the right hemisphere.

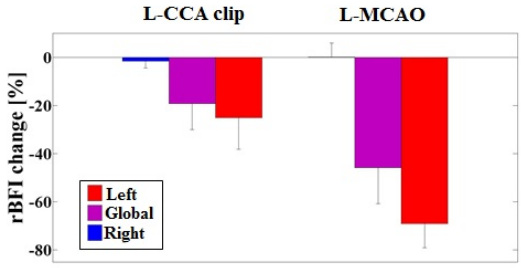

FIG. 7. The average of the relative blood flow index (rBFI) changes measured by laser Doppler flowmetry (LDF) and diffuse speckle contrast analysis (DSCA) in the left hemispheres of three rats during left common carotid artery (L-CCA) clipping and left middle cerebral artery occlusion (L-MCAO).

IV. CONCLUSION

In conclusion, we have demonstrated the potential of a DSCA system using a 1 × 2 optical switch to monitor CBF in the rat brain during transient MCAO surgery. The DSCA results for rat CBF measurements were correlated strongly with the LDF measurements. DSCA was less sensitive to motion artifacts and enabled flow measurement in deeper tissue without the need for a unique apparatus or complicated measurement procedures. The simultaneous monitoring of regional CBF and global-CBF realized real-time visualization of cerebral hemodynamics following transient ischemic stroke. We hope that DSCA can be used to assess proper arterial occlusion and estimate neurological tissue damage. The BFI reduction of >50% may be taken as a threshold to assess the success of MCAO. In a future study, we will consider longitudinal changes in CBF after MCAO surgery to provide information relevant to therapeutic intervention.

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