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Prompt, Objective, and Accurate Measurement of Rat Abdominal Flap Blood Flow Using Laser Speckle Flowgraphy

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Background: The indications for free flap procedures have expanded, with recent technical advances enhancing procedural safety. However, few objective indicators exist to monitor flap status during and after the operation. This experimental study assessed laser speckle flowgraphy (LSFG) as a prompt and accurate indicator of free flap blood flow.

Methods: After elevating bilateral lower abdomen flaps with superficial inferior epigastric artery (SIEA) and superficial inferior epigastric vein vasculature in Wistar rats, the right flap with the SIEA was cut (ischemic group) or the superficial inferior epigastric vein was cut (congestive group), and the unaltered left flaps were monitored using LSFG every 5 minutes for a 30-minute period. Flap survival or necrosis was assessed after 7 days.

Results: In the ischemic group, LSFG measurements were significantly lower after cutting the SIEA than beforehand (74% at 5 minutes and 72% at 30 minutes). Similar findings were seen in the congestive group (63% at 5 minutes and 55% at 30 minutes). LSFG measurements were significantly lower in the congestive group than in the ischemic group. Seven days afterward, whereas all right-side flaps with cut vessels were necrotic, all unaltered left-side flaps had survived.

Conclusions: Our preliminary results demonstrated that LSFG could objectively identify abnormal blood flow in skin flaps as early as 5 minutes into surgery and predict graft survival. LSFG may potentially enable quick and objective assessment of flap blood flow and reduce the risk of complications and flap loss. *(Plast Reconstr Surg Glob Open 2024; 12:e6062; doi: 10.1097/GOX.0000000000006062; Published online 9 August 2024.)*

INTRODUCTION

The free flap technique has been established as a safe and effective surgical procedure.[1,2] Because flap loss may occur as a result of poor blood flow,[3] the accurate evaluation of flap blood flow is essential for favorable surgical results. Despite advances in surgical methods, however, the prediction of successful surgery depends largely on the surgeon's experience, the subjective appearance of the skin, and the bleeding pattern of the flap during the operation.[4] An objective, two-dimensional, noninvasive blood flow evaluation technique for free flaps has not yet been established.

Laser speckle flowgraphy (LSFG) is a recent device that objectively evaluates skin blood flow. We earlier reported significantly lower LSFG values in patients with peripheral arterial disease than in patients with nonperipheral arterial disease.[5,6] Other experiments with rats have suggested that LSFG can detect reduced blood flow in compressed skin.[7] However, it remains unknown how LSFG perceives changes in flap skin blood flow under congestion or ischemia. The present experimental study aimed to evaluate the LSFG measurement changes in ischemic and congested flaps in the free flap technique.

MATERIALS AND METHODS

Ethics

All animals were housed under standard conditions. The Shinshu University School of Center for Animal Research approved all experimental protocols (No. 020036).

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Animal Model

Male Wistar rats 10.6 ± 0.7 weeks of age and weighting 340–525 g (mean: 413 g) were divided into the ischemia group (N = 6) and the congestion group (N = 6). All rats were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan).

Each rat was anesthetized by hypodermis injection into the back. Medetomidine (0.6 mg/0.6 mL), midazolam (8 mg/1.6 mL), and butorphanol (10 mg/2 mL) were combined in a physiological salt solution (5.8 mL), and 0.25 mL the agent was used per 100 g of rat.

Flaps of approximately 5 × 3 cm in size on both sides of the lower abdomen, which were vascularized by the superior inferior epigastric artery (SIEA) and the superior inferior epigastric vein (SIEV), were raised after abdominal hair removal (Fig. 1A).[8] The lower border was at the pubis and the inguinal fold. The medial border was 5 mm lateral to the abdominal midline. The SIEA and SIEV were separated on both sides (Fig. 1B), and the flaps were left to rest for 5 minutes. Both side flaps were then sutured to the surrounding skin. In the ischemic group, the SIEA was cut in the right-side flap only. This was similarly done to the SIEV in the congestion group. Operations were performed on an operating table with a warming device for small animals. After LSFG measurements of the abdominal flaps, the rats were returned to their cage after the administration of atipamezole (0.3 mg/kg).

Measurements

Skin blood flow in the flaps was assessed using LSFG (LSFG-PFI; SoftCare Co., Ltd., Japan) before cutting the vessel and at 5–30 minutes afterward at 5-minute intervals (Fig. 2A). Surface temperature was determined by an infrared thermometer (Bing Zun, China).

LSFG Device

The LSFG device is approximately 11 cm in width × 22 cm in depth × 9 cm in height. The camera unit has a weight of 1.5 kg and is mounted on a wagon by an arm for stability to prevent excess motion from interfering with measurement values.

LSFG irradiates the skin with a near-infrared laser (wavelength: 830 nm) from a distance of 24 cm, and a charge-coupled device camera captures the reflected light.[9] Operating the LSFG instrument is simple via applications installed on a laptop computer and by adjusting the distance between the camera and the object by matching two laser indicators together. Regarding safety, LSFG has been approved as a class 3R laser by the International Electrotechnical Commission-1. During the LSFG measurements, the examiner and subject must be careful not to look at the laser directly, although special goggles for eye protection are not needed. Measurement time is 4–6 seconds, and maximum measurement range is 20 × 15 cm. The results are displayed on a monitor in pseudocolor, with warm colors for high skin blood flow areas and cool colors for low skin blood flow areas, expressed as a two-dimensional map.

The principle of LSFG is that laser irradiation of the skin forms a random speckled pattern as reflected light is

Takeaways

Question: How does skin blood flow in abdominal rat skin flaps objectively change on laser speckle flowgraphy when the feeding or drainage vessel is cut?

Findings: This study used right- and left-side abdominal flaps with connecting vessels in Wistar rats. After cutting the artery or vein on the right side only, relative skin flap blood flow was measured using laser speckle flowgraphy. All areas in the surgically treated flaps decreased to 80% or less at 5 minutes after vessel cutting and were necrotic 7 days later.

Meaning: Flaps with blood flow less than 80% on laser speckle flowgraphy are at risk of necrosis.

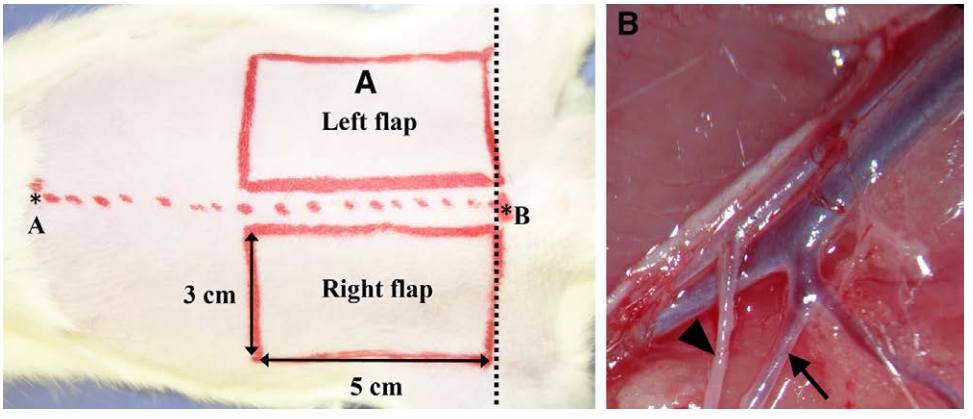

Fig. 1. The flap design and the nourish vessels. A, Right and left flaps (5 × 3 cm) were traced on each side of the lower abdomen. The caudal line of the flap was set on a perpendicular line to the midline, which passed the pubic symphysis. The inner line of the flap was set 5 mm away from the midline. The midline was set as a line passing through the xiphoid process (A) and the pubic symphysis (B). B, Connecting vessels of the skin flap in this study. The arrowhead indicates the SIEA diverted from the femoral artery. The arrow indicates the SIEV diverted from the femoral vein.

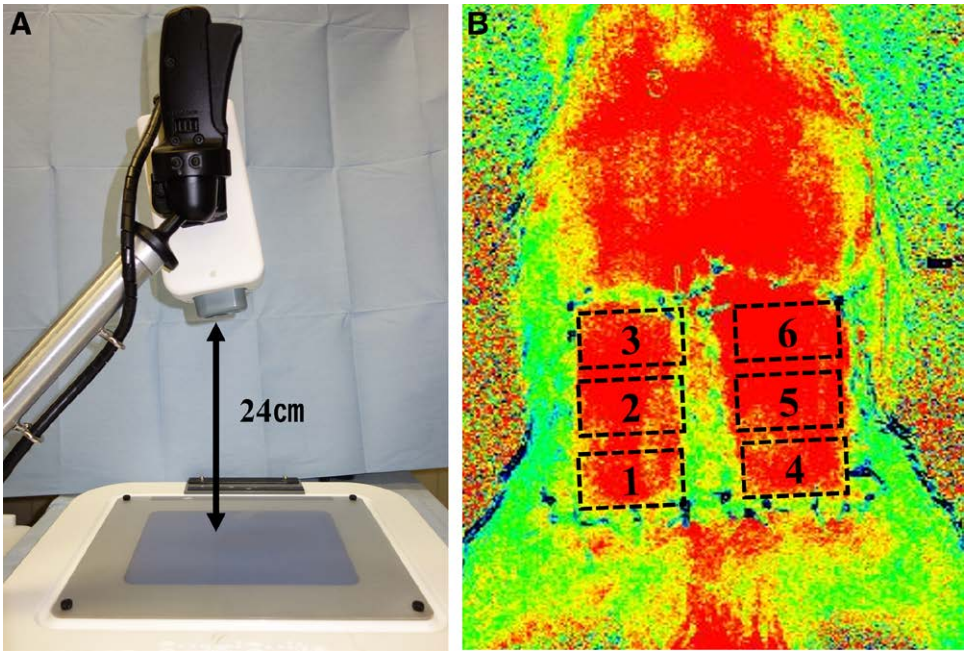

Fig. 2. The LSFG system and 2 dimensional mapping. A, The laser speckle flowgraphy system irradiates near-infrared light 24 cm away from the object and captures the reflected and scattered light with a charge-coupled device camera. Measurement time is 4–6 seconds. B, Measurement results are displayed on a monitor as a two-dimensional map using pseudocolor. The average value within the designated ROI is displayed as the measured value. In this study, ROIs 1–6 were set on the skin flaps by dividing them into 3 equal parts craniocaudally.

scattered by erythrocytes. This speckled pattern changes with the movement and velocity of erythrocytes located 1–2 mm under the skin. The input and output points are at different points in LSFG measurement. The path length corresponds to the distance that passes through the skin beneath from the input to the output, and is reportedly 5 mm under 830 nm near-infra-red irradiation to the skin.[10] Therefore, the depth of LSFG is estimated to be 1–2 mm.

In terms of limitations, there is a possibility that LSFG cannot accurately measure moving targets, such as from respiratory fluctuation or tremor, areas of dark pigmentation, and uneven surfaces. When the distance between the camera unit and the object is not constant, the reflection time from the object changes, which may result in inconsistent measurements. Also, because LSFG is a device that measures flat areas, it is unable to evaluate intraoral or buried flaps.

Evaluation

First, the preoperative skin blood flow in both flaps was measured by LSFG before vessel cutting. After cutting the SIEA or SIEV in the right flap, skin blood flow in both flaps was again evaluated by LSFG every 5 minutes for 30 minutes. The region of interest (ROI) was set as the proximal part, middle part, and distal part of each flap (right flap: ROI 1, 2, and 3, and left flap: ROI 4, 5, and 6, respectively) (Fig. 2B). Based on LSFG measurements, the rates of change in ROIs 1–6 were plotted for each time point and compared between the ischemic and congestive groups. The temperature of the skin flap surface was measured similarly during the testing period for comparisons of the degree of change. Next, the flaps were judged as either achieving engraftment or necrosis at 7 days after the flap operation.

In addition to the LSFG measurements, the flaps were examined histologically to explore the mechanistic reasons for the LSFG changes. Three flaps for each group were harvested at 5 minutes after vessel cutting. These specimens were fixed and stained with hematoxylin and eosin, and then divided into the proximal, middle, and distal parts. The ratio of blood vessel cross-sectional area in one field of view (100× magnification) was measured using image analysis software (Image J version 1.54; National Institutes Health, Bethesda, MD). The five sections in which the calculated cross-sectional area was highest were selected for statistical analysis.

Statistical Analysis

Data are presented as the mean (interquartile range) and compared using paired and nonpaired *t* tests along with the Games–Howell test. Statistical significance was set at *P* value of less than 0.05. All statistical analyses were performed using IBM SPSS version 23.0 software (IBM Corp., N.Y.).

RESULTS

The LSFG measurements for ROIs 1–3 had dropped significantly to 74%, 80%, and 83%, respectively, at 5 minutes after cutting the SIEA (all $P < 0.01$, paired *t* test) (Fig. 3). These values then plateaued and were 72%,

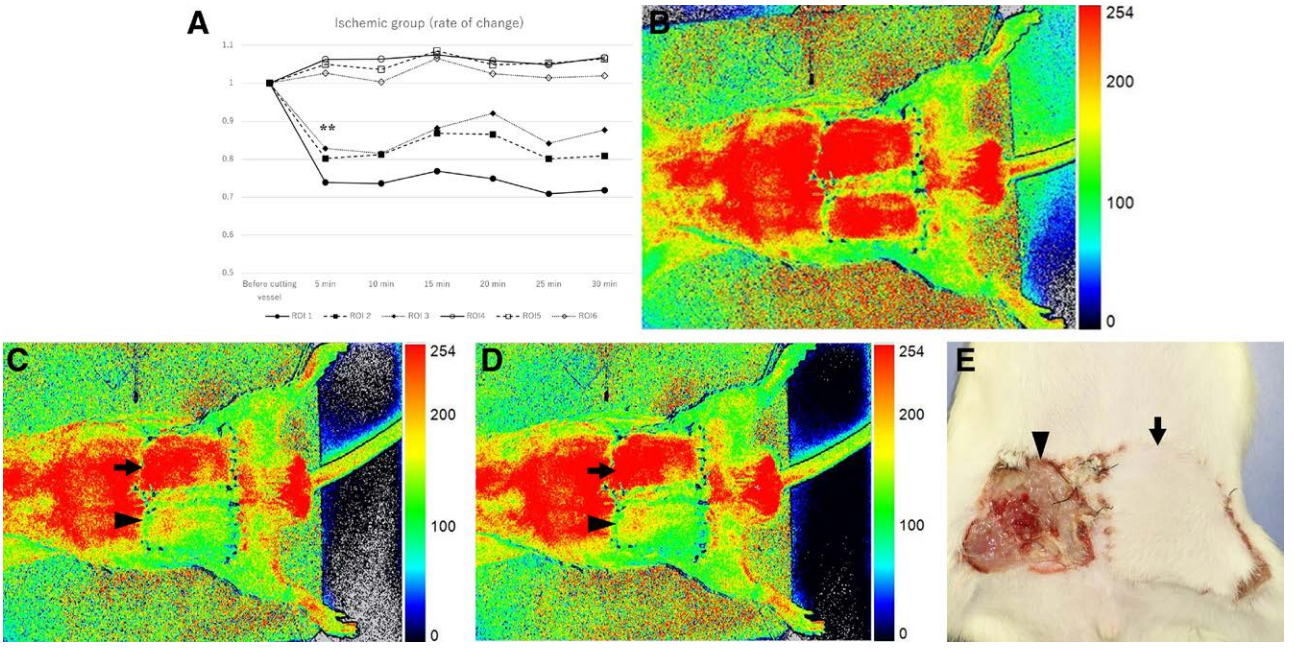

Fig. 3. The results of LSFG and flap in the ischemic group. A, Ratio of laser speckle flowgraphy value divided by the value before cutting the superficial epigastric artery at each time point. The ratios at ROIs 1–3 had become significantly lower at 5 minutes after cutting vs beforehand (proximal ROI: 74%, middle ROI: 80%, and distal ROI: 83%). The ratios then plateaued until the study end point. The ratios at ROIs 4–6 were comparable from before cutting the vessel to 30 minutes afterwards. $^{**}P < 0.01$. SD range for ROIs 1–3 was 3.9–17 and for ROIs 4–6 was 5.7–10. B, Two-dimensional laser speckle flowgraphy-generated map before cutting the right SIEA shows a similar pseudo-color pattern in both flaps. C, Two-dimensional laser speckle flowgraphy-generated map at 5 minutes after cutting the right SIEA shows the right skin flap as a cold color and the left skin flap as a warm color. D, Two-dimensional laser speckle flowgraphy-generated map at 30 minutes after cutting the right SIEA shows the right skin flap as a cold color and the left skin flap as a warm color. E, The entire right flap became necrotic and the left flap had survived at 7 days after the right SIEA was cut. *Arrowheads indicate the SIEA cut side, and arrows indicate the normal side.

81%, and 88%, respectively, at 30 minutes (Fig. 3). The LSFG measurements for ROIs 4–6 showed no remarkable changes from precutting to 30 minutes after cutting the SIEA (Fig. 3A–D). The entire right flap in the ischemic group was necrotic at 7 days after the operation in all rats, with engraftment in the entire left flap in all rats (Fig. 3).

Similarly, the LSFG readings for ROIs 1–3 had decreased significantly to 63%, 72%, and 79%, respectively, at 5 minutes after cutting the SIEV ($P < 0.01$, $P < 0.01$, and $P = 0.04$, respectively, paired t test) (Fig. 4). However, these values had gradually fallen to 55%, 63%, and 71%, respectively, at 30 minutes (Fig. 4). The LSFG readings for ROIS 4–6 showed no remarkable changes from precutting to 30 minutes after cutting the SIEV (Fig. 4). Necrosis and engraftment were observed in the entire right flap and left flap, respectively, of all animals at 7 days postoperatively (Fig. 4).

In the proximal ROI 1, the congestive group showed a significantly higher decrease rate at 5 minutes after cutting the vessel than did the ischemic group ($P = 0.02$, nonpaired t test). The same observation was made in the middle the ROI 2 at 15 minutes after vessel cutting ($P = 0.02$, nonpaired t test) and in the distal ROI 3 at 20 minutes after vessel cutting ($P = 0.01$, nonpaired t test).

In the ischemic group, the respective temperatures of the right and left flaps were 36.1°C and 36.1°C before cutting the SIEV and 36.5°C and 36.6°C 30 minutes later, respectively. In the congestive group, these values were 36.1°C and 36.0°C in addition to 36.4°C and 36.4°C. No significant differences were seen between the right and left flaps in either group at 30 minutes after cutting the vessel (ischemic group: $P = 0.4$, congestive group: $P = 0.4$).

Figure 5A shows the ratio of blood vessel cross-sectional area in one field of view. The cross-sectional area in each section was significantly larger in the congestive group than in the other two groups (proximal part: $P < 0.01$, middle part: $P < 0.01$, and distal part: $P < 0.01$, respectively). No significant differences were observed between the ischemic group and control group (proximal part: $P = 0.798$, middle part: $P = 0.991$, and distal part: $P = 0.562$). Histologically, erythrocytes were relatively sparse within the SIEA in the ischemic group, with the flat-shaped SIEV filled with erythrocytes (Fig. 5B). In the congestive group, both the SIEA and SIEV were dilated and filled with erythrocytes (Fig. 5C). In the control group, the oval-shaped blood SIEA and SIEV were also filled with erythrocytes (Fig. 5D).

DISCUSSION

The present study identified the potential of LSFG to objectively evaluate changes in blood flow toward predicting flap survival. Five minutes after cutting the selected vessel in the right flap, LSFG values were significantly decreased in both test groups at all ROIs on the flap. We observed two clues to potentially help distinguish between

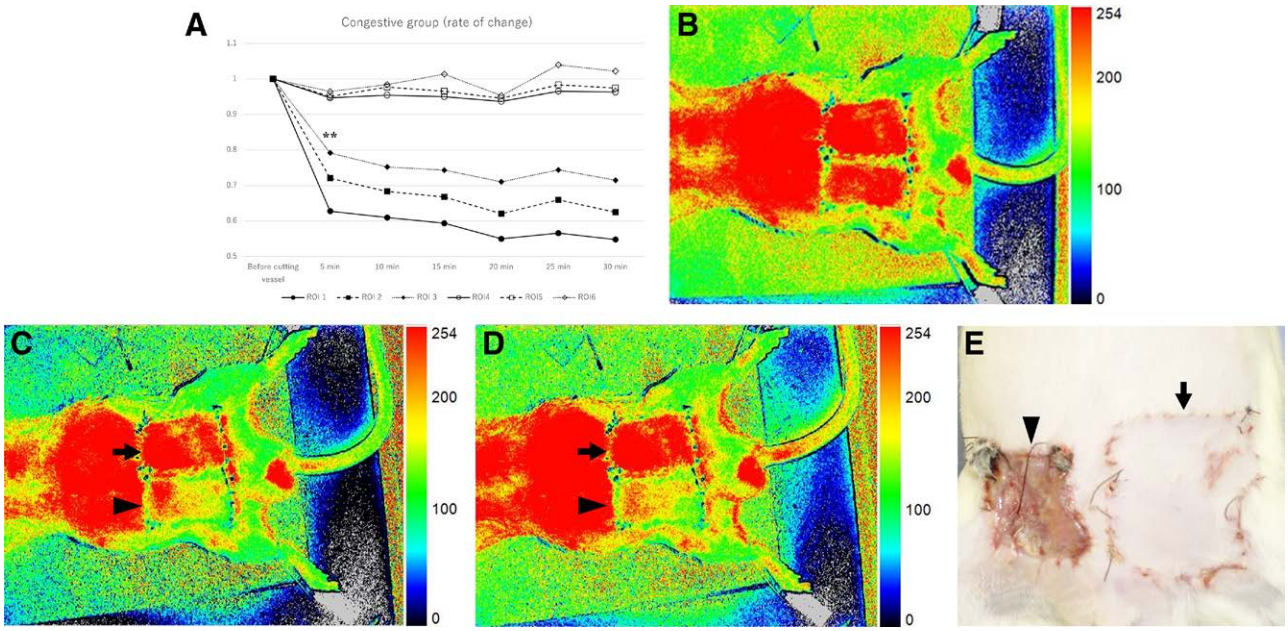

Fig. 4. The results of LSFG and flap in the congestive group. A, Ratio of laser speckle flowgraphy value divided by the value before cutting the superficial epigastric vein at each time point. The ratios at ROIs 1–3 had become significantly lower at 5 minutes after cutting vs beforehand (proximal ROI: 63%, middle ROI: 72%, and distal ROI: 79%). The ratios then gradually decreased until the study end point. The ratios at ROIs 4–6 were comparable from before cutting the vessel to 30 minutes afterward. $^{**}P < 0.01$. SD range for ROIs 1–3 was 5.7–21 and for ROIs 4–6 was 5.0–12.8. B, Two-dimensional laser speckle flowgraphy-generated map before cutting the right SIEV shows a similar pseudocolor pattern in both flaps. C, Two-dimensional laser speckle flowgraphy-generated map at 5 minutes after cutting the right SIEV shows the right skin flap as a cold color and the left skin flap as a warm color. D, Two-dimensional laser speckle flowgraphy-generated map at 30 minutes after cutting the right SIEV shows the right skin flap as a cold color and the left skin flap as a warm color. E, The entire right flap became necrotic and the left flap had survived at 7 days after the right SIEV was cut. *Arrowheads indicate the SIEV cut side, and arrows indicate the normal side.

ischemia and congestion. First, the change rate in the congestive group was significantly lower than in the ischemic group. Especially in the proximal ROI 1, LSFG readings were significantly lower in the congestive group at 5 minutes after cutting the vessel. The second clue was the change rate trend following 5 minutes; although in the ischemia group it plateaued, in the congestion group it fell gradually. No significant differences in surface temperature were seen between the right and left flaps in either group during the 30 minutes after cutting the vessel. All skin flaps that had decreased to 83% or less at 5 minutes after cutting the vessel became necrotic at 7 days after the operation. These findings suggested that LSFG could accurately monitor skin flap blood flow and prognosticate necrosis.

In the histological examination, the blood vessel cross-sectional area in the congestion group was significantly larger than in the other two groups. These blood vessels were filled with red blood cells because erythrocyte movement was presumably suppressed. On the other hand, we witnessed no significant difference in blood vessel cross-sectional area between the ischemic and control groups, although measurements tended to be slightly smaller in the ischemic group. Some erythrocytes were scattered in the SIEA, but many were observed within flat-shaped SIEV.

In an earlier study, the measurement of skin flap blood flow with laser speckle contrast imaging, which is similar to LSFG, revealed that decreased values resulted in necrosis in a rat abdominal flap model.[11] The measurements showed little change at 6 hours after artery ligation compared with preligation, and then decreased until 24 hours after ligation. In a much shorter time span, LSFG values were significantly decreased at 5 minutes after the respective vessels were cut in this experimental study. We considered LSFG to be a useful measuring instrument, as it could evaluate the sluggish movement of erythrocytes in skin flaps with aberrant blood flow in both congested and ischemic conditions after only 5 minutes at any ROI in the flap. Histological examination also showed discernable changes at 5 minutes after blood vessel cutting.

Gazyakan et al[12] described that laser Doppler examination showed a 67.7% reduction in flap blood flow at the time of artery clamping and a 26.6% reduction at the time of vein clamping, which indicated a much lower flow rate for artery clamping than for vein clamping. The merits of laser Doppler flowmetry are convenience, lower costs, and a high true positive rate for detecting flap compromise.[13] Implantable Doppler is especially useful for monitoring buried flaps.[14] The drawbacks of this technique include patient discomfort in the form of sound and physical contact, the influence of physiological changes unrelated to the flap, delayed detection of decreasing blood flow, and measurement at only one point.[13] Moreover, external Doppler is not specific for vessels of interest.

In the current study, LSFG-determined flap blood flow decreased to 74% and 63% after artery and vein cutting,

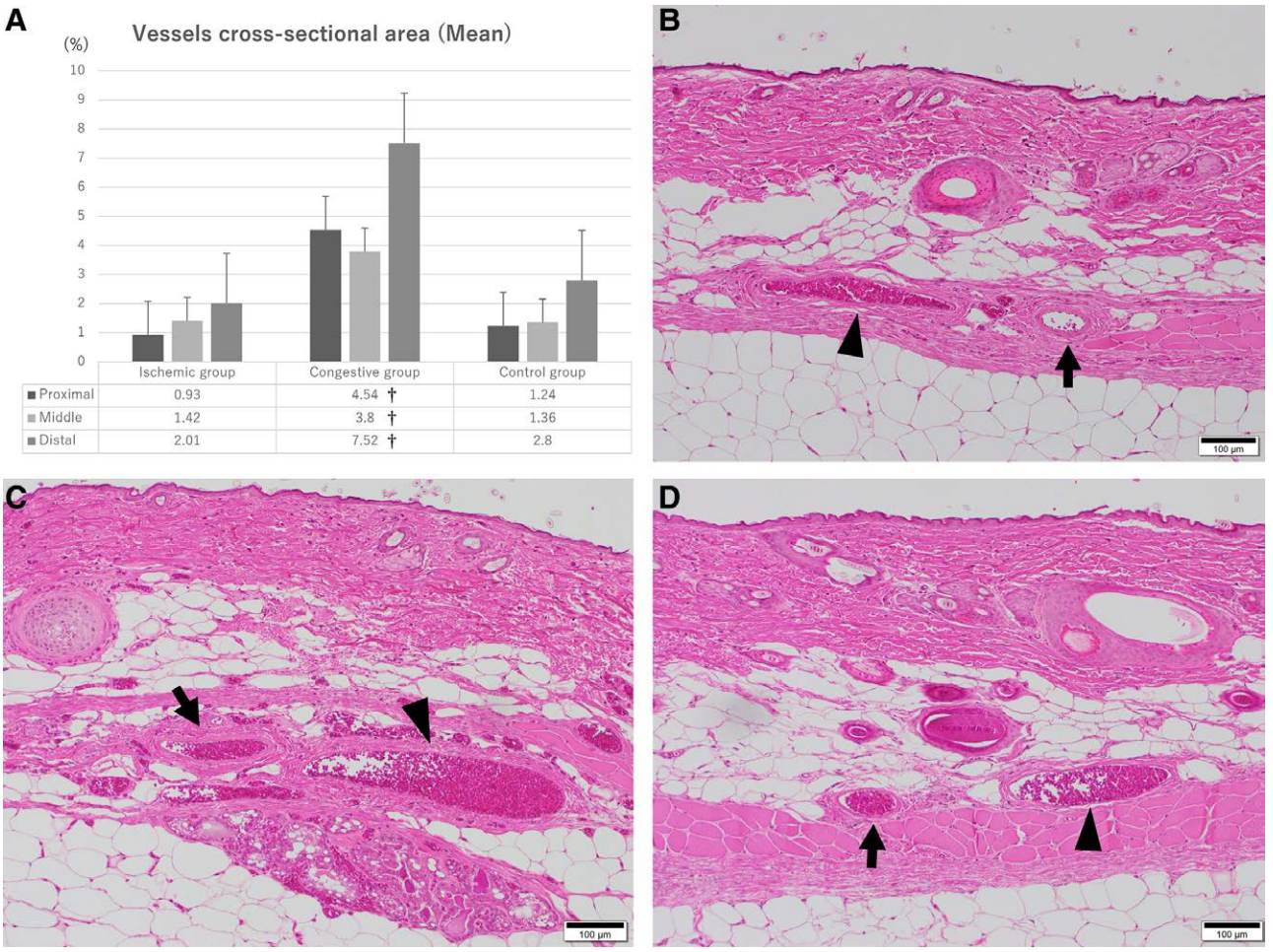

Fig. 5. The histological results. A, Ratio of the cross-sectional area of blood vessels in one field of view. In the congestive group, the ratio of the cross-sectional area of blood vessels was significantly larger (†) than in both the ischemic and control groups. There was no significant difference in cross-sectional area between the ischemic and control groups. Bars indicate the standard error of the mean. B, In the ischemic group, there were fewer erythrocytes in the SIEA and abundant erythrocytes in the SIEV. C, In the congestive group, abundant erythrocytes were visible in the SIEA and SIEV. Both the SIEA and SIEV were dilated. D, In the control group, abundant erythrocytes were detectable in both the SIEA and SIEV. No excessive dilation of either vessel was observed. *Arrows indicate the SIEA, and arrowheads indicate the SIEV.

respectively, in the proximal ROI 1. Histologically in the congestion group, the vessel cross-sectional area was significantly larger than in the ischemic group. Many erythrocytes were observed in the SIEA and SIEV, whereas in the ischemic group, fewer erythrocytes were noted, especially in the SIEA. There were several possibilities as to why the LSFG measurements were lower in the congestive and ischemic groups. In the former, erythrocytes were increased in blood vessels due to the outlet being blocked, but their movement as detected by LSFG was restricted. In the ischemic group, erythrocytes were decreased in the SIEA due to the supply being stopped, and so the blood was stagnant. Variation in the degree of erythrocyte movement suppression might have influenced the differences in LSFG measurement results. The potential ability of LSFG to differentiate between ischemia and congestion merits further investigation in larger cohorts.

In line with these findings, necrosis was observed in all test animals whose LSFG was lower than 83% after vessel cutting. Therefore, in the case of free skin flaps, LSFG measurements of less than 83% after vascular anastomosis compared with immediately postflap elevation could represent an important indication for prompt reanastomosis. Particularly in the proximal area, when the rate of change is approximately 70% after 5 minutes, there is a possibility of arterial trouble, whereas a change rate of roughly 60% indicates a problem with veins. However, the precise cutoff of flap survival for LSFG between 83% and 100% could not be demonstrated in this study.

Smit et al[15] determined that fluorescence imaging (FI) and laser Doppler flowmetry were objective and reliable methods of flap perfusion. No comparisons were made with FI or laser Doppler imaging in our study, and the precise correlations need further investigation. The advantages of FI include fewer reoperations, lower costs, and better identification of problematic flaps.[16] However, the disadvantages of FI are the injection of a contrast agent and a low true positive rate for detecting flap compromise.[13] FI

is also difficult in patients with allergies, liver disease, and renal disease[17] because the contrast agent is injected into blood vessels. The amount of contrast medium that can be administered daily is limited as well.[18] This contrast agent spreads over time, which makes it challenging to determine the location of safe areas.

The mechanisms of LSFG reflecting blood flow in skin flaps remain in the exploratory stages. However, significant correlations have been reported between LSFG and laser Doppler measurement values for microcirculation in the optic nerve head.[19] In the skin region, LSFG readings were found to increase after distal bypass surgery, similarly to an increase in skin perfusion pressure.[20] These reports corroborate the notion that LSFG measurement values are related to blood flow. The potential clinical advantages of LSFG are the identification of problematic flaps, no discomfort to the patient, and early detection of changing flap blood flow. LSFG may also be a useful tool to determine the operative success or failure of vascular anastomosis promptly and objectively. In addition, no burns or skin damage, including inflammatory pigmentation, were observed in this study or other prior reports.[5,6] However, attention is needed to biological noise, such as respiratory and blood pressure fluctuations.[21] Another strong advantage of LSFG is that it can be measured any number of times because it is noncontact and noninvasive. As this was a pilot study on the potential to obtain numerical values for skin blood flow with LSFG for skin flap monitoring and the estimation of flap engraftment, our findings need further investigation.

Limitations

This study could not pinpoint the LSFG cutoff value for skin flap engraftment. Moreover, it reflected a condition in which the blood vessel was completely cut, which might differ from those of partial occlusion, kinking, or spasms. The authors have been measuring skin blood flow in Asian patients and in Wistar rats with LSFG, and so the results for other skin tones are unknown. Because this study targeted rats, additional research is required to determine whether similar measurement results can be obtained in actual clinical settings. Comparative definitive confirmation by other methods, such as laser Doppler and FI, are needed to validate our results.

CONCLUSIONS

LSFG could detect abnormal blood flow in skin flaps from as early as 5 minutes. The device could also potentially distinguish between ischemia and congestion during abnormal blood flow at 5 minutes in the proximal area. Skin flap necrosis may occur when LSFG values drop to below 83%. Prompt and objective skin blood flow assessment with LSFG may be of potential clinical use to monitor intraoperative results and reduce the risk of flap loss.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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