

Correlation between blood flow, tissue volume and microvessel density in the flap

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ABSTRACT

The purpose of this study was to assess the correlation between tissue volume and blood flow of the flap in an animal model. Using animal model, tissue volume can be attenuated, and precise change of blood flow could be evaluated. We further investigate the relationship between blood flow and vascular density in the tissue. In this study, we assessed flap conductance (ml/min/mm Hg) as to evaluate the conductivity of blood flow into the flap. Japanese white rabbit was used (n=7) for this study. The amount of blood flow of jejunal and latissimus dorsi muscle (LD) flaps was measured while removing the distal portion of the flap sequentially. Conductance at each time was calculated from blood pressure and blood flow volume. The tissue volume at each time was also measured. The correlation between conductance and volume was analyzed using a linear mixed model. Immunohistochemical evaluation of microvessel densities (MVD) in these tissues was also performed for CD31/PECAM1 positive area. Conductance and tissue volume were significantly correlated in both jejunal and LD flaps. As the volume increases by 1 cm³, the conductance increased significantly by 0.012 ml/min/mm Hg in jejunum, and by 0.0047 ml/min/mm Hg in LD. Mean MVD was 1.15±0.52% in the jejunum and 0.37±0.29% in the LD muscle. In this study, we revealed that flap conductance is proportional to volume and proportional constant is different between the type of tissue. It suggests that the difference of MVD creates the unique conductance of each tissue.

Keywords: conductance, free flap transplantation, vessel resistance, blood flow, microvessel density

Abbreviations:

MVD: microvessel densities

LD: latissimus dorsi muscle

SBP: systolic blood pressure

DBP: diastolic blood pressure

Ht: hematocrit

T: body temperature

P: change in blood pressure

ABP: arterial blood pressure

VBP: venous blood pressure

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Res: vascular resistance
 Q: amount of blood flow
 C: conductance

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INTRODUCTION

It is beneficial for surgeons to understand the blood flow of the flap to be transferred.¹ In clinical practice, we often experience that the blood flow of flaps varies between cases. A flap with high blood flow is advantageous for a site lacking blood flow or one in which wound healing is inefficient. Flaps with high blood flow are known to control infectious wounds² and improve the circulation of ischemic limbs³ or irradiated ulcers.⁴ Those with inadequate blood flow are not resistant to infection.⁵ Flaps with excessive blood flow, in contrast, need drainage. If the flap is subjected to blood flow surpassing the drainable amount, congestion follows. In clinical cases, venous congestion often occurs when a flap is anastomosed to a vein with insufficient capacity but is improved by changing to a vein with sufficient capacity. In that situation, appropriate recipient vein selection is important.

In previous clinical research, our group demonstrated that the blood flow of the flap varied between tissue types and that it increased in respect to the tissue volume.^{6,7} This is consistent with another report describing that vascular resistance is related to the tissue volume in muscle flaps.⁸ However, the validity of these clinical studies was limited by variations in patient backgrounds and flap characteristics (e.g. a consistent tissue type and volume could not be standardized) that cannot be disregarded. Factors that determine blood flow within the tissue are yet to be fully elucidated. Vascular density is a leading possibility, but it remains difficult to prove clinically.

The purpose of this study was to test the claim that tissue volume and flap conductance are correlated in an animal model where tissue volume can be controlled, and to investigate further the relationship between blood flow and vascular density in the tissue. Hemodynamics consists of three factors: blood flow, vessel resistance and conductance. Because blood flow into the flap (ml/min) is greatly affected by central blood pressure, it is inappropriate to evaluate only the

Conductance

$$C_{\text{Total}} = C_1 + C_2 \dots + C_{X-1} + C_X$$

Resistance

$$\frac{1}{R_{\text{total}}} = \frac{1}{R_1} + \frac{1}{R_2} \dots + \frac{1}{R_{X-1}} + \frac{1}{R_X}$$

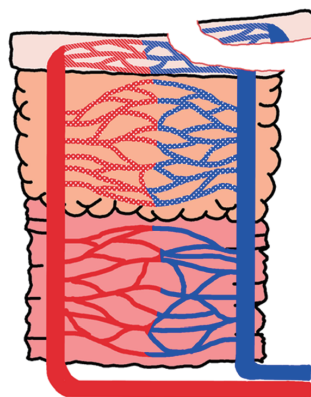


Fig. 1 The calculation formula and schema of the remaining flap when a part of the flap is resected. The total conductance of the flap is determined by the sum of each component. The total resistance of the flap is determined by the sum of the reciprocal of each component. When a part of the flap is excised, the conductance of the remaining flap can be calculated simply by subtracting the conductance of the resected part.

individual flap blood flow. Previous reports used vascular resistance as the evaluation criterion of blood flow to the flap. In this study, we utilized conductance (ml/min/mm Hg), obtained by standardizing the blood pressure of the feeding vessel, for its ease in calculation and comprehension (Fig. 1). It represents the conductivity of blood flow in an individual flap.

MATERIALS AND METHODS

Experimental Animals and General Settings

Experiments were conducted in compliance with regulations on animal experiments upon approval from the Nagoya University Animal Experiment Committee. Seven adult (16 weeks or older) male Japanese white rabbits with body weights ranging from 3.1 to 3.4 kg were used. The gross health condition of the animal was examined by blood test. General anesthesia was induced with 0.5 mL/kg of ketamine HCl and isoflurane (5%) and maintained with isoflurane (1.5–2.5%). The rabbits breathed unaided throughout the experiment while their percutaneous oxygen saturation and femoral artery blood pressures were monitored.

Blood Flow Measurement

Under general anesthesia, jejunal flaps (n=4) and latissimus dorsi (LD) muscle flaps (n=3) were elevated. The vascular pedicle was exposed, a hook-shaped probe was applied to the artery, and the blood flow (ml/min) was measured using a transit-time ultrasound flowmeter (HT323 Surgical Flowmeter, Transonic Systems, Ithaca, USA) by calculating the difference between the upstream and downstream integrated transit times of the blood.⁶ To preclude errors of measurement from vasospasm, lidocaine (4%) was applied to the pedicle at least 10 minutes prior to

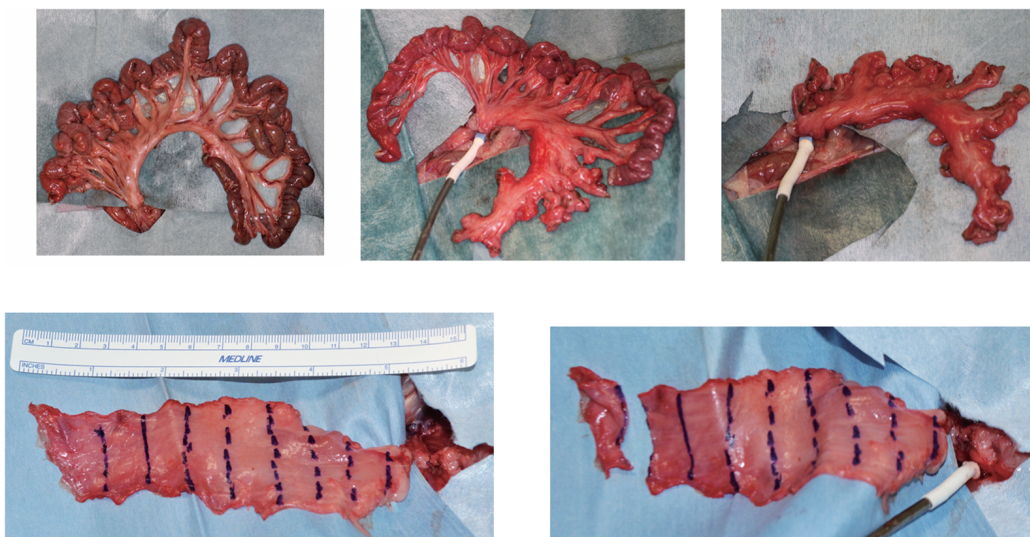


Fig. 2 Measurement of flap blood flow

(Above, left) Anterior mesenteric vessels supply the entire jejunum in a rabbit intestine. The jejunal flaps were completely isolated on a single pedicle and a 1 cm segment of the pedicle was dissected free for probe application. (Above, center) The distal part of jejunum was dissected from the mesentery and resected. The rest of the flap's blood flow was measured. (Above, right) These steps were repeated until all jejuna were resected. (Below) The same procedure was performed on latissimus dorsi muscle flaps. The distal part of the flaps was resected sequentially.

the measurement.

In a rabbit intestine, the entire jejunal flap is supplied by the anterior mesenteric artery and vein.⁹ The jejunal flaps were isolated on a single pedicle, a 1 cm segment of the pedicle was dissected for probe application, and the measurements were carried out as follows.

- i. measure blood flow of flap
- ii. resect small distal segment of jejunum
- iii. measure blood flow of rest of flap

These steps (i–iii) were repeated until all of the jejunum was resected and the mesentery part was left. For each measurement, blood flow was recorded for 2 minutes to ensure accuracy.

Jejunal flaps were ultimately divided into from 9 to 15 portions (average 11.8). The same procedure was performed on LD muscle flaps with thoracodorsal arteries and veins in the pedicle. The distal part of each flap was resected sequentially and only the pedicle of the flap was left. LD muscle flaps were divided into from 7 to 10 portions (average 8) (Fig. 2).

Flap Conductance Calculation

Conductance is influenced by amount of blood flow, systolic blood pressure (SBP), diastolic blood pressure (DBP), hematocrit (Ht), and body temperature (T). The conductance of the flap is the reciprocal of its resistance. We calculated vascular resistance as described in previous studies.^{10,11} Vascular resistance is inversely proportional to the amount of blood flow.

$$\text{Res}_{\text{uncorrected}} = \Delta P / Q \text{ (mmHg/ml/min)}$$

where ΔP (change in blood pressure) = ABP (arterial blood pressure) – VBP (venous blood pressure), Res = vascular resistance, and Q = amount of blood flow.¹⁰ We set ABP as the mean arterial blood pressure (MAP).

$$\text{MAP} = (\text{SBP} + 2 \times \text{DBP}) / 3 \text{ SBP}$$

Venous blood pressure was estimated to be 0 mmHg. The vascular resistance was adjusted further with Hct and T.

$$\text{Res}_{\text{corrected}} = \text{Res}_{\text{uncorrected}} \times 1.025^{(T-T_0)} \times (1 + 0.025 \times \text{Hct}_0 + 0.00735 \times \text{Hct}_0^2) / (1 + 0.025 \times \text{Hct} + 0.00735 \times \text{Hct}^2)$$

where $T_0 = 37^\circ\text{C}$ and $\text{Hct}_0 = 38\%$. We set standard Hct as 38% and T as 37°C . Finally, we calculated flap conductance (C).

$$C = 1 / \text{Res}_{\text{corrected}}$$

Tissue Volume Measurement

For the measurement of jejunal volume, food debris in the lumen was washed out and then the wet weight of each tissue segment was measured. Using Archimedes' principle, we calculated the volume of each tissue segment by submersion in serine (Fig. 3).¹² After resecting all of the conduit part, the mesentery of the flap was cut at the pedicle and its volume was measured in the same way. The volume of the flap at each point of blood flow measurement was calculated by subtracting the volume of the resected part from that of the entire jejunal flap. Volume measurement of the latissimus dorsi muscle flap was also carried out in the same way.

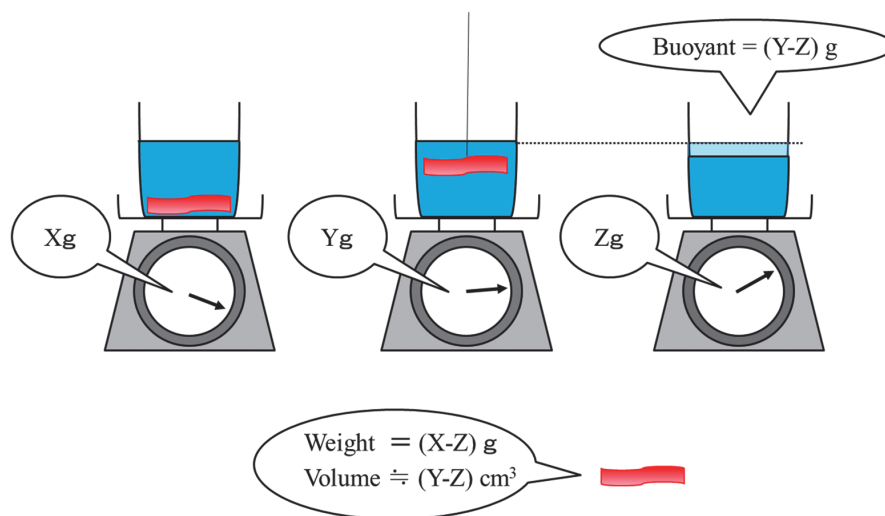


Fig. 3 Measurement of flap volume by Archimedes' principle

(Above) Each tissue segment was immersed in serine. The increased weight representing the weight of serine displaced by the segment equals the buoyant force.

(Below) Serine's specific gravity is 1.004, so the buoyant force approximated the volume of the jejunum segment.

Microvessel Density Measurement

Samples of jejunum ($n=4$) and LD muscle tissue ($n=3$) were fixed with 4% paraformaldehyde immediately after retrieval and embedded in paraffin. The embedded specimens were sectioned 5 μm . Immunostaining for vessels was performed using rabbit polyclonal antibody against CD31/PECAM1 (1:300, Dianova GmbH, Hamburg, Germany). Signal detection was performed with avidin-biotin horseradish peroxidase (HRP) with diaminobenzidine (DAB) (Vector Laboratories, Burlingame, USA) and counter-stained with hematoxylin. Color images were captured $\times 150$ in 20 fields per sample using DP2-BSW (Olympus Corporation, Tokyo, Japan). All image processing was carried out by Image J (NIH, Bethesda, USA).¹³ The area of each blood vessel was obtained by accentuating and extracting the CD31/PECAM1-positive area with a binary process and filling its lumen. Vessels with a diameter larger than 200 μm (arterioles) were excluded from the area of investigation since they do not contribute to conductance. The ratio of total blood vessel area to entire tissue (%) was calculated and regarded as the microvessel density (MVD).

Statistical Analysis

To assess the association between the volume and conductance in each flap, a simple linear regression analysis was performed and the significance was assessed by Pearson's chi-square test. Further, the conductance was analyzed with a linear mixed model that included the volume as a fixed effect and individuals (i.e. rabbits) as a random effect.

For the assessment of the MVD in both the jejunum and LD, Welch's t -test was performed. The MVD was also analyzed by means of a linear mixed model with the same parameters used previously to assess conductance. All statistical analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, USA). A P value less than 0.05 was considered indicative of statistical significance.

RESULTS

Correlation between Conductance and Tissue Volume

Conductance and tissue volume were significantly correlated in both jejunal and LD flaps as shown in Figure 4. Slopes of the regression line in each sample varied between 0.0067–0.030 (0.020 ± 0.011) for jejunal flaps and 0.0037–0.0097 (0.0059 ± 0.0033) for LD flaps.

The conductance was further analyzed using a linear mixed model that included the volume as a fixed effect and the individual effect minimized. The result showed that conductance increased significantly by 0.012 (95% CI; 0.0070–0.016) ml · min/mmHg in jejunal flaps and 0.0047 (95% CI; 0.0032–0.0062) ml · min/mmHg in LD flaps, while volume increased 1 cm³.

Microvessel Density Analysis

MVD assessment was carried out in 60 microscope areas in both the jejunum and LD (Fig. 5). Mean MVD was 1.15 ± 0.52% in the jejunum and 0.37 ± 0.29% in the LD. Statistical analysis showed that the MVD of the jejunum was significantly higher than that of the LD. Further analysis with a linear mixed model also showed that the MVD of the jejunum was significantly greater than that of the LD by 0.78% (95% CI; 0.63–0.93) (Fig. 6).

We set volume as a fixed effect and individual difference as a random effect. All the data were integrated by a linear mixed model analysis. Conductance and volume were closely correlated in both jejunal and LD flaps. The conductance increased significantly by 0.012 (95% CI; 0.0070–0.016) ml/min/mm Hg with a 1 cm³ volume increase. Single regression analyses between V and C in LD flaps are presented (*Right*); No. 5 (red), No. 6 (blue), No. 7 (yellow). All the data were integrated by a linear mixed model analysis. The correlation was presented

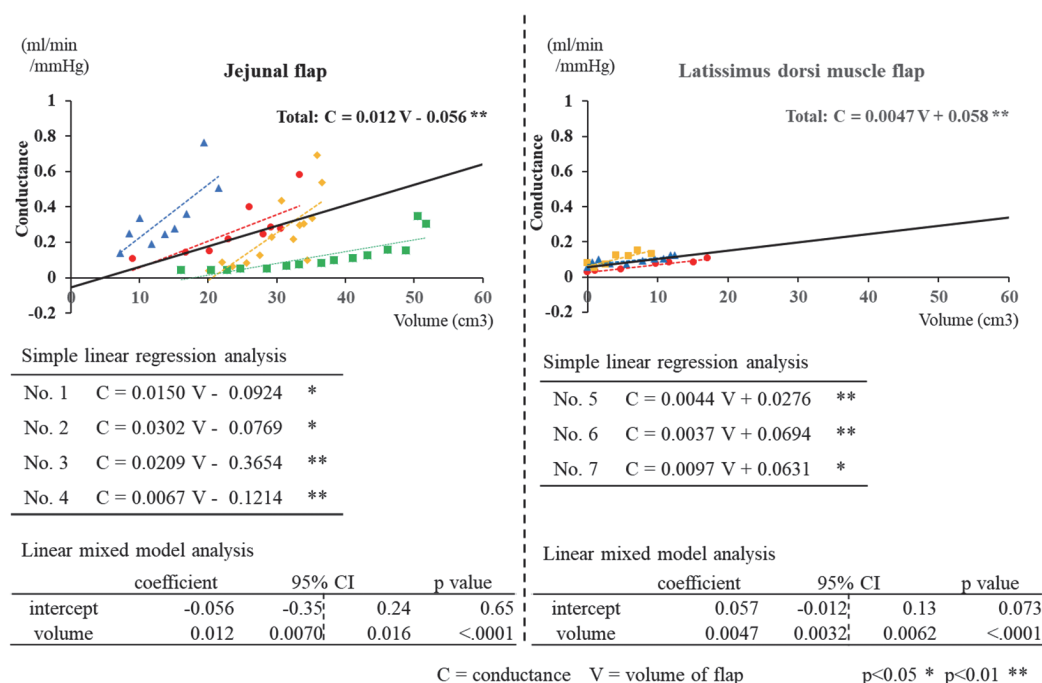
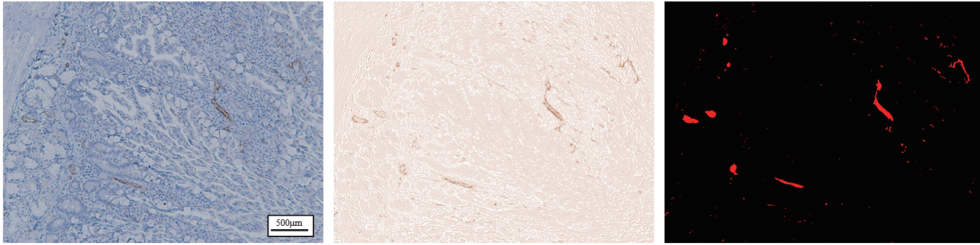


Fig. 4 Single regression analyses between total volume (V) and conductance (C) in jejunal flaps are presented (*Left*); No. 1 (red), No. 2 (blue), No. 3 (yellow), No. 4 (green).

Hemodynamics in the flap

Jejunum



Latissimus dorsi

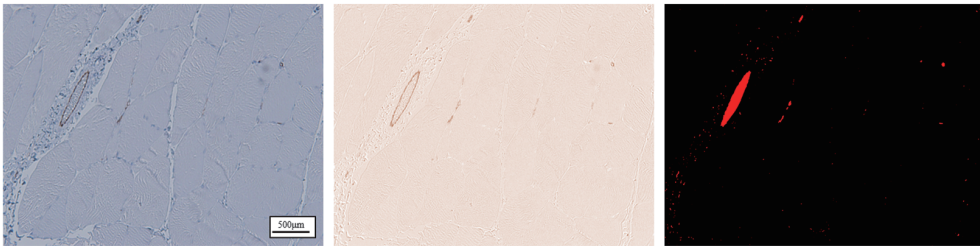


Fig. 5 Microvessel density measurement

Tissue samples of jejunum (*above*) and LD muscle (*below*) were immunostained with rabbit polyclonal antibody against CD31/PECAM1 (1:300, Dianova GmbH). Signal detection was performed with avidin-biotin horseradish peroxidase (HRP) with diaminobenzidine (DAB) (Vector Laboratories) and counter-stained with hematoxylin (left). The blood vessel area was obtained by accentuating and extracting the CD31/PECAM1-positive area using a binary process (*center*) and filling its lumen (*right*).

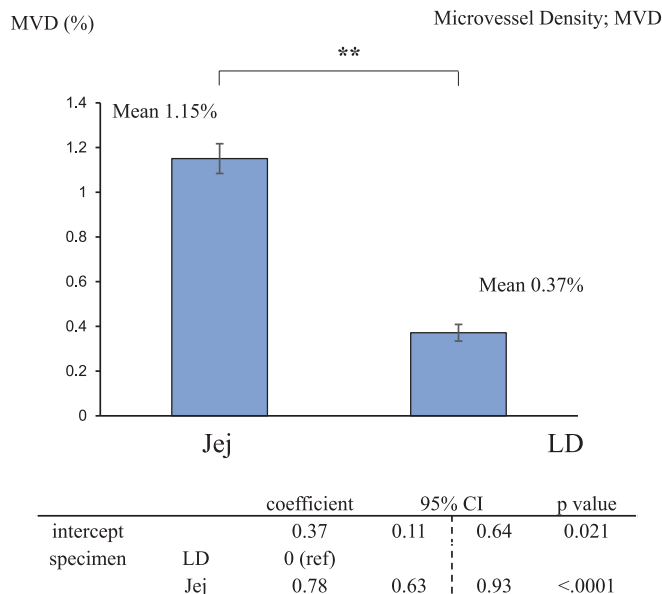


Fig. 6 Microvessel density analysis. Mean MVD was $1.15 \pm 0.52\%$ in jejunal flaps, and $0.37 \pm 0.29\%$ in LD flaps. Statistical analysis showed that the MVD of the jejunum was significantly higher than that of the LD ($p < 0.01$). Linear mixed model analysis showed that the MVD of the jejunum was significantly higher than that of the LD by 0.78 (95% CI; 0.63–0.93)%.

as a straight black line on the graph. The conductance increased significantly by 0.0047 (95% CI; 0.0032–0.0062) ml/min/mmHg with an increase in volume of 1 cm³.

DISCUSSION

Understanding the conductance of flaps contributes to safer free flap transplantation. If the same free flap is transferred to either of two different recipient vessels, the flap survival area will be greater in the recipient artery with higher blood flow.¹ If multiple free flaps are transferred to the same recipient vessel, the flaps with better conductance will have higher blood flow. Several reports show that a free flap transfer has the potential to increase the blood supply of the ischemic limb,³ which suggests that replacing low-conductance tissue with high-conductance tissue helps increase vascular blood flow. However, it should be noted that transplantation with a high-conductance flap produces a large amount of blood flow in the transplanted tissue, consequently necessitating its drainage. If the flap is subjected to blood flow exceeding the drainable amount, congestion of the flap ensues. In clinical cases, venous congestion often occurs when the flap is anastomosed to a vein with insufficient capacity but is improved by shifting to a vein with sufficient capacity. In that situation, appropriate recipient vein selection is important for high-conductance flaps. We speculate that flap conductance could be decreased by reducing the quantity of flap components.

We previously reported in a clinical free flap analysis that vascular resistance differs between the flap type and composition of tissue components.^{7,8} However, in the clinical setting, neither patient background nor flap characteristic could be standardized. In the current study, by utilizing an animal model with two single-pedicled flap types (jejunum and LD), variations of patient and flap characteristics were excluded and measuring the blood flow of the flap multiple times while decreasing the volume became possible. We demonstrated that a significant correlation between volume and conductance existed both in jejunal and LD muscle flaps, which means that is substantially determined by the tissue itself. This is consistent with our previous clinical findings.^{6,7} Furthermore, jejunal flaps had a conductance approximately three times greater than LD muscle flaps. This is also consistent with our clinical findings that the blood flow in jejunal flaps is about four times higher than that in LD flaps.⁷ Interestingly, the MVD of the jejunum was three times higher than that of LD muscle in this rabbit model. These results imply that the conductance of the tissue is regulated by the MVD of the tissue. To our knowledge, this is the first report that shows a correlation between conductance and vascular density in tissue.

In systemic circulation, about two thirds of the total resistance to blood flow is produced by small-sized vessels including arterioles (100–50 µm), metarterioles (20–10 µm) and capillaries (10–5 µm).¹⁴ The vascular network runs in parallel and creates the conductance of the blood flow,¹⁵ thus the resistance of each tissue is considered an aggregate of the resistance of each vascular network within it. As Turek et al verified, jejunal capillary density was higher than that of skeletal muscle in a mouse model.¹⁶ This is consistent with our findings in the present study, and it suggests that the distribution of the capillary network in organs and tissues varies based on the metabolic activity (e.g. intestine is greater than muscle, muscle is greater than fat/skin). It is understandable that tissue with a higher demand for blood circulation has a higher vascular density.

Other factors that affect conductance should also be mentioned. In tissue in which blood vessels are not uniformly distributed, such as those that include an area supplied via choke vessels or arterio-venous shunts, conductance may be influenced.¹⁷ Neural regulation also plays an important role in tissue conductance.¹⁸ It causes the constriction of vessels, particularly resistance vessels.

However, in clinical free flaps as well as those in our animal model, there is no neural regulatory effect because the tissue is disconnected from the neural circuit. Circulating soluble factors including systemic humoral factors, autocrinal or paracrinal agents, and the effect of vasoactive drugs should be considered as well. Eley et al reported that a dobutamine infusion increases flap conductance, while norepinephrine decreases flap conductance and decreases conductance of other tissues significantly, ultimately increasing blood flow of the flap.¹⁹ Further investigation will provide a better understanding of conductance in flap hemodynamics.

Although only two tissue types (intestine and muscle) were investigated in this study, together with our clinical experience, we postulate that this finding is applicable to other types of tissue (e.g., skin, fat, or bone) or composites of these tissues (e.g., face transplantation). This report contributes to a better understanding of a basic factor in achieving successful tissue transfer outcomes.

CONCLUSIONS

We demonstrated significantly greater conductance within jejunal flaps than in LD flaps and the same tendency was identified in the microvessel density which produced most of the total resistance to blood flow. Our results indicate that the difference in microvessel density regulates the unique conductance of each tissue. These findings may contribute to a better understanding of factors that determine blood flow of flaps and to achieving more successful tissue transfers.

SOURCE OF SUPPORT THAT REQUIRE ACKNOWLEDGMENT

This study was received Nagoya University Hospital Institutional review board approval. We obtained consent of both cases to the publication of information about a patient.

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CONFLICT OF INTEREST

The authors have no conflicts of interest, whether they are financial or related to any other relationships, to disclose.

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