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Original Article

Anatomical analysis of the periosteal blood supply system of the fibula using fresh cadavers ☆

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ABSTRACT

A vascularized free fibula flap is often used to reconstruct bone defects. However, bone resorption within the osteotomized segment is often observed. This may be attributed to damage to bone blood flow supplied by nonpenetrating periosteal vessels (NPPVs); however, there are few studies on NPPVs in the fibula. In this study, we investigated dissection methods to assess the vascular network in the fibula and performed a detailed anatomical investigation of NPPVs using fresh cadavers provided by the Clinical Anatomy Laboratory at the Keio University. Three dissection methods were compared to assess the vascular network, and data on the branching, distribution, and number of NPPVs from the peroneal artery were collected. A method involving the elevation of the periosteal bone flap was found to be the most acceptable for assessing fibular NPPVs with less vascular damage. A total of 13 limbs from 7 male and 2 female cadavers were dissected. The number of detected NPPVs was 12-23 per limb (median: 17). No nutrient vessels were detected 5 cm from the proximal and distal ends of the fibula. Fibular NPPVs were distributed in the anterior and poste-

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rior directions along the peroneal artery trunk, with more NPPVs toward the posterior. Among the osteotomized segments of 1.0 cm, 30% did not contain any NPPVs, whereas segmentations of 1.5, 2.0, and 3.0 cm resulted in 87%, 95%, and 99% of the segments with at least one NPPV, respectively. These findings for the vascular network in the fibula may help to improve the graft blood supply and prognosis.

Introduction

Artificial materials or biomaterials can be used to reconstruct bone defects caused by tumor resection or trauma. Biomaterials are superior to artificial materials because they are more biocompatible and cause fewer infection, and vascularized free bone grafts are the gold standard treatment due to these properties.[1,2] The fibula is widely used as a biomaterial because it has sufficient length to be used in the reconstruction of long bone defects and there is little effect on the motor function of the doner leg after harvesting.[3] In recent years, a vascularized free bone flap, which maintains blood flow to the bone, has been widely used to prevent graft infection and resorption after transplantation.[4] The grafted bone is manufactured to match the recipient defect to permit functional and cosmetic reconstruction. However, post-operative imaging sometimes reveals bone resorption over time in each osteotomized segment of the bone graft, which may be due to altered blood flow to each segment.[5-8] Therefore, there is a need for minimal osteotomies and procedures that do not damage the blood supply to the bone.

There are 2 routes of blood supply to the bone: nutrient vessels (NVs) through the nutrient foramen and nonpenetrating periosteal vessels (NPPVs) from the periosteal arterial plexus.[7] The NPPVs form a nutrient system that maintains and develops the compact bone on the bone surface.[7] Studies on micro-blood supply routes in the skin, subcutaneous tissue, and muscle have led to clinical applications such as the free perforator flap,[9-11] but there are few reports on the micro-blood supply to the bone and periosteal tissue.[7] Thus, further studies are required to understand blood flow from the NVs and NPPVs to the grafted bone or periosteal arterial plexus when the bone graft is highly processed and molded.

In this study, we used fresh cadavers to first investigate the dissection methods to assess the distribution and branching of NPPVs in the fibula. The optimal method was chosen to examine the NPPV network in the fibula, which is frequently used as a biomaterial for bone reconstruction. The results permitted the evaluation of the appropriate length of osteotomies and segmentations for the preservation of the bone vascular system. In addition, we examined whether a photoacoustic imaging system, which has recently been applied clinically, could be used to delineate peroneal NPPVs.

Materials and methods

Anatomical analysis was conducted using unfixed fresh cadavers provided by the Clinical Anatomy Laboratory at the Keio University between March 2015 and November 2019. The cadavers used in this study were donated to the Keio University School of Medicine after obtaining consent from their families. All investigations were performed in accordance with the guidelines of the Japan Surgical Society and Japanese Association of Anatomists for cadaver dissection in education and research in clinical medicine. This work was approved by the Keio University medical research ethics committee (approval number: 2007-0026). Dissection was performed by a certified plastic surgeon (EM). Three

Table 1
Details of the cadavers.

Case (n)	Age (years)	Sex	Limb
1	81	Male	Left
2	79	Male	Both
3	89	Male	Right
4	85	Female	Both
5	90	Male	Both
6	75	Female	Right
7	95	Male	Both
8	79	Male	Right
9	80	Male	Right

modified methods were compared for assessing the bone and periosteal vascular system in the fibula: elevating the skin and periosteal flaps, myocutaneous-periosteal flap, and periosteal bone flap.

Angiography was performed using the method reported by Imanishi et al. for investigation of the vascular network of skin and soft tissue.[9,10] Briefly, barium latex was injected from the femoral artery, which made the blood vessels detectable grossly, and the bone or periosteal flap was elevated. The vascular distribution was then determined grossly and radiographically. Stereoimaging was also used to analyze the distribution of blood vessels in 3 dimensions to assess the direction of branching. Data were collected for the location of the first perforator, presence and location of bifurcation of the main trunk of the peroneal artery, and location and distribution of the peroneal periosteal branches. PAI-05 photoacoustic imaging system was used[12] to visualize the vascular system in the fibula and surrounding periosteum. Statistical analysis was performed using the *t*-test in GraphPad Prism ver. 10.1.0 (Boston, MA, USA), with $P < 0.05$ considered to be significant.

Results

Patients background

Thirteen limbs from 9 cadavers (7 male, 2 female) with confirmed perforating branches were included in the study, after excluding limbs with poor angiography results. Age at death ranged from 79 to 95 years (median: 81 years). Ten of the dissected limbs were from males and 3 were from females (Table 1). Clinical information for height, weight, smoking history, and presence of vascular diseases in the lower limbs was not available, but vascular disease or thrombosis was not the direct cause of death in any cadaver.

Dissection method for investigation of the vascular distribution in the fibula

A schematic anatomy of the fibula and surrounding tissue is shown in Figure 1A. First, dissection was performed by creating a sequential free skin and periosteal flap with a peroneal artery (skin + periosteum (SP) method; Figure 1B). In this method, the periosteal vessel branches and periosteum were often damaged during elevation of the periosteum from the bone. To solve this problem, we considered a different method in which the periosteum is elevated with the muscle, and the muscle is removed after radiography (skin + muscle + periosteum (SMP) method; Figure 1C). However, similar to the SP method, it was challenging to elevate the periosteum from the bone and obliterate muscle branches. Thus, the bone + periosteum (BP) method was tested (Figure 1D). In this method, the bone and periosteum were subjected to angiography together, and then the bone was split in half and angiography was performed again. The BP method caused a lower rate of damage to the periosteal branches and NVs were easier to detect. Therefore, the BP method was used in further investigation.

The direction of bone incision in the BP method was examined to obtain images that allowed vessel evaluation. Angiographic radiography and stereoimaging were performed on the dissected fibula to assess 2D and 3D vessel distribution. The results showed that the peroneal arteriovenous vein ran

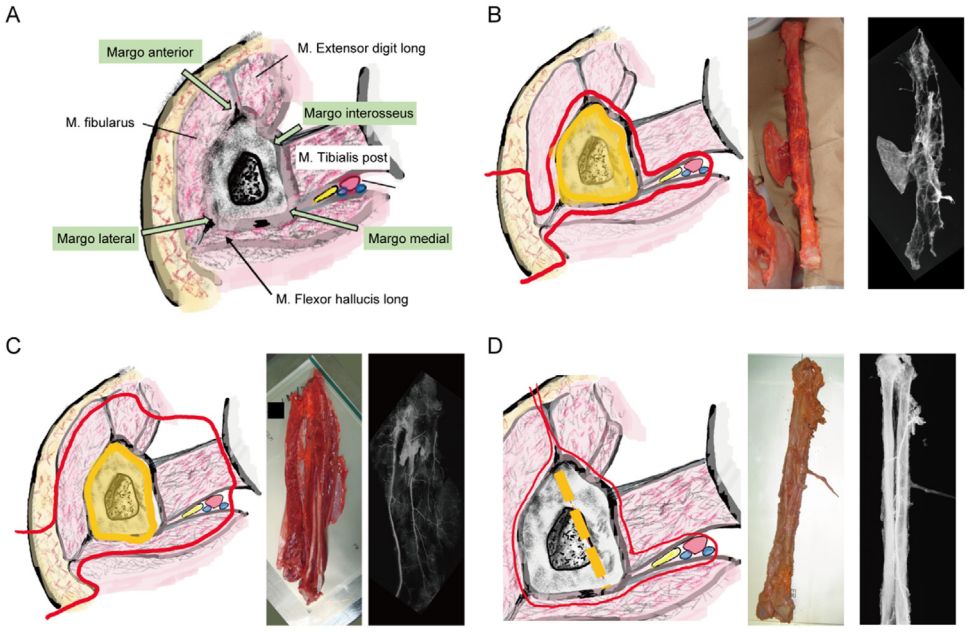

Figure 1. Schemas of the dissection methods. A) Anatomy of the fibula and surrounding tissue. In panels B-D, the resection line (solid red line), dissected fibula macro image, and angiographic image are shown from left to right. B) Skin + periosteum method. In this method, the periosteum and periosteal branches of the blood vessels are frequently damaged when the periosteum is detached from the bone. C) Skin + muscle + periosteum method. With this method, vascular branches that are not from the peroneal artery could be detected. Moreover, it is difficult to detach the periosteum from the bone, and muscle branches could remain when muscle tissue was removed. D) Bone + periosteum method. The periosteum is not detached from the bone. In this method, the fibula is hemi-incised and angiography is performed (yellow dashed line: half split line).

along the medial border of the fibula, and NPPVs were branched from the peroneal artery to the posterior and interosseous borders of the fibula. Based on these findings, a bone hemi-incision was made at the anterior border toward the posterior of the fibula. The periosteum of the posterior surface of the fibula was preserved by this hemi-incision. Radioangiography and stereoimaging of the unfolded hemi-incised bone were then used to examine the 2D and 3D vascular network.

Distribution of NVs and NPPVs in the fibula

The distributions of NVs and NPPVs are summarized in Table 2. The peroneal length was 30-38 cm (median: 32 cm). The first perforator, presumed to be the primary NV, was identified at 6-15 cm (median: 10 cm) from the proximal end of the fibula. Peripheral branches of the main fibular artery trunk were found in 11/13 limbs (84.6%), located 17.6-29 cm (median: 21 cm) from the proximal end of the fibula. No NVs were found in the proximal and distal 5 cm of the fibula. The number of NPPVs was 12-23 per limb (median: 17). The main trunk of the peroneal artery ran along the medial border of the fibula, and the NPPV from the main trunk branched in the anterior (interosseous) and posterior direction. Posterior direction branches predominated in 9 out of the 13 limbs.

In a clinical setting, a fibular bone graft is modified by osteotomies to fit the bone defect. Therefore, the number of perforating blood vessels within each osteotomized segment was counted to assess the segment length required to maintain the blood supply system in the fibula. The results are summarized in Table 3. With segmentation of 1.0 cm, 30% of the segments contained no perforating branches, whereas segmentations of 1.5, 2.0, and 3.0 cm resulted in 87%, 95%, and 99% of segments containing at least one perforating branch, respectively. This finding is consistent those in previous reports.[13-15]

Table 2
Data for nutrient vessels and non-penetrating periosteal vessels (NPPVs).

Case	Limb	Fibula length	Location of first perforator		Branch point of peroneal artery		Number of NPPVs	Number of NPPV branches	
		(cm)	(cm)	(%)	(cm)	(%)		To anterior	To posterior
1	L	32	10.5	32.8	19	59	14	8	6
2	L	34	13.7	40.3	20.4	60	15	9	6
	R	34	11.5	34.0	24	70.6	17	7	10
3	R	30	8.5	28.3	20	66.7	12	5	7
4	L	30	10.0	33.3	22	73.3	18	8	10
	R	30	10.5	35.0	17.6	58.7	18	7	11
5	L	32	6.0	18.7	NA	NA	17	8	9
	R	32	7.6	25.0	21	65.6	17	9	8
6	R	30	10.0	29.0	NA	NA	15	10	5
7	L	35	10.0	28.5	19.8	57	15	5	9
	R		10.0	29.0	29	82.8	14	7	8
8	R	38	15.0	39.0	26.5	69.7	23	6	8
9	R	34	14.0	41.0	26.5	78	18	6	12
Maximum		38	15	41	29	82.8	23	10	12
Minimum		30	6	18.7	17.6	57	12	5	5
Median		32	10	32.8	21	66.7	17	7	8

Table 3
Percentage of segments of different lengths including non-penetrating periosteal vessels (NPPVs).

Segment length (cm)	Segments with 0, 1, or ≥2 NPPVs (%)		
	0	1	≥2
1	29.6	40.9	29.6
1.5	13.0	38.9	48.1
2	5.7	35.2	60.0
3	1.4	14.3	85.7

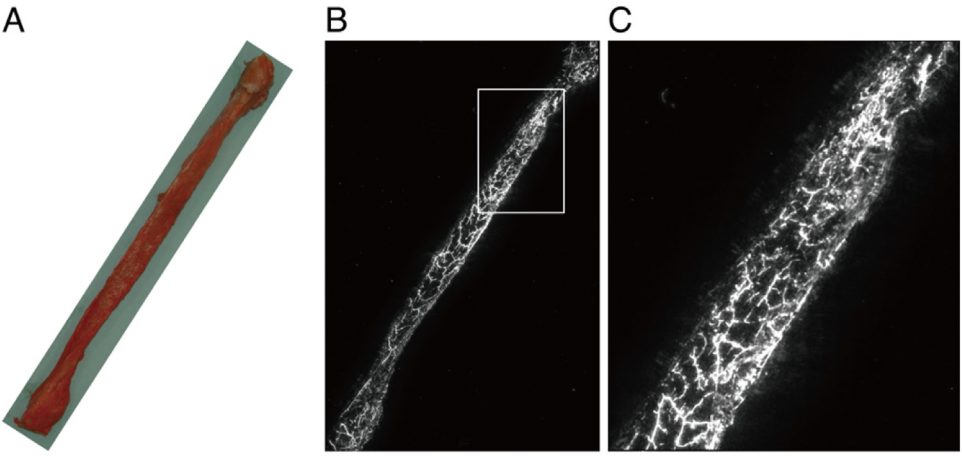

Figure 2. Identification of an non-penetrating periosteal vessels (NPPVs) by photoacoustic imaging. A) Gross findings of the dissected fibula. B) Photoacoustic image of the periosteal nutrient vessels (low magnification). C) Partially magnified image. The photoacoustic imaging system provides NPPV imaging quality similar to that with X-ray angiography.

Detection of NPPVs using a photoacoustic imaging system

A photoacoustic imaging system has recently been used to detect shallow lymph vessels in clinical settings because it is less invasive than angiography using contrast agents. This technique was found to detect NPPVs of the fibula with visualization similar to those obtained using X-ray angiography (Figure 2).

Discussion

Segmentally osteotomized fibulas are used to reconstruct bone defects caused by tumor resection or trauma, and post-operative imaging sometimes reveals bone resorption in these segments.[1,2] This is considered to be due to a deficiency of blood flow in the segment.[5-7] Clinical applications associated with the micro-blood supply system in the subcutaneous skin and muscle have been developed, but understanding of this system in the bone and periosteum is limited.[7,11,15-17] Computed tomography angiography (CTA), digital subtraction angiography, and magnetic resonance angiography (MRA) have been used before the surgery to determine the osteotomy position to improve blood flow in grafted bone after transplantation.[13] However, there are few studies of the bone vascular network using fresh cadavers.[15] Therefore, the establishment of a dissection method for observing the peroneal vascular system and data obtained for the NPPV distribution in the current study may be beneficial for determining the position and length for segmentation of a fibular graft.

By using the fibula as a vascularized free flap, approximately 5 cm of the proximal and distal ends are left in the donor leg during harvesting.[18] This procedure is performed to preserve the function

of the knee and ankle joints in the donor leg. Although the current study was not conducted with consideration of blood flow to the grafted fibular bone flap, no NV was identified within 5 cm of the proximal and distal ends of the fibula, suggesting that the current clinical procedure is unlikely to cause problems in maintaining blood flow in the harvested fibula. Consistent with our findings, Morsy et al. described an anastomotic vascular network at the proximal end of the fibula in 28 dissected limbs, with this network formed by the branches of the inferior lateral genicular and anterior tibial arteries.[19]

Bifurcation distal to the main trunk of the peroneal artery was found in 84.6% of the limbs in the current study. This distribution has not been previously reported, as far as we are aware. The locations of bifurcation were more distal, rather than at the center of the fibula. These findings suggest that osteotomy closer to the proximal end of the fibula is associated with a lower risk of nutrient vessel injury. In addition, for a vascularized free fibula flap, the bifurcation may be used to create a chimeric graft. After implantation of an osteotomized vascularized free flap, blood flow from the NV through the bone marrow is considered lost, and the flap blood supply is thought to depend on NPPVs. In our study, approximately 30% of the 1-cm segments did not contain any NVs. This finding is consistent with that of a previous study on the assessment of the distribution of blood vessel networks using CTA and angiography in fresh cadavers.[15] As grafts with preserved blood flow have better bone remodeling, slower bone resorption, and better prognosis,[4] avoidance of osteotomies with segments <2 cm may improve the graft prognosis.

Examination of the post-bifurcation branching direction of NPPVs showed more branching in the posterior direction (flexor halluces longus attachment) than the anterior direction (extensor digitorum longus and tibialis posterior attachment), viewed from the medial border of the fibula. Similar findings have been reported for the location and number of nutrient foramen in the fibula.[14] In clinical practice, the graft periosteum is removed to fix the plate. Our findings suggest that periosteum dissection in the anterior border direction is appropriate, especially on the lateral surface where the peroneus muscle attaches.

NPPVs of the fibula are minute and their detection is continues to be challenging. The detection of fibular NPPVs using CTA and MRA has been reported,[20,21] and a photoacoustic imaging system has recently been applied clinically to identify blood vessels and lymphatic vessels.[22,23] In the current study, this imaging method could detect peroneal NPPVs in a limb at a level similar to that of angiography. Because photoacoustic imaging is less invasive, identifying vessels using this method may be feasible and helpful in improving the surgical outcomes when using a vascularized free fibula flap.

There are some limitations in this study. As fresh cadavers were used, it was impossible to assess blood flow, which made it challenging to determine if the identified NPPVs were functional vessels. Moreover, the number of NPPVs may be more or fewer than in reality due to the sensitivity of the imaging method used in the study. In fact, more perforating branches were detected than that in a previous report.[13] Furthermore, bias in the data cannot be ruled out owing to the lack of clinical information for the cadavers. Within these limitations, the results revealed the detailed distribution of NVs and NPPVs in the fibula. These findings provide useful information for the optimization of osteotomies during fibula bone graft segmentation that may improve the graft blood supply and graft prognosis.

Conflict of interest

The authors have no conflicts of interest to declare that are directly relevant to the content of this article.

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Ethical approval

Keio University Medical Research Ethics Committee (approval number: 2007-0026).

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