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의학석사 학위논문

복직근과 근육내 주행하는 심하복벽동맥 천공지의 관계에 대한 조직학적 연구

Histologic Analysis of the Relationship between Rectus Abdominis muscle and Intramuscular Perforator of Deep Inferior Epigastric Artery

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이 논문을 의학석사 학위 논문으로 제출함

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영문 요약

Background: Deep inferior epigastric artery (DIEA) perforator free flap has gained significant popularity in breast reconstruction, and the use of the intramuscular perforator has acquired major interest among microvascular surgeons. However, attempts have not yet been made to analyze the intramuscular course of DIEA perforator from the histologic perspective. We investigated the histologic relationship between the intramuscular course of DIEA perforator and rectus abdominis (RA) muscle.

Methods: From one cadaveric and 15 human subjects who underwent breast reconstruction following mastectomy, 17 RA tissue blocks including DIEA perforator were harvested (area, 1 cm^2; depth, 1 cm). All samples were stained using hematoxylin-eosin and Masson's trichrome. Six to nine serial images were processed on a personal computer for generating a larger merged image. Histologic components were evaluated under light microscopy. We categorized 15 human samples into three groups according to the course of the intramuscular perforator: longitudinal (parallel to the muscle), oblique (obliquely to the muscle), and transverse (perpendicular to the muscle). Cross-sectional mean arterial diameter (DIEA perforator vessel) and cross-sectional area (perivascular adipose tissue) were calculated from each specimen at ×40 magnification. These two values were compared and correlated among three types of vessels using Kruskal-Wallis tests and regression analysis, respectively.

Results: RA muscle fibers are divided into multiple fascicle bundles by their perimysium (fibroadipose septa), including DIEA perforator vessels, nerves, and lymphatic channels. The perimysium comprises a main framework (fibroadipose septum), parallel to the RA muscle arrangement, and its ramifying fibroadipose branches. The main DIEA perforator was included in the fibroadipose septum (main framework of perimysium, running parallel to the arrangement of RA muscle). These structures are consistently found in all human and cadaveric samples. Mean arterial diameter of DIEA perforators was significantly longer in longitudinal, compared with transverse types (822 vs. 564 μm, respectively; $P < 0.05$), No significant difference was noted between oblique (706 μm) and other types. Cross-sectional area (perivascular adipose tissue) was large in the order of longitudinal, oblique, and transverse types (3.12, 2.81, and 2.64 mm^2). Statistically significant difference was noted between the cross-sectional area of Longitudinal and transverse types. No significant correlation was noted between cross-sectional area of perivascular adipose tissue and mean arterial diameter of DIEA perforators.

Conclusion: The main framework of RA perimysium includes major DIEA perforator vessels, running parallel to the muscle fiber arrangement. Minor neurovascular branches are included in its ramifying fibroadipose branches. Better understanding of the histologic relationship between the RA muscle and DIEA perforator may help surgeons in performing pedicle isolation under microscopy.

Keywords: Deep inferior epigastric artery perforator, Histology, Rectus Abdominis muscle

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Introduction

Over the past decade, the deep inferior epigastric artery (DIEA) perforator flap has gained significant popularity in breast reconstruction.[1,2] By isolating musculocutaneous perforators throughout their course, thereby allowing the harvest of skin and subcutaneous fat alone, a reliable blood supply can be preserved while simultaneously minimizing the risk of donor site dysfunction. Despite these advantages, a limiting factor of the usage of the DIEA perforator flap comprises increased operative time required for meticulous dissection of the intramuscular course of the perforators. Among several factors that affect the difficulty of intramuscular pedicle dissection, including length of the pedicle, course of the perforators, number of branches, and other individual characteristics, the relationship of the pedicle to the muscle is of the most interest during pedicle dissection.[3]

The intramuscular course of the DIEA has been described by Rozen et al.[4] as a substantial transverse and longitudinal expedition by the DIEA perforators traversing through the muscle belly. While the longitudinal course of the perforator, easily freed by blunt dissection, may necessitate only muscle splitting, the transverse course of the perforator may necessitate the transaction of muscle fibers.[4] Provided that the muscles are encased in fascia and that fat planes often surround their respective perforators, the dissection plane during pedicle isolation is at the level between the loose connective tissue layer surrounding the perforator and the muscle fiber.[5] The multiple perimysial septations between the muscle fibers are sequentially divided while looking for the chosen perforator.[6] These intramuscular septae are attached to the muscle fascia and carry the perforating vessels. In our experience, dense fibrous attachment was frequently found at the side of the pedicle during circumferential dissection around the intramuscular perforator, which necessitate coagulation or clipping to be completely freed from the surrounding tissue, not merely by blunt dissection. Identifying these structure from histologic perspectives would be helpful for dissection of the perforating vessels from the rectus abdominis (RA) muscle.

Despite previous studies on intramuscular anatomy of the DIEA perforator, to our knowledge there have been no clinically significant histological studies on the intramuscular perforator during dissection of the perforating branches through the rectus abdominis (RA) muscle. We aimed to histologically elucidate the relationship between RA muscle fiber and DIEA perforator and the difference in perivascular structure among the course of the perforator.

MATERIALS AND METHODS

The protocols of this study were approved by the institutional review board of our institution

.

Histologic Study in Cadaveric Sample

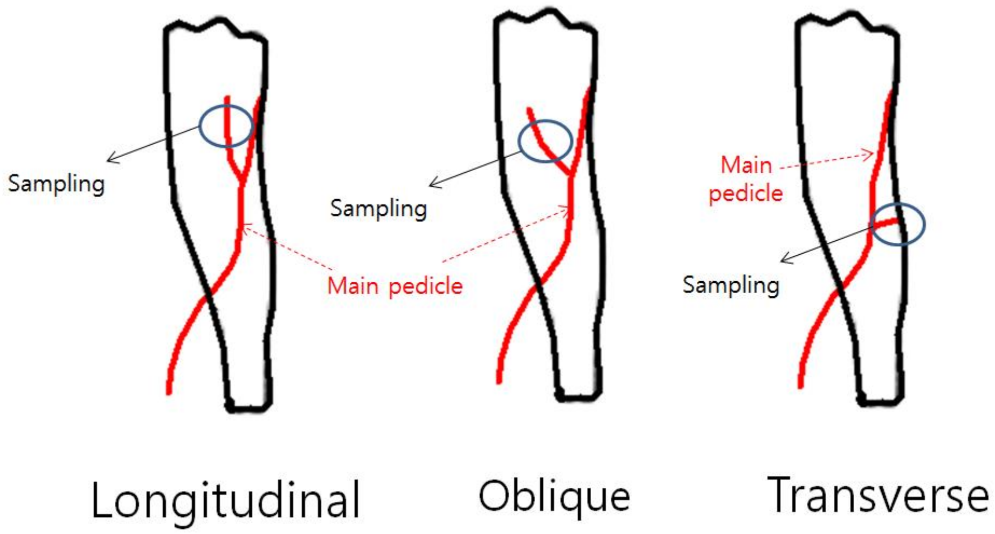
Histologic analysis was performed on a cadaveric (60-year-old male) hemiabdominal wall. The DIEA was identified on the deep surface of the specimen, and the peritoneum and posterior wall of the rectus sheath were carefully dissected. The DIEA was traced to the point of penetration from the RA muscle, and two samples of RA muscle including DIEA perforator vessel were harvested with an area of 1 cm^2 and depth of 1 cm. The samples were fixed by immersion in 4% neutral buffered formaldehyde, maintained in 10% neutral formaldehyde solution for 7 days, dehydrated in ascending alcohol concentrations, and embedded in paraffin. Multiple 12-μm thick sections were obtained from the specimens, which were stained with hematoxylin-eosin using the Weigert van Gieson technique and Masson's trichrome. Histologic components of each sample were evaluated using light microscopy.

A series of image fields (×40 objectives) were scanned to visualize the histologic structure between the RA muscle and DIEA perforator vessel. Six to nine serial images were transferred to a personal computer and processed using Adobe Photoshop (Adobe Systems, Inc., San Jose, CA) to generate a larger merged image. The protocols of this study were approved by the institutional review board of our institution.

Histologic Study in Human Samples

Patients scheduled for breast reconstruction with a DIEA perforator flap were included in the study group. Exclusion criteria comprised subjects younger than 18 or older than 65 years, with history of abdominal surgery or trauma. A total of 15 RA muscle samples including DIEA perforator vessels were harvested from 15 patients during microsurgical breast reconstruction using abdominal tissue. The samples were categorized into three groups according to the course of the intramuscular perforator: Longitudinal (parallel to the muscle), oblique (obliquely to the muscle), and transverse (perpendicular to the muscle) types (Fig. 1). Five samples (area, 1 cm^2; depth, 1 cm) were harvested for each type of vessel.

Fig. 1. Rectus Abdominis (RA) muscle including deep inferior epigastric artery (DIEA) perforator vessel was harvested (area, 1 cm^2; depth, 1 cm). The samples were categorized into three groups according to the course of the intramuscular perforator: Longitudinal (parallel to the muscle), oblique (obliquely to the muscle), and transverse (perpendicular to the muscle) types.



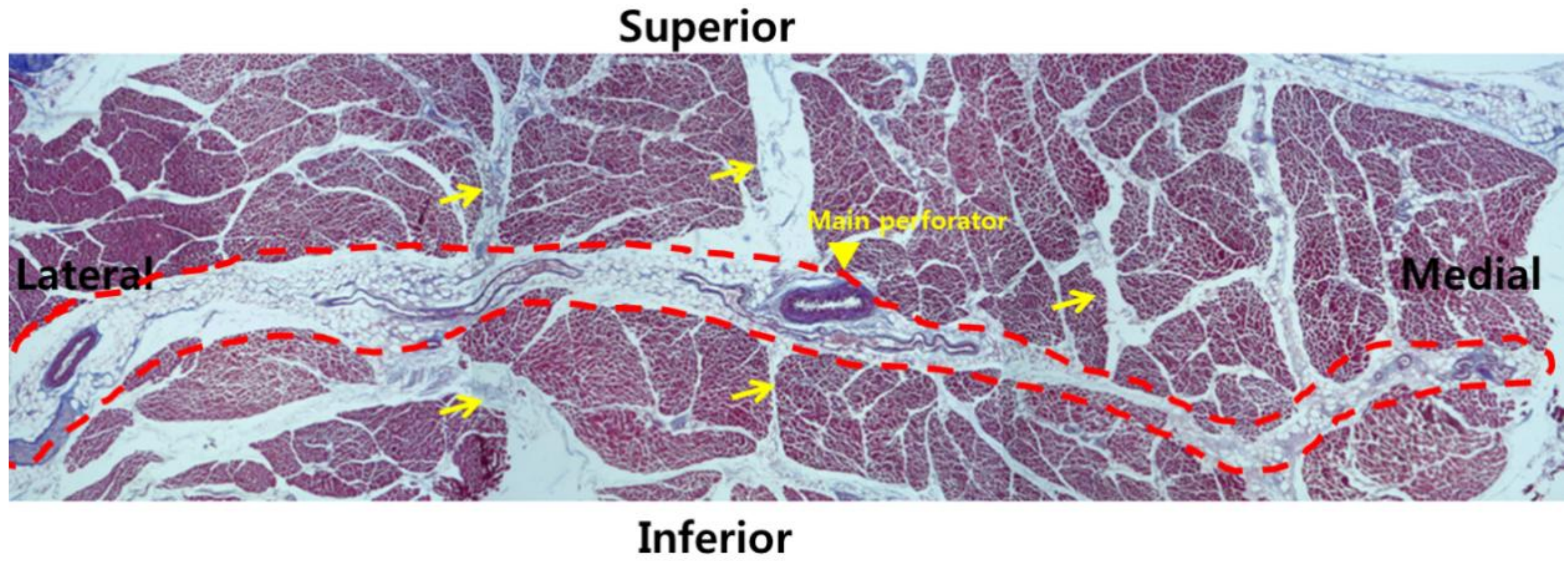
The samples were processed and stained in the same manner as in the cadaveric study. Histologic components of each sample were evaluated under light microscopy. Six to nine serial images were merged according to each type of vessel.

For histomorphologic analysis, the cross-sectional mean arterial diameter of DIEA perforator vessels and cross-sectional area of perivascular adipose tissue (in mm^2) were calculated from each specimen at ×40 magnification. These two values were compared among the three types of vessels using the Kruskal-Wallis test. Regression analysis of the correlation between cross-sectional mean arterial diameter of DIEA perforators and cross-sectional area of perivascular adipose tissue was performed. A P value of <0.05 was considered statistically significant.

RESULTS

The histologic specimen was obtained from a 60-year-old male cadaveric hemiabdomen and 15 female human hemiabdomens without a history of abdominal surgery or vascular pathology. Average patient age was 48.2 years (range, 34–61 years). The specimens were collected such that the DIEA perforator vessels were incorporated within the RA muscle. Representative images from cadaveric and human samples merged by software are depicted in Figures 2 and 3, respectively.

Fig. 2. Histologic examination of cadaveric sample. (A) The perimysium consists of a relatively dense fibroadipose plane parallel to the muscle fiber (*red dot*), and its ramifying fibroadipose branches, spreading out into spaces between fascicles (*arrow*). These structures comprise an integrated network, that is, the small perimysial branches stretch out from the main perimysium. It is remarkable that the major DIEA perforators are included in the main perimysium (*arrowhead*). Serial ×40 magnified images were merged to generate a gross view.



(B) Small branches of perimysium contain fibroadipose tissue, fibroblasts, neurovascular structure, and extracellular matrix. (×40 magnified image).

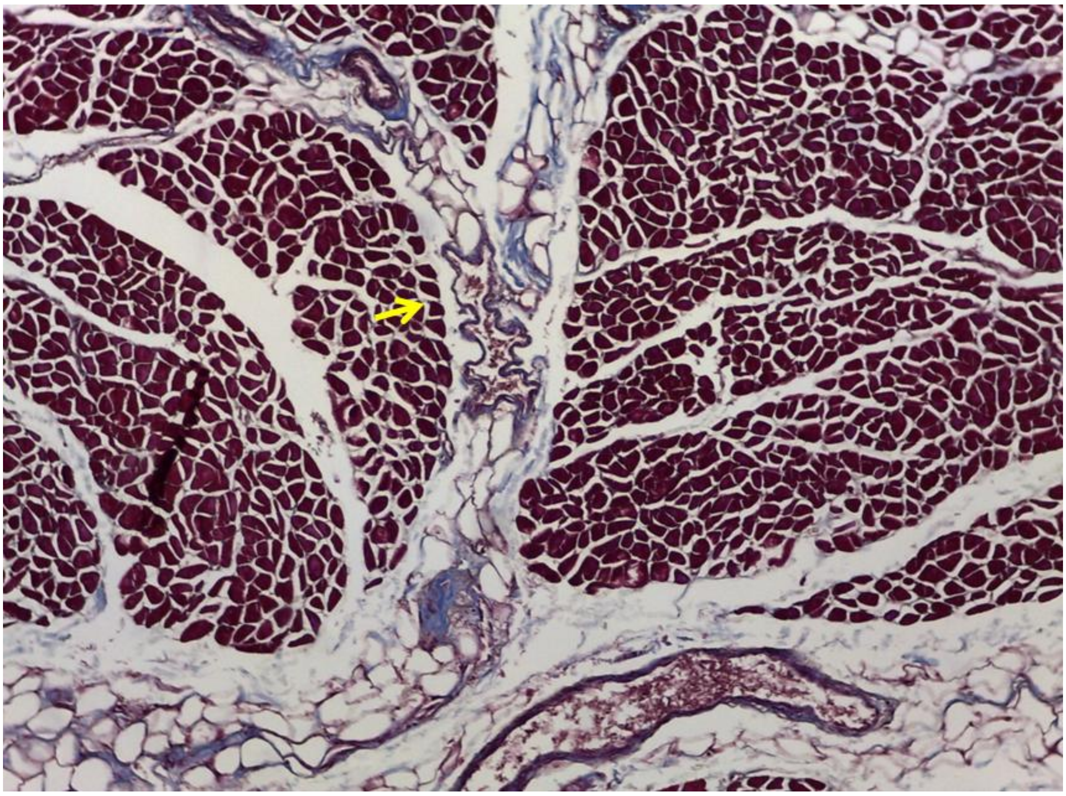
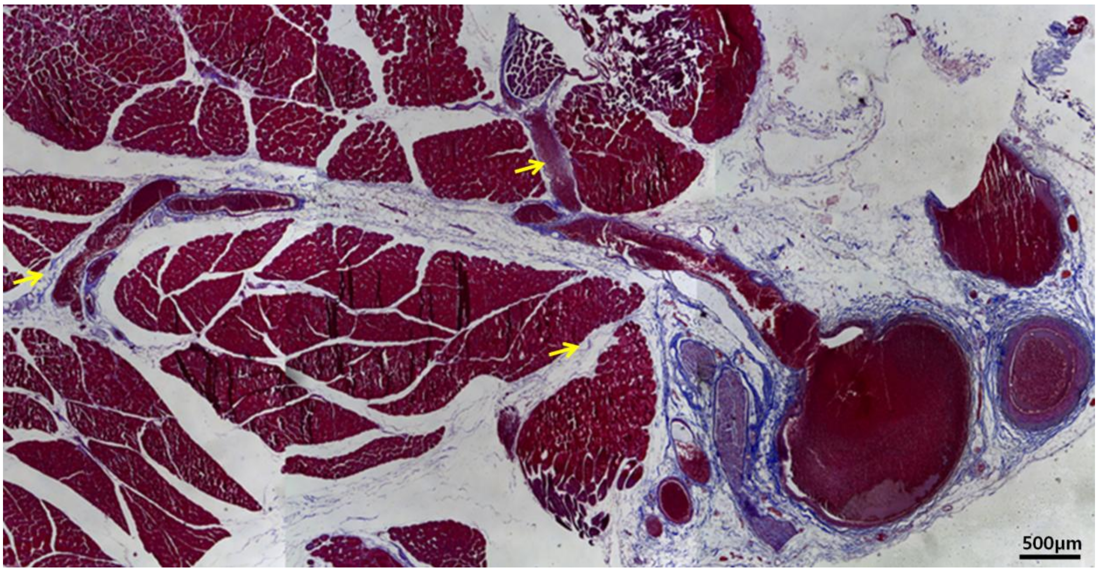
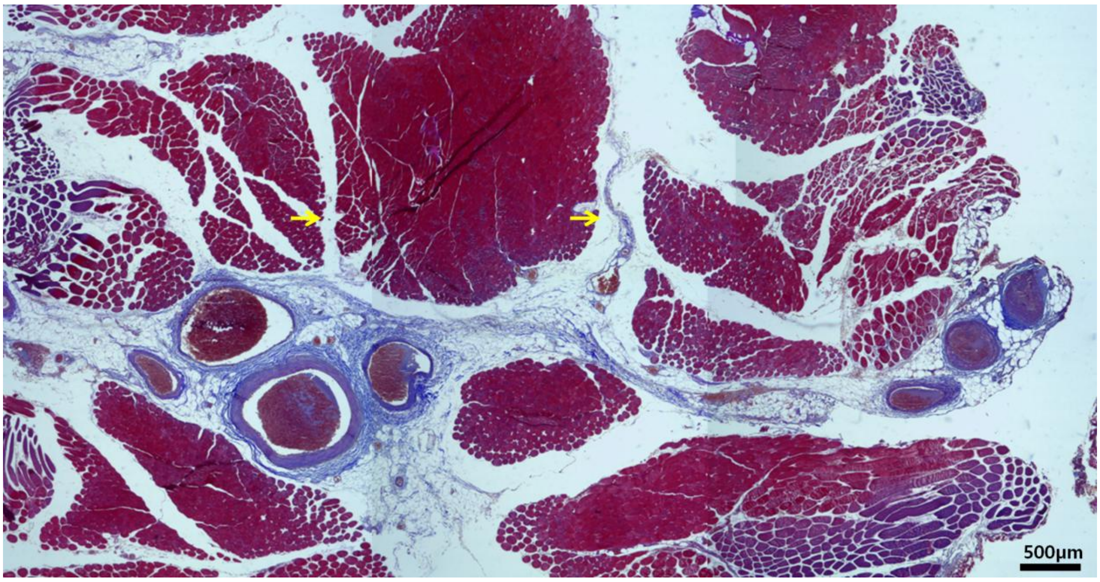


Fig. 3. Histologic examination of the human sample. Serial scans of ×40 magnified images were merged to generate a gross view. The network structure consists of main perimysium and its branches were visualized in each vessel type.

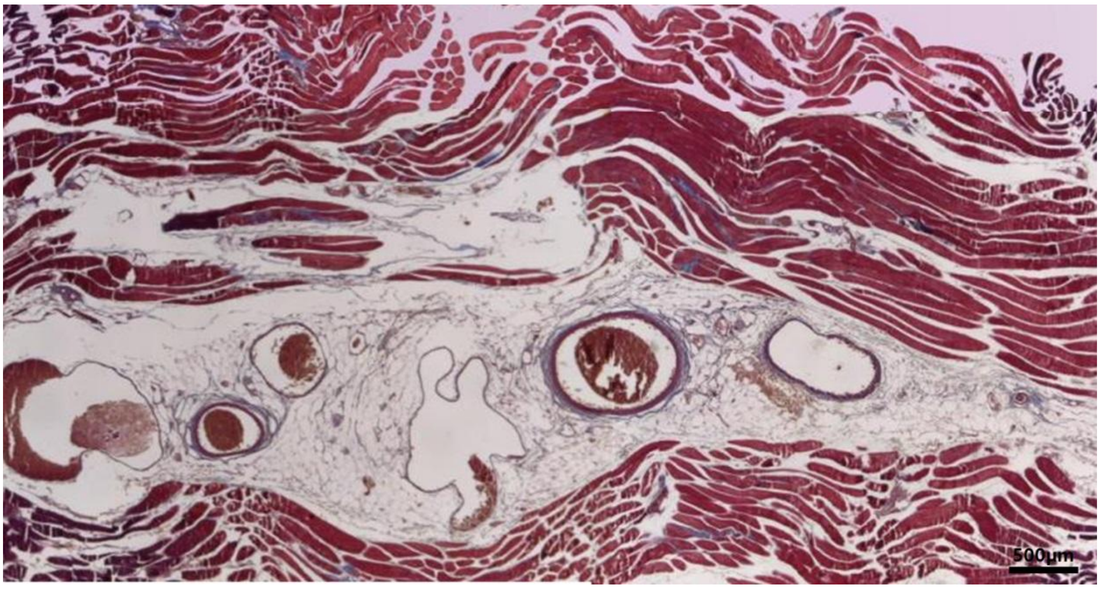
(A) Longitudinal type DIEA perforator vessels incorporated with the RA muscle.



(B) Oblique type DIEA perforator vessels incorporated with the RA muscle.



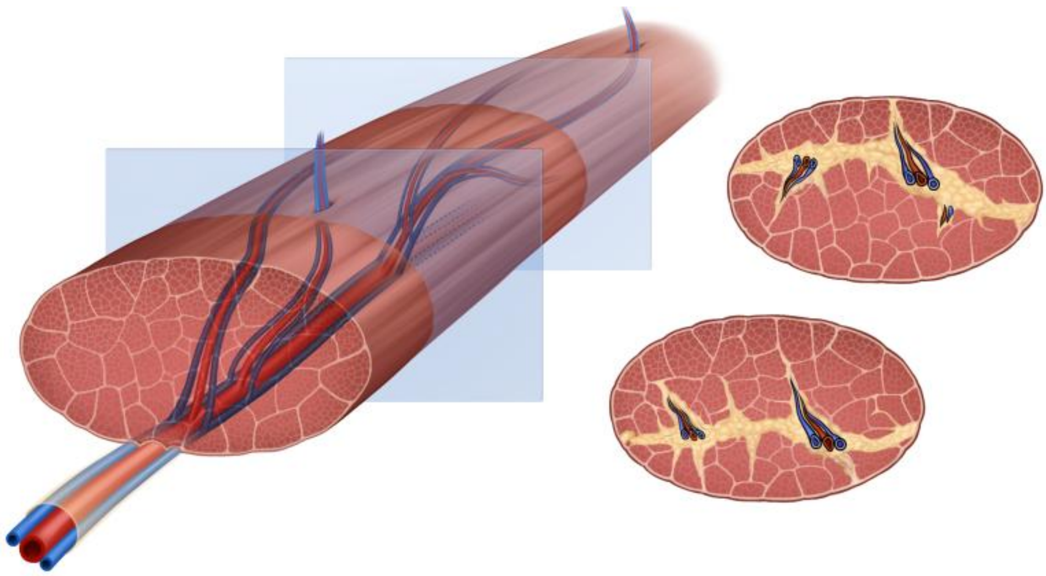
(C) Transverse type DIEA perforator vessels incorporated with the RA muscle.



The perimysium, referred to as the fibroadipose septum, was identified, which comprised a sheath of connective tissue grouping muscle fibers into bundles. The perimysium consisted of a relatively dense fibroadipose plane parallel to the muscle fiber, and its ramifying branches, spreading out into spaces between fascicles. These perimysial structures resemble an integrated crossroad, that is, the small perimysial branches stretch out from the main perimysium. This perimysial collagen network is organized in continuity throughout the arrangement of the muscle fiber.

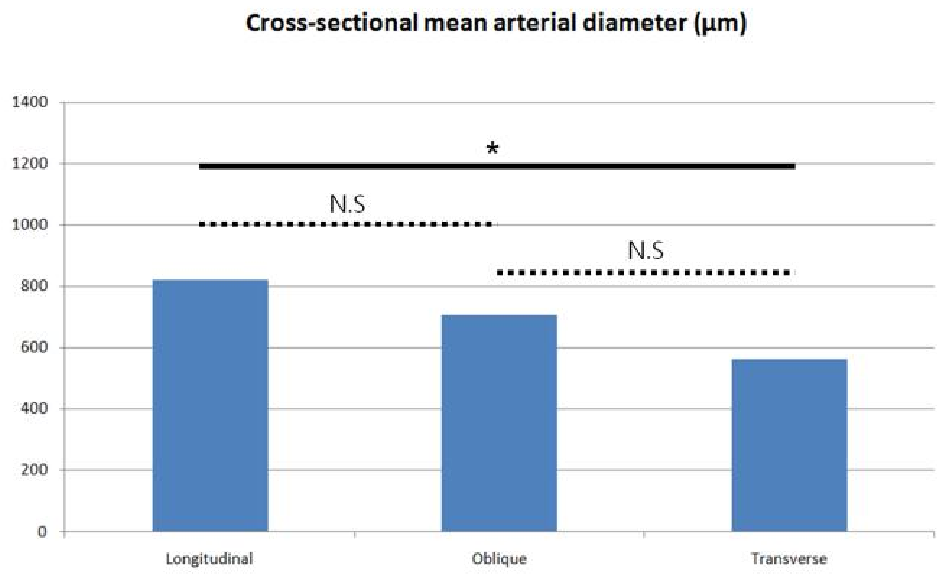
Between the RA muscle and DIEA perforator, three histologic features are observed. The neurovascular structures, whether major or minor, always exist in the perimysium network. Especially, the major DIEA perforators are included in the main perimysium and small neurovascular branches and lymphatic channels are present in the small perimysial branches. Second, there was no direct contact between the muscle fiber and perforator vessel. The neurovascular structures are enveloped within the fibroadipose tissue, which comprised the perimysial network. Third, the main perimysium was distributed parallel to the vascular axis formed by the major DIEA perforator vessel; that is, one artery and two venae comitans. It appeared as horizontal dense fibroadipose tissue, parallel to the pedicle vessel, in the transverse section of the RA muscle. This structure is depicted in Figure 4.

Fig. 4. Illustrated image of the network structure resembling the crossroad, as the major DIEA perforators are included in the main perimysium, and small neurovascular branches are present in the small perimysial branches.

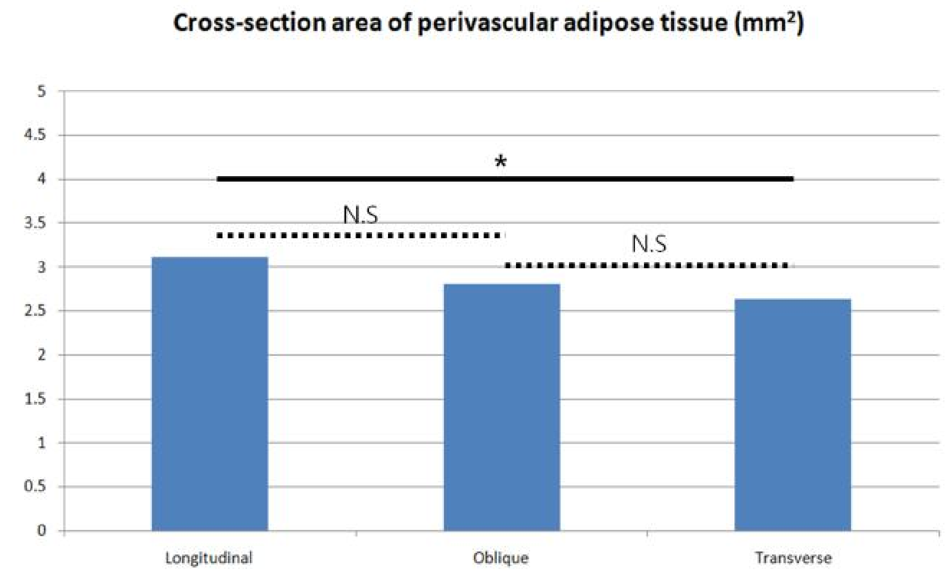


The histomorphologic analysis was performed in 15 human samples of DIEA perforator vessels incorporated with RA muscle, including five samples of each vessel type. The cross-sectional mean arterial diameter of DIEA perforator vessels was significantly higher in the longitudinal type (822 μm), compared with that of the transverse type (564 μm), even though these values were not significantly different compared with that of the oblique type (706 μm). The cross-sectional area of perivascular adipose tissue was large in the order of longitudinal, oblique, and transverse types (3.12, 2.81, and 2.64 mm^2). Statistically significant difference was noted between the cross-sectional area of the Longitudinal and transverse types. No correlation was noted between the cross-sectional area of perivascular adipose tissue and mean arterial diameter of DIEA perforators (Fig. 5).

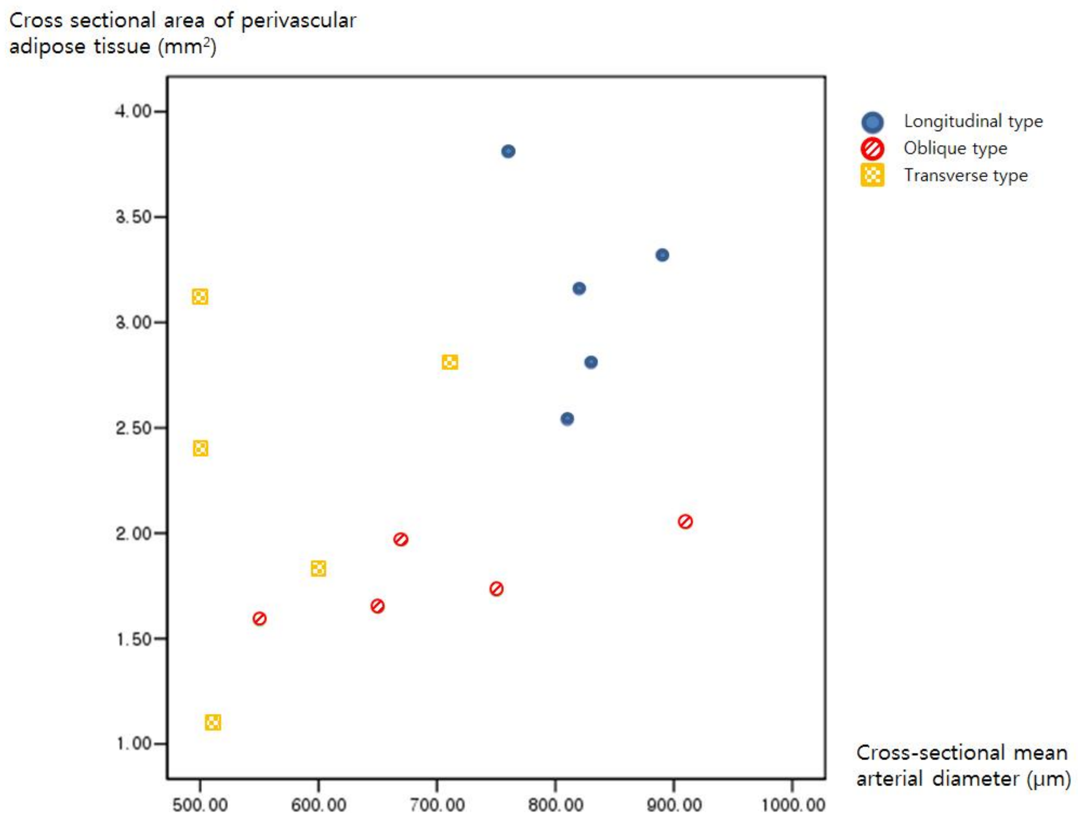
Fig. 5. Histomorphologic analysis among the types of DIEA perforator. (A) Cross-sectional mean arterial diameter of DIEA perforator vessels showed the highest value in the Longitudinal type and the lowest in the Transverse type.



(B) The cross-sectional area of perivascular adipose tissue showed the largest dimension in the longitudinal type and the smallest in the transverse type.



(C) No significant correlation was noted between the cross-sectional area of perivascular adipose tissue and mean arterial diameter of DIEA perforators.



DISCUSSION

With recent advancement in the understanding of DIEA perforator flaps, microvascular surgeons have focused on the intramuscular course of the DIEA perforator.[4,7,8] However, inadequate research is present on the intramuscular perforator of DIEA from the histologic perspective. When the DIEA perforator, which is incorporated within the RA muscle fiber, was harvested without pedicle isolation, the detailed structure at the dissection plane could be visualized in our study.

Previous histologic studies conducted on RA muscle have depicted various types of collagen fiber forming a network structure.[9,10] Collagen is an important component of muscles and fasciae that provides strength to the structures. Types I and III collagen are frequently found in the loose connective tissue comprising fibroadipose and vascular structures by forming a continuous network structure separating and supporting muscle fiber.[10] This network, referred to as perimysium, is a sheath of connective tissue that groups muscle fibers into bundles or fascicles. Our findings confirm the distribution of neurovascular structures in the perimysial fibroadipose networks, which is comprised of a main perimysium and small perimysial branches, resembling a crossroad. Especially, the major DIEA perforators are included in the main perimysium and small neurovascular branches and lymphatic channels are present in the small perimysial branches.

Our findings on the histologic relationship between the DIEA perforator and RA muscle can help in understanding the process of intramuscular dissection of the DIEA perforator from a microscopic perspective. The intramuscular perforators are initially dissected by “deroofing” or exposing the vessel, and splitting the muscle fibers above the perforator. In the meantime, small perimysial branches must be released, which necessitates coagulation or the use of hemoclips, not just sharp dissection. Then, the remaining attachment at the side of the pedicle, that is the main perimysium, should be released and this also necessitates careful ligation. Finally, the underside of the perforator is freed from the surrounding fibroadipose septum in a same perspective as in the deroofing process.

In fact, there is no direct contact between the muscle fiber and perforator vessel. The perforator vessel can be easily released from the muscle fiber by blunt dissection. What we actually dissect during the isolation of the pedicle, however, are the multiple perimysial septations between the muscle fiber, which comprise the main and small branches of perimysium. If any resistance is encountered during circumferential dissection around the perforator, the presence of fibroadipose septum should be considered and completely freed from the surrounding tissue by coagulation or clipping, not merely by blunt dissection. It is most remarkable at the side of the pedicle where a relatively dense attachment exists in the main perimysium.

There have been descriptions of the course of the DIEA perforator, which often depict a longitudinal, oblique, and transverse course as the perforator superiorly passes through the muscle.[4,7] In this study, findings of histomorphologic analysis revealed the histologic differences among these types of vessels. The longitudinal type perforator showed a larger arterial diameter compared to the transverse perforator. Similarly, an abundant quantity of perivascular adipose tissue was found in the longitudinal type perforator. Considering the intramuscular course of the DIEA perforator, transverse and oblique type perforators branches from longitudinal type perforator. This may affect the size and amount of the perivascular tissue depending on the perforator type, which result in smaller values in branched vessel. In order to take ease and reliability of the pedicle dissection, therefore, longitudinal component of DIEA perforator should be included as much as possible.

This study has several limitations. The sample size of each type of vessel was too small to represent the full set of characteristics of this group. The size and location of harvested vessels cannot be standardized owing to the unexpected course of the DIEA perforator. Although a larger vessel would be better for histologic examination, it may increase donor site morbidity and raise ethics issues. Limited visualization of cross-sectional area is another issue, which was confined to only 1 cm^3-sized tissue samples. Further studies performing serial section of whole RA muscle along its longitudinal axis in large-group cadaveric samples may clarify our hypothesis.

CONCLUSION

Our clinical experience demonstrated that the remaining attachment at the side of the pedicle during circumferential dissection around the intramuscular perforator may be explained by the presence of perimysial fibroadipose septum, which consists of a main and small branches of perimysial network, resembling a “crossroad.” Comprehensive understanding of the histologic relationship between the RA muscle and DIEA perforator may help surgeons to perform pedicle isolation from microscopic perspectives.

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국문요약

배경: 유방재건 분야에서 심하복벽동맥 천공지 피판이 널리 사용되면서 근육내 주행하는 천공지의 경로에 대한 해부학적인 연구가 많이 시행되었으나, 이에 대한 조직학적 연구는 시행된 바 없다. 본 연구는 근육내 주행하는 심하복벽동맥 천공지와 복직근 사이의 조직학적 관계에 대한 분석을 시행하였다.

환자 및 방법: 1 구의 사체 및 유방암 절제 후 유방재건술을 시행한 15 명의 환자의 반복벽 조직에서 심하복벽동맥 천공지를 포함하는 17 개의 복직근 조직을 채취하였다. 모든 검체는 Hematoxyline-eosin 및 Masson's trichrome 방법으로 염색을 시행하였고, Adobe Photoshop software 을 이용하여 40 배 확대 영상을 6-9 장 내외로 병합하여, 거시적인 관점에서 복직근과 심하복벽동맥 천공지 사이의 조직학적 구조를 관찰하였다. 인체로부터 채취한 15 건의 검체는 근육내 주행하는 천공지의 방향에 따라 각 5 개의 세로형, 사선형, 그리고 가로형으로 구성되었다. 분류된 천공지의 유형에 따라 심하복벽동맥 천공지의 평균 직경(D)과 혈관주위 지방조직의 면적(A)을 비교하였고, D 와 A 사이의 통계학적 상관관계가 있는지 검증하였다.

결과: 복직근은 섬유지방조직으로 구성된 격막구조(Fibroadipose septa)의 근주막(Perimysium)에 의해 근다발(fascicle)이 나뉘어지며, 근주막 내부에 천공지 혈관 및 신경, 임파조직을 포함하는 형태를 보였다. 근주막은 복직근의 배열과 평행하게 분포하는 주요 근주막(Main perimysium)과 여기서 분지되는 작은 가지 (Small perimysial branches) 구조로 이루어져 있었다. 주요 천공지들의 경우 주요 근주막

부위에 포함되어 복직근의 배열과 평행한 면에서 나란히 주행함을 1 구의 사체 조직 및 15 명의 인체조직에서 동일하게 관찰하였다. 심하복벽동맥 천공지의 평균 직경은 가로형에 비해 세로형에서 유의하게 큰 값을 보였고 (세로형, 822μm; 가로형, 564μm; $p<0.05$), 사선형과 다른 두 유형과의 비교에서는 통계학적인 유의성이 없었다. (사선형, 706μm) 혈관주위 지방조직의 면적은 가로형에 비해 세로형에서 유의하게 큰 값을 보였다 (세로형, 3.12mm^2; 가로형, 2.64mm^2; $p<0.05$). 사선형과 다른 두 유형과의 비교에서는 통계학적인 유의성이 없었다. (사선형, 2.81mm^2) 심하복벽동맥 천공지의 평균 직경과 혈관주의 지방조직의 면적은 통계적으로 유의한 상관관계를 보이지 않았다.

결론: 복직근의 근주막은 복직근의 배열과 평행하게 분포하는 주요 근주막 (Main perimysium) 및 여기서 분지되는 작은 가지 구조(Small perimysial branches)의 섬유지방 조직 격막의 형태를 보인다. 심하복벽동맥 천공지는 복직근의 근주막의 주요 근주막에 포함되어 주행하며, 이로 인해 천공지의 박리시에 주요 골격 부위의 양쪽을 분리하는 것 뿐만 아니라, 근주막의 작은 가지 부위를 분리하는 과정을 요한다. 본 연구를 통해 심하복벽동맥 천공지의 주행 방향에 따른 조직학적 특성의 차이를 이해함으로써 근육내 천공지의 박리시에 미시적인 안목을 얻을 수 있다.

중심 단어: Deep inferior epigastric artery perforator, Histology, Rectus Abdominis muscle