

# The Superficial Inferior Epigastric Artery Fascia Flap in Rats

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## Abstract

**Background** An adipofascial flap in the rat may provide new options for adding vascularization to scarred or nonvascularized beds for a variety of research studies. Current literature lacks sufficient description for a simple reproducible flap model for a vascularized pedicled flap in rats, in particular for neovascularization of allograft nerves for the reconstruction of sciatic nerve defects. The purpose of this study was to describe a surgical technique and determine long-term survivability for the pedicled superficial inferior epigastric artery fascial (SIEF) flap in the rat to meet requirement for a tunneled adipofascial flap to add vascularization to the sciatic nerve area.

**Methods** The technique and use of a 4 × 3-cm SIEF flap are described. Twenty Lewis rats underwent the technique to determine feasibility. The flap was wrapped around processed allograft nerve reconstructions and viability of the flap was evaluated after 12 and 16 weeks. To visualize vessels, nerve grafts were harvested at 12 weeks and stained with hematoxylin-eosin and an antibody against microvessels (CD-34).

**Results** All flaps remained viable after survival of 12 and 16 weeks. Complications included one hematoma formation and two lymphocele formations that did not have any impact on the flap. Immunohistochemistry confirmed an increase in microvessels and Schwann cell nuclei in the SIEF group compared with nerve samples from the unoperated, contralateral side.

**Conclusion** A pedicled adipofascial flap model in the rat to provide a vascular bed for sciatic nerve reconstruction is detailed with long-term survivability evaluation of the flap. This flap is technically simple to be harvested and is suitable for revascularization procedures of various tissues in the lower abdomen, genital area, thigh, or upper limb of the rat.

## Keywords

- ▶ sciatic nerve vascularization
- ▶ vascularized bed
- ▶ pedicled flap
- ▶ animal models

It has been postulated that the application of either vascularized nerve grafts or a vascularized flap wrapped around the nerve graft will improve outcomes of nerve grafts in severely scarred tissue beds.<sup>1–6</sup> A well-vascularized bed becomes more critical as length and caliber of a nerve graft increase.<sup>6</sup> The literature regarding pedicled fascial flaps in animals is scarce. The free superficial inferior epigastric artery (SIEA) flap in rats was first described in 1967 by Strauch and Murray.<sup>7</sup> This free tissue transfer could be designed in various sizes and has been

applied in different investigational and training models (e.g., distal flap necrosis,<sup>8–12</sup> ischemia reperfusion injury,<sup>13–16</sup> and microvascular training<sup>17</sup>). In the rat, no previously described facial flaps have been described or used to wrap around the sciatic nerve.<sup>18,19</sup> One study had been performed elevating a SIEA flap and evaluated for short-term viability after 7 days.<sup>19</sup> While this pedicled flap was demonstrated to be safe and avoided the potential risk of free flap failure (microvascular anastomosis complications), long-term viability was never

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assessed.<sup>19,20</sup> Another study described pedicled flaps to vascularize nerve grafts in an intratemporal facial nerve defect model and focused on histological outcomes.<sup>18</sup> The sciatic nerve defect model is a well-established model to investigate multiple outcomes varying from functional motor outcomes to histology.<sup>21–23</sup> A pedicled flap model in rats to provide vascularization to the nerve wound bed has not been described and requires validation prior to measurement of other outcomes. Therefore, the purpose of this study was to describe a reproducible surgical technique and determine long-term survivability for the pedicled superficial inferior epigastric artery fascial (SIEF) flap in the rat to meet requirement for a tunneled adipofascial flap to add vascularization to the sciatic nerve bed.

## Methods

### Animals

The study was approved by our Institutional Animal Care and Use Committee (IACUC A3348–18). Twenty male adult Lewis rats, weighing 250 to 300 g (Envigo; Madison, WI) had a unilateral 10-mm sciatic nerve gap repaired with an optimized processed allograft (OPA).<sup>24</sup> Ten Sprague–Dawley rats (Envigo; Madison, WI) weighing 250 to 300 g served as major histocompatibility mismatch donors.<sup>25,26</sup> A 15-mm segment of the sciatic nerve was harvested bilaterally. The nerves were cleaned from external debris and decellularized using a 5-day previously described decellularization protocol.<sup>24</sup> The nerves were sterilized using  $\gamma$ -irradiation and stored in a sodium phosphate buffer (PBS) at 4°C until surgery. During the survival period, the Lewis rats were housed individually with a 12-hour light-dark cycle and ad libitum access to food and water. Flap viability was evaluated after 12 ( $n = 10$ ) and 16 weeks ( $n = 10$ ).

### Anesthesia

Rats were anesthetized in an isoflurane chamber, shaved, prepped, and positioned in the nosecone to maintain anesthesia throughout the procedure. Body temperature was maintained at 37°C with a heating pad. Preoperatively, the following were administered subcutaneously; 5 mL of NaCl 0.9% to prevent dehydration, buprenorphine SR (Buprenorphine SR-LABORATORY, ZooPharm pharmacy, 0.6 mg/kg) for pain control and enrofloxacin (Baytril; Bayer, Germany, 10 mg/kg) providing infection prophylaxis. Postoperatively, the rats were kept warm in towels. Rats were observed daily until completion of the experiment. During sacrifice, rats were euthanized with 1 mL intraperitoneal injection of pentobarbital sodium (Fatal Plus; 390 mg/mL, Vortech, Dearborn, MI).

### Surgical Procedure

The SIEF flap is a pedicled flap supplied by superficial inferior epigastric (SIE) vessels including both the arteries and the accompanying veins. These vessels arise from femoral vessels close to branches of the popliteal and saphenous vessels, and are direct branches of the cutaneous arteries and veins. The main trunk of the SIE vessels divides into two branches. The main, lateral trunk branches from the femoral vessels in the

groin and enters the abdominal wall skin. The smaller medial branch extends toward the medial abdominal skin to collateralize with a branch in the internal mammary vessel<sup>27</sup> (**►Fig. 1**).

The SIEF flap measured 4 × 3 cm and was designed in the ventral abdomen. After a 4-cm paramedian incision, on the ipsilateral side of the nerve reconstruction, the femoral artery was identified in the groin (**►Fig. 2**).

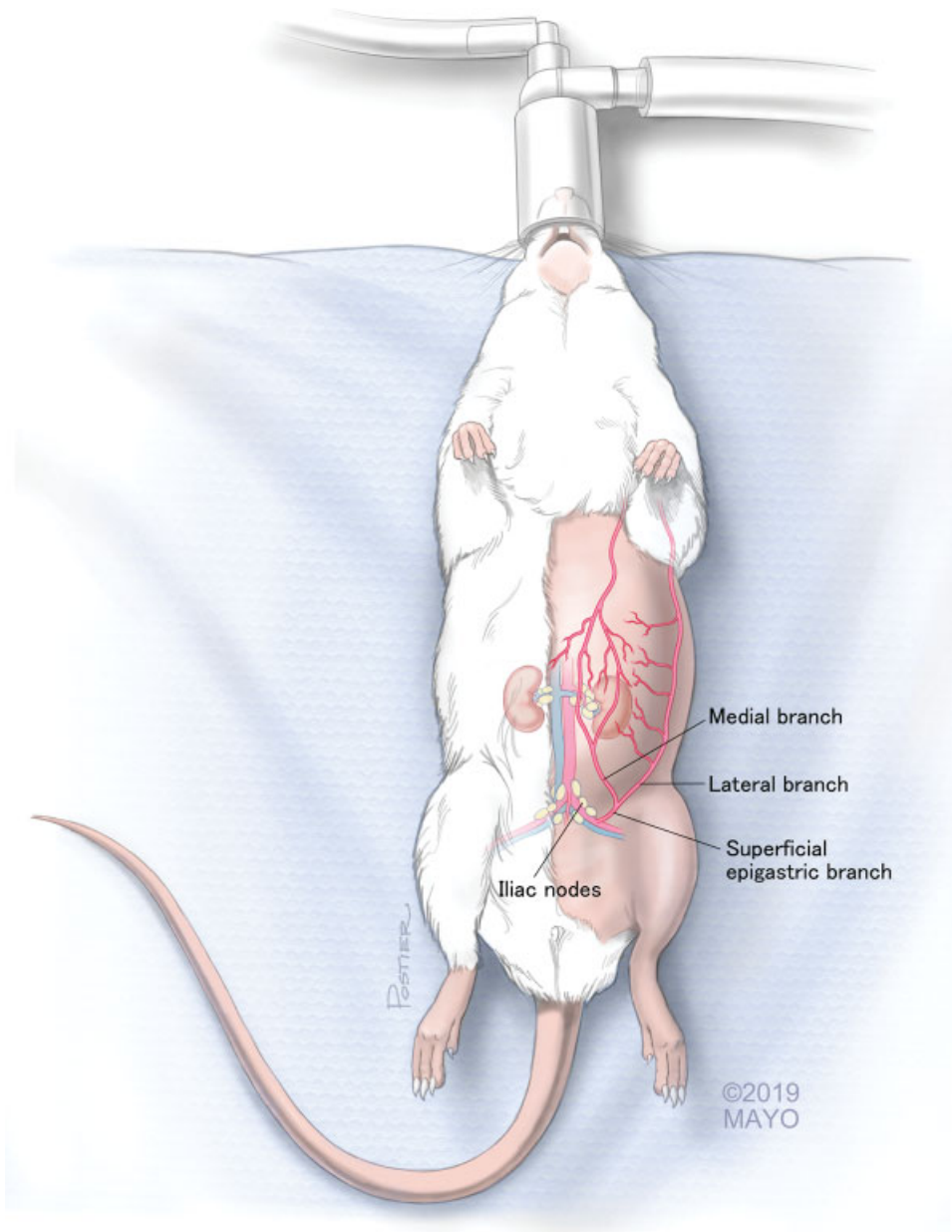
The SIE lateral vessels were exposed proximally and protected with a vessel loop. The flap was dissected distally, starting on the medial side. The superficial- and deep membranous layers of subcutaneous tissue (Camper fascia and Scarpa fascia) were separated from the abdominal muscles, leaving the fascia intact. Using microsurgical scissors, the flap was raised toward the proximal branch of the SIE vessels (**►Fig. 3**).

This dissection was performed under surgical loupe magnification to avoid damage to the SIE vessels. While dissecting, lymph nodes in the inguinal area were seen and preserved. Small vessel branches were anticoagulated using a bipolar as needed to prevent postoperative hematomas. The epigastric nerve was consistently encountered running in conjunction with the SIE vessels and transected in all cases. The flap was raised to the level of the bifurcation of the femoral artery and then kept moist in gauze until the nerve reconstruction was finished.

The sciatic nerve was fully exposed proximally from the inferior margin of the piriformis muscle to approximately 5 mm distal to the bifurcation under an operating microscope (Zeiss OpMi 6, Carl Zeiss Surgica, Oberkochen, Germany). A 10-mm segment of the sciatic nerve was excised by sharp transection with microsurgical scissors and bridged with a 10-mm OPA with six 10–0 nylon (10–0 Ethilon; Ethicon Inc., Sommerville, NJ), epineural interrupted sutures on either side of anastomosis.

A 2-cm linear incision was made from the patella toward the pelvis to develop a wide subcutaneous tunnel from the distal aspect of the nerve reconstruction toward the femoral artery. A hemostat was passed through the distal incision into the inguinal region, and the flap was delivered through the tunnel, with approximate 100 degrees rotation clockwise about its original axis (**►Fig. 4**). Caution was taken to prevent vascular twisting. The 4 × 3-cm SIEF flap contained subcutaneous fat, inguinal fat, femoral vasculature and SIE vessels, and was tunneled subcutaneously toward the nerve reconstruction without torsion of the pedicle (**►Fig. 5A**).

The flap was wrapped around the nerve allograft with the SIE vessels in line with the nerve and reaching both the proximal and distal anastomoses, as shown in **►Fig. 5B**. The flap edges were trimmed to fit the defect (**►Fig. 5C**). Care was taken to ensure that there was no tension on the nerve anastomoses while positioning the flap under the reconstructed nerve. After ensuring that there was no pedicle compression in the subcutaneous tunnel, the vascular pedicle remained freely mobile with full ranging of the leg and that the nerve anastomoses were without tension, two loosely tied 10–0 nylon sutures (10–0 Ethilon, Ethicon Inc.,



**Fig. 1** Schematic drawing of the rat superficial vascular abdominal anatomy. The superficial epigastric artery originates from the femoral artery and divides into two branches to supply the abdominal fascia: the lateral and the medial branches. Close to the bifurcation lie the iliac nodes. (Reproduced with permission of Mayo Foundation for Medical Education and Research. All rights reserved).

Sommerville, NJ) were placed through the SIEF flap (► **Fig. 5D**). Wounds were closed in layers, with muscle approximated with 5-0 absorbable sutures (5-0 Vicryl Rapide; Ethicon Inc., Sommerville, NJ), and the skin of the leg and the abdomen was closed subcutaneously using the same suture.

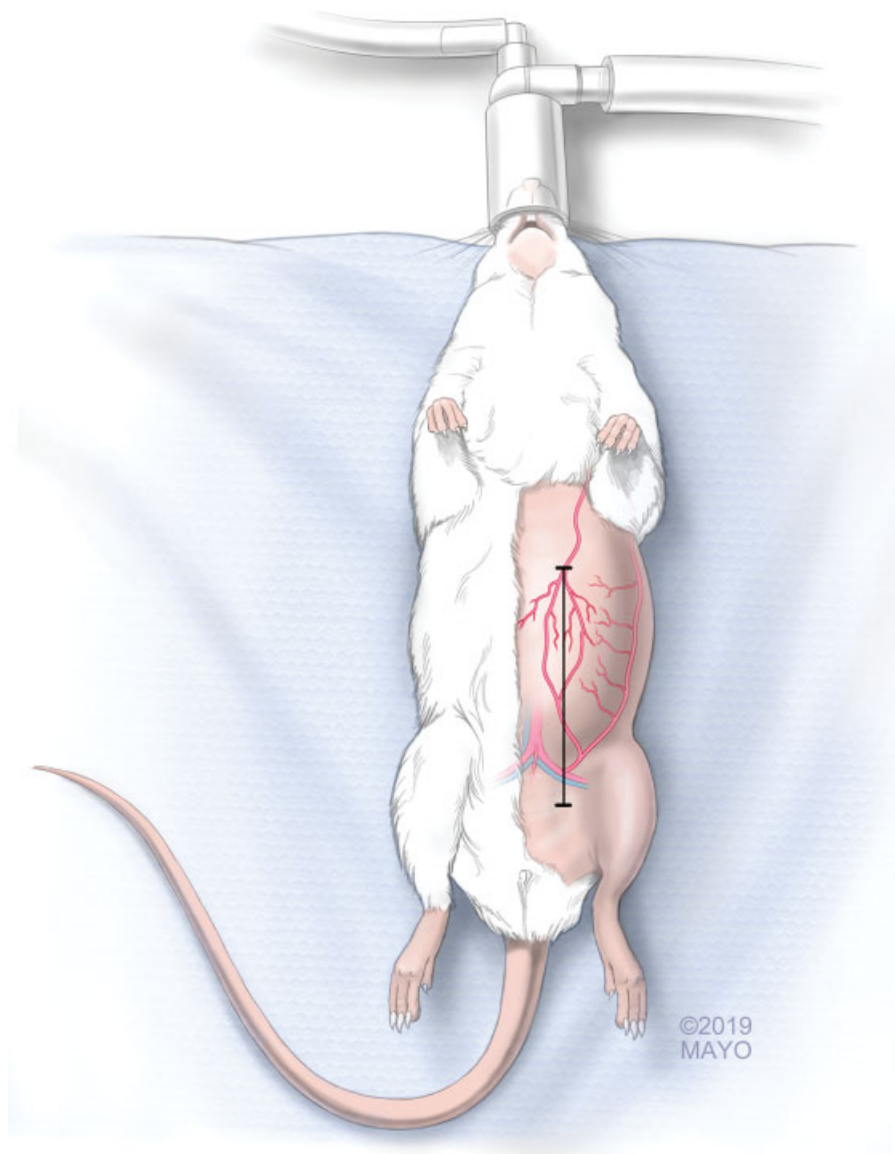
#### Evaluation SIEF Flap

The viability of the SIEF flap was evaluated at sacrifice by 12 and 16 weeks. This was performed using the milking patency test.<sup>28</sup> The SIEA was found and the vessel was occluded with forceps distal to the flap. The other forceps was placed just distally to the first. The vessel would be milked a few millimeters away from the flap. Thereafter, the proximal forceps would be released. Rapid filling from proximal to

distal would indicate that the artery was not occluded (1); if no filling occurred, the test would be scored a (0). Viability of the flap was also characterized by color of the flap and active bleeding at the edges of the flap.

#### Immunohistochemistry

At 12 weeks, immunohistochemical staining of the nerves was obtained to confirm revascularization of the nerve. After sacrifice, the nerve grafts were harvested and fixed in 10% formalin (Fisher Scientific, NH) for 48 hours, transferred to 70% ethanol, and stored at 4°C. After embedding in paraffin, serial sections (5 µm) were obtained from distal parts of the nerve grafts. These sections were stained with hematoxylin and eosin (H&E) and primary antibody rabbit anti-rat CD34 (1:4000; Abcam, Cambridge, MA) to visualize



**Fig. 2** Schematic drawing of the 4 cm paramedian incision. This incision allows for harvesting the flap without damaging the SIE vessels or other vessels supplying the abdominal skin. (Reproduced with permission of Mayo Foundation for Medical Education and Research. All rights reserved). SIE, superficial inferior epigastric.

vascularization and fibrosis in the nerve graft. CD34 is a transmembrane phosphoglycoprotein and established as a marker of hematopoietic cell types, including vascular endothelial progenitors and extensively expressed on blood vessels.<sup>29</sup> Contralateral nerve samples were stained as control. Immunohistochemical digital photographs were taken at  $\times 40$  magnification with a microscope (Nikon Eclipse 50i) equipped with a digital camera. Images were qualitatively assessed.

## Results

### Evaluation SIEF Flap

Successful flap transfer was accomplished in all rats. After 12 and 16 weeks, rats were sacrificed and flap viability was investigated. The patency of the artery was checked with the milking patency test at sacrifice and all arteries were patent

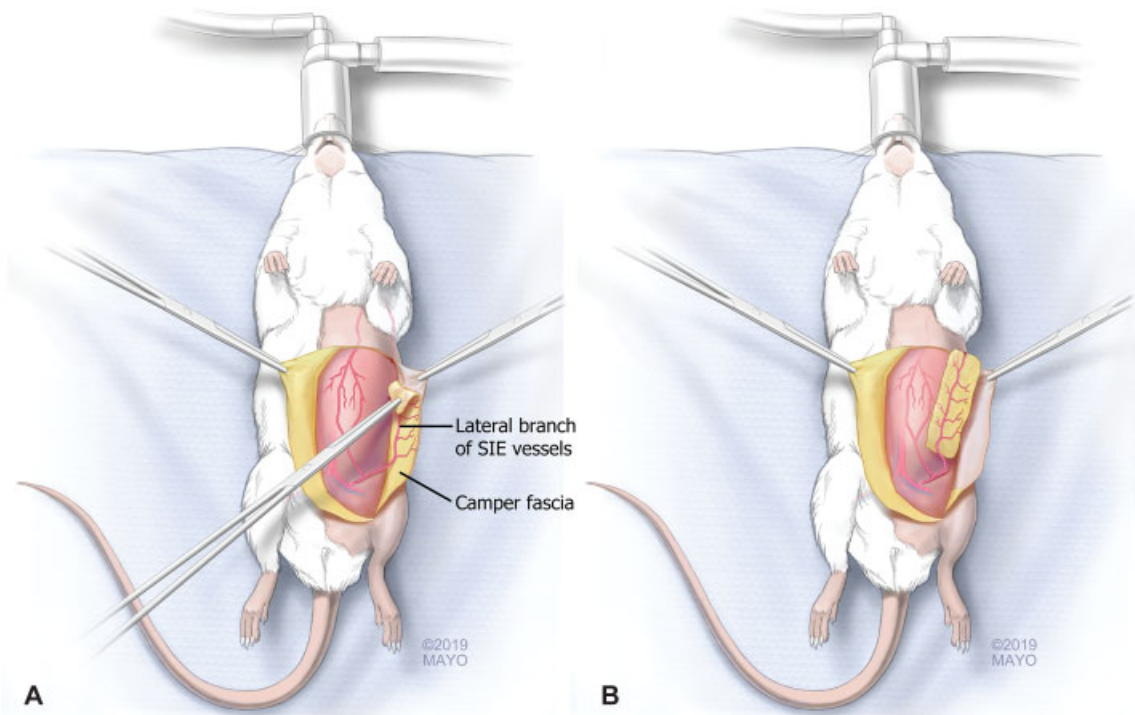
after 12 and 16 weeks of survival. The flap was well vascularized and demonstrated active bleeding at its margins. The vessels were in line with the nerve graft (**Fig. 6**).

No flap necrosis occurred and no infections were seen. Of the 12-week survival rats, lymphedema occurred in two which did not infect or complicate recovery and resolved by itself. In one rat, a small subcutaneous hematoma was seen on the abdominal side at 4 weeks, which did not increase size during follow-up and did not have any impact on the SIEF flap. The 16-week survival rats did not show any complications. The SIEF flap had an acceptable and aesthetic donor site scar without any observable loss of function.

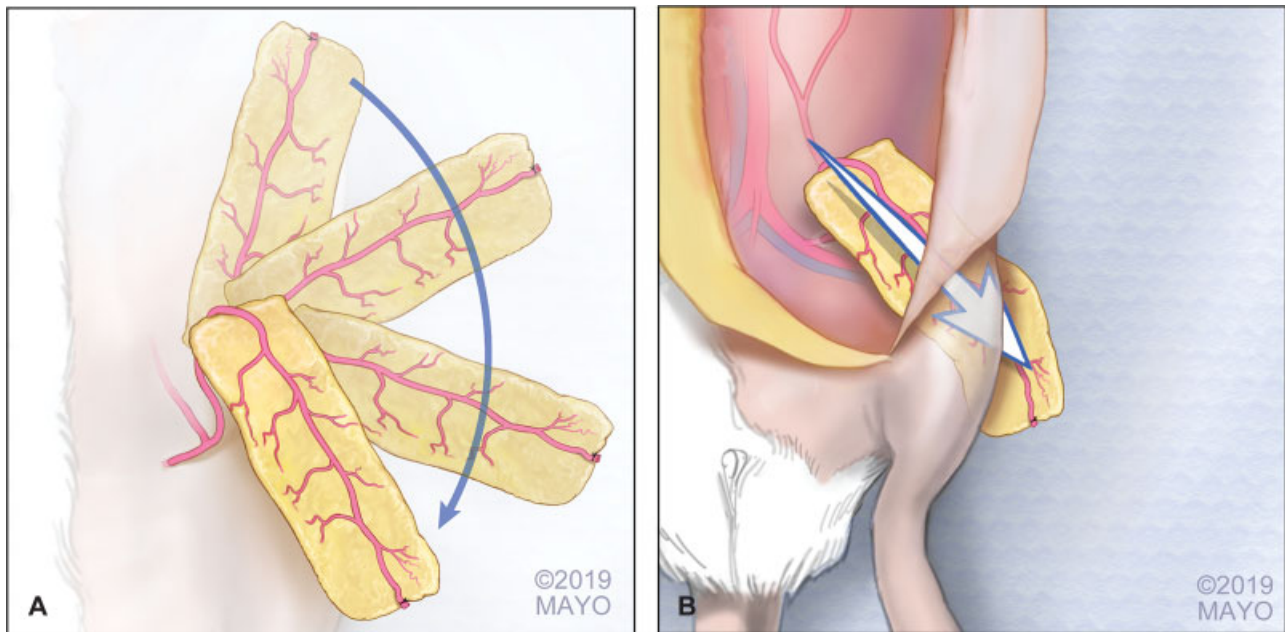
### Immunohistochemistry

To assess the effect of the SIEF flap on nerve allograft samples, nerve samples were stained for H&E and anti-CD34. Results of H&E staining in cross-sections of the nerve





**Fig. 3** Schematic drawing of SIEF flap harvest. Depicted is elevating the flap from distal to proximal (A), providing a 4 × 3-cm adipofascial flap (B) with lateral branch of the superficial inferior vessels. (Reproduced with permission of Mayo Foundation for Medical Education and Research. All rights reserved). SIEF, superficial inferior epigastric fascia.

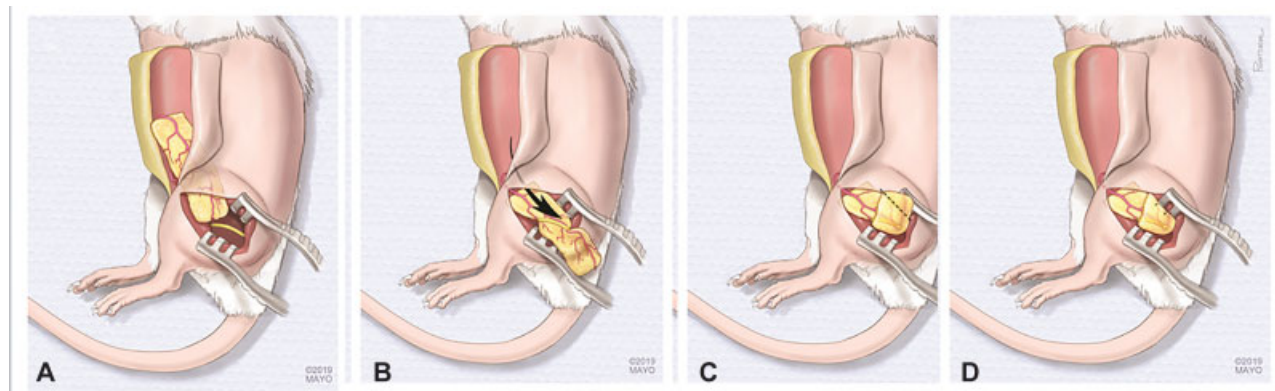


**Fig. 4** Schematic drawing of flap rotation (A) and tunneling (B). The vessels were kept ventrally and the flap was tunneled without vascular twisting of the epigastric trunk. (Reproduced with permission of Mayo Foundation for Medical Education and Research. All rights reserved).

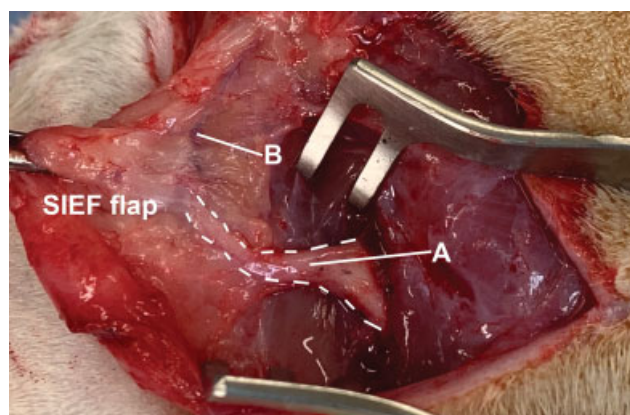
showed no infiltration of inflammatory cells in either group at 12 weeks. As can be concluded from the H&E staining, no fibrosis was seen. In the SIEF group, an increase in vessels and Schwann cell nuclei was evident in the H&E stained samples. This increase in vessels was consistent with the notable increase of CD34-positive microvessels in nerve grafts that were surgically revascularized at 12 weeks compared with unoperated nerve samples (► Fig. 7).

## Discussion

The surgical technique for the rat SIEF flap harvest was described and evaluated. While this flap has been reported previously in a few animal studies,<sup>17,19,20</sup> long-term viability has not been adequately assessed or determined. Additionally, a succinct and detailed description of this technique in rats has not been illustrated or reported.



**Fig. 5** Schematic drawings of SIEF flap positioning. The SIEF flap was tunneled subcutaneously toward the nerve reconstruction (A) without torsion of the pedicle and wrapped around the nerve graft reaching both anastomoses (B). The flap edges were trimmed (C) and two 10–0 nylon sutures were placed to secure the position of the SIEF flap (D). (Reproduced with permission of Mayo Foundation for Medical Education and Research. All rights reserved). SIEF, superficial inferior epigastric.



**Fig. 6** Relation SIE vessels and nerve after 16 weeks. The white dashed line surrounds the nerve allograft (A). Depicted is the close relationship between the nerve allograft (A) and the SIE vessels (B) after 16 weeks of survival. Also, flap viability could clearly be seen. SIE, superficial inferior epigastric.

The main findings of this study were that the SIEF flap is an easy flap to raise and remains viable in all rats after either a 12 or 16-week survival period. Complications included two lymphoceles and one hematoma acutely, but no long-term consequences at 12 and 16 weeks.

The flap demonstrated to have 100% success rates after elevation without flap failure or necrosis at the donor site. One major advantage is that the SIE vessels of the rat are approximately 0.5 mm in diameter,<sup>30</sup> which categorizes it as relatively large and makes them to be easily dissected under loupe magnification. By only including the lateral branch, necrosis at the donor site is prevented. This flap design can be applied to other rat strains as well as rats of different sizes as the anatomical branches are easily recognized. The pedicled flap eliminates the need for microvascular anastomosis and minimizes flap failures secondary to surgical techniques. This pedicled flap allows for a technically simple elevation without intramuscular dissection and a relatively short operation time. An additional benefit is that the transplanted adipofascial tissue can improve blood flow in adjacent tissues such as bone, nerve, and muscles and be a readily

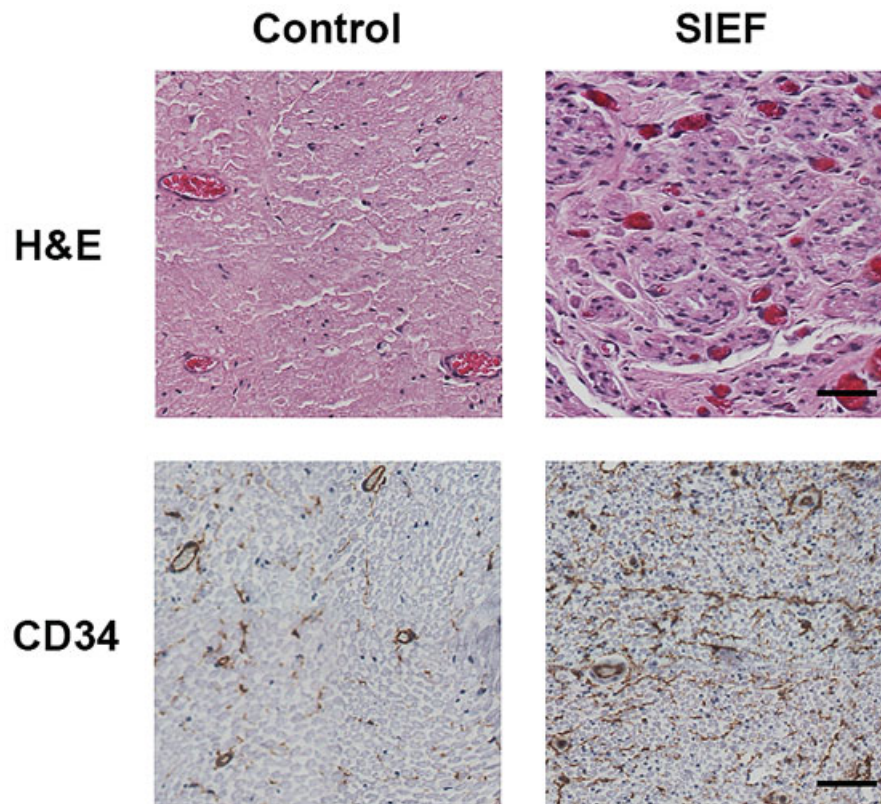
applied pedicle flap for studies on vascularization.<sup>20,31</sup> The inclusion of adipofascial tissue in the flap decreases intra-vascular resistance of the bundle, resulting in improved blood flow in the flap which decreases the risk of thrombosis.<sup>20,31</sup>

Immunohistochemical qualitative analysis of the nerve samples confirms the increase in vascularity in the SIEF group (vascularized flap wrapped around the processed nerve allograft) in both H&E and anti CD-34 sections at 12 weeks. Vasculature is known to play a crucial role in supporting nerve regeneration following injury.<sup>32</sup> A lack of blood supply could lead to nerve hypoxia and damage, leading to nerve fibrosis.<sup>33,34</sup> At time of a nerve injury, Wallerian degeneration is activated causing Schwann cell proliferation distal to the nerve injury.<sup>35,36</sup> This process causes blood vessels to precede Schwann cell migration and to stimulate axonal extension, describing an important interaction between Schwann cells and blood vessels.<sup>34,37</sup> Schwann cells are known to produce neurotrophic factors to support neurite outgrowth.<sup>38</sup> After nerve injury, these cells and their secreted neuro-supportive factors enhance axonal growth.<sup>39</sup> Although comparison of nerve allograft in a well-vascularized bed to the contralateral unoperated nerve is not an adequate comparison of nerve tissue, the increase of vessels and Schwann cell nuclei in the nerve allografts confirm the relationship between blood supply and nerve regeneration and suggest that addition of a well-vascularized bed to the nerve area may enhance nerve regeneration. This study describes a pedicled adipofascial flap model in rats and validates its use in future investigations in the rat sciatic nerve model.

## Conclusion

This study demonstrated a total success rate of SIEF flap viability without necrosis at 12 and 16 weeks, providing evidence that this flap is durable and can be used for future studies in a rat model. We recommend this simple technique to add vascularization to various tissues in the lower abdomen, genital area, thigh, and upper limb of the rat.





**Fig. 7** Immunohistochemical digital photographs of nerve samples. Representative images of H&E staining in control (contralateral unoperated nerve) and SIEF (vascularized flap wrapped around the processed nerve allograft) samples at 12 weeks. Purple nuclei denote the Schwann cell nuclei. Digital photographs were taken at  $\times 40$  magnification. Scale bars, 200  $\mu\text{m}$ . H&E, hematoxylin and eosin; SIEF, superficial inferior epigastric fascia.

**Note**

The study was performed at Mayo Clinic, Rochester, United States. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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**Conflict of Interest**

A.Y.S. reports grants from National Institutes of Health during the conduct of the study; all the other authors report no conflict of interest.

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Artwork of **►Figs. 1 to 5** was created by Jim Postier, Mayo Clinic, Rochester, MN, United States.

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