A Novel Model of Perifascial Areolar Tissue Transplant in Rats

Junya Oshima[1]   Kaoru Sasaki[1]   Yoichiro Shibuya[1]   Mitsuru Sekido[1]

[1] Department of Plastic, Reconstructive, and Hand Surgery, University of Tsukuba, Tsukuba, Japan

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Address for correspondence Junya Oshima, MD, Department of Plastic, Reconstructive, and Hand Surgery, University of Tsukuba, 1-1-1Tennodai, Tsukuba, Ibaraki 305-8577, Japan (e-mail: ooshima-tuk@umin.ac.jp).

Abstract

Background Perifascial areolar tissue (PAT) transplant is a method of transplanting loose connective tissue harvested in a sheet form from above the fascia to the wound bed and is effective for wounds with exposed ischemic tissue. However, the engraftment mechanism is unknown, and no animal models of PAT transplant for wound healing exist.

Methods   In this study, we harvested connective tissue from the backs of Wistar rats in a sheet form to simulate a human PAT transplant. The PAT was affixed to exposed bone of the head.

Results   In the PAT(+) group, the wound areas gradually decreased due to epithelialization and contraction. The wound area of the PAT(+) group was significantly smaller than that of the PAT(−) group.

Conclusions   This clinically relevant rat model is useful for elucidating the mechanism of the PAT transplant and establishing a reliable surgical method.

Keywords
► perifascial areolar tissue
► loose connective tissue
► model
► grafts
► bone exposure

Introduction

Treatment of wounds involving exposed ischemic tissue (bones and tendons) or artificial materials is difficult. Even if the ischemic tissue will be covered with granulation for secondary healing, this prolonged treatment is problematic in terms of infection risk and treatment cost. Skin grafts do not take directly on ischemic tissue. Although flap surgery is often performed in actuality, drawbacks such as surgical invasion and related costs, difficulty of the procedures, and donor complications persist.

The perifascial areolar tissue (PAT) transplant, as reported by Kouraba, relocates loose connective tissue harvested in a sheet form from above the fascia to the wound bed.[1] Wound bed preparation can, thus, be achieved early, and the graft harvesting is minimally invasive, technically simple, and carries a short operation time. Other advantages of PAT are abundant tissue volume and self-organization that reduces the cost of artificial materials. Although several clinical reports exist on this technique, basic research on wound treatments and their mechanisms and appropriate surgical indications are unknown. The purpose of this study, therefore, was to create a PAT transplant model that would be useful to elucidate PAT survival mechanisms, indications, and expansion of therapeutic indications.

Materials and Methods

To establish the model, six female Wistar rats (CLEA Japan, Tokyo, Japan), aged 11 weeks and weighing between 180 and 200 g, were used. The rats were housed under specific pathogen-free conditions with one rat per cage under a 12-hour light/dark cycle with ad libitum access to food and water. All the procedures were performed in accordance with
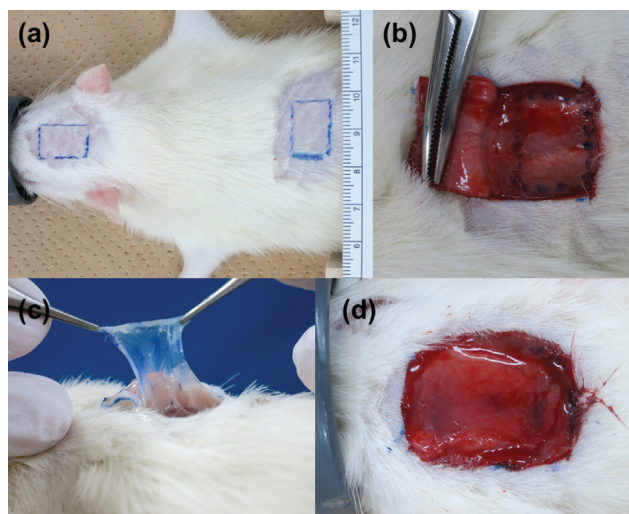
Fig. 1 (a) Marking of the PAT harvesting area and bone-exposure creation area. (b) The back was incised in a U shape and the PAT to be harvested, marked. (c) The harvested PAT. (d) The PAT was placed on the exposed bone.

Fig. 2 Comparison of the wound areas of the two groups.

the guidelines of our institutional animal care and use committee (approval number 2019-401). For surgery, general anesthesia was administered via inhalation of 4% isoflurane through a rodent ventilator (TK-5, Biomachinery, Chiba, Japan) in an induction box.

Anesthesia was maintained via inhalation of isoflurane vaporized in fresh 96 to 100% air at 500 mL/min through a handmade facemask. The isoflurane gas concentration was adjusted to 2 to 4% with visual respiratory monitoring, including signs of chest and abdominal motions. The wound bed to which the PAT was transplanted used a previously reported bone exposure model of the head.[2] A 10×15-mm rectangle was marked in the space behind the orbit and between the ears (►Fig. 1a) before square soft tissue defects with exposed bone were created by removal of a 10×15-mm slice of cutaneous tissue and cranial periosteum.

The backs of the rats received a 15×10-mm square, U-shaped incision (►Fig. 1a). Cutaneous flaps were raised between the loose connective tissues, including the panniculus carnosus muscle. Within the wound, thin connective tissue was visible in the shallow layer of the thoracolumbar fascia (►Fig. 1b). This was harvested in a sheet form and used as the PAT (►Fig. 1c). A size of 15×10 mm was chosen to unify the tension when transplanting and fixing (PAT[+] group, $n = 3$). The PAT was affixed to the bone surface of the wound bed (►Fig. 1d). As an initial dressing, the PAT was covered with a 0.5-mm silicon sheet (Wakomu Seisakusyo, Saitama, Japan) and fixed with 10 stiches using 5-0 monofil-
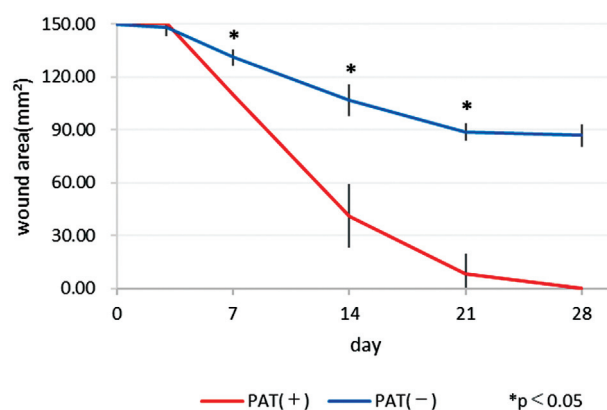
ament nylon sutures (Keisei Medical, Tokyo, Japan).[2] The control rats (PAT[−] group, $n = 3$) did not receive PAT harvested from the back, and the exposed-bone head wound was directly covered with a silicon sheet.

After the surgery, the rats were again housed with one rat per cage, and the initial silicon sheet dressing was removed from all the rats 3 days after the surgery. The degree of wound healing was recorded at 3, 7, 14, 21, and 28 days after the surgery. At the established endpoints, the wound areas (square millimeters) were measured by the tracing of the wound margins on the photograph followed by the calculation of the pixel data by means of ImageJ software (National Institutes of Health, Bethesda, MD, USA). For histologic evaluation, the entire rat dorsal layer before and after PAT harvesting was excised to prepare the slices. These 4-μm sagittal slices were stained with Elastica van Gieson (EVG) before being mounted on glass slides.

In the statistical analyses, all results were presented as means ± standard deviations. The data were normally distributed; comparisons between two groups (i.e., PAT[+] vs. PAT[−]) in the wound areas were performed using the t-test, and probability values less than 0.05 were considered significant (►Fig. 2, ►Table 1).

Results

Wound Area Results
In the PAT(+) group, all the PATs took, and the wound areas gradually decreased owing to epithelialization and contraction, resulting in these rats healing by day 28 (►Fig. 3a). In the PAT(+) group, the wound area steadily decreased to 109.7 ± 0.6 mm^2 on day 7, 41.2 ± 22.4 mm^2 on day 14, and 8.19 ± 14.2 mm^2 on day 21. On day 28, the wound area of all the rats in this group was 0 mm^2.

Table 1 Wound areas (mm2) of the two groups

	0 d	3 d	7 d	14 d	21 d	28 d
PAT(+) group	150.0 ± 0	150.2 ± 4.8	109.7 ± 0.6	41.2 ± 22.4	8.19 ± 14.2	0 ± 0
PAT(−) group	150.0 ± 0	148.0 ± 4.8	130.9 ± 5.3	106.7 ± 11.1	88.5 ± 6.2	86.7 ± 6.3

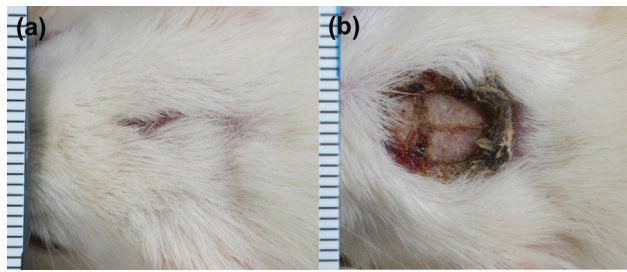Abbreviation: PAT; perifascial areolar tissue.

Fig. 3 (a) PAT(+) 28 days after surgery. (b) PAT(−) 28 days after surgery.

On the contrary, in the PAT(−) group, the wound area was slightly reduced, to 130.9 ± 5.3 mm^2 on day 7, 106.7 ± 11.1 mm^2 on day 14, and 88.5 ± 6.2 mm^2 on day 21. The wound area on day 28 was 86.7 ± 6.3 mm^2, and the rat still had an exposed bone and no closure (►**Fig. 3b**). At postsurgery days 7, 14, and 21, the difference in the wound area between the 2 groups was significant ($p < 0.05$).

Histologic Results

Histologically, before PAT harvesting, the backs of the rats had a loose layer between the latissimus dorsi muscle and the panniculus carnosus muscle that stained yellow with EVG (►**Fig. 4a**). This layer consisted mainly of reticulated collagen fibers and blood vessels (►**Fig. 4b**) that contracted noticeably after PAT harvesting but maintained the layered structure of connective tissue (►**Fig. 4c**).

Discussion

PAT transplant for wounds, as reported by Kouraba,[1] relocates loose connective tissue harvested in a sheet form from above the fascia to the wound bed. The effectiveness of this technique has been shown in several reports.[3–8] However, all of these were clinical reports, and the size and depth of the wound, the exposed tissue, the presence or absence of surrounding blood flow, the presence or absence of irradiation, the site of the wound, and so forth are diverse. The appropriate surgical indications are unknown. In addition, no basic research has so far been reported, and the mechanism of PAT engraftment and the reason why PAT transplant is effective for wound healing remain unclear. To date, no animal model of PAT transplant for wounds is available.

Therefore, we thought that an animal model was needed to make the wound conditions uniform and to elucidate the mechanism of PAT.

The PAT is located under the deep adipofascial layer and directly on the deep fascia. It is a loose connective tissue and part of a group of structures known as the lubricant adipofascial system reported by Nakajima et al.[9] Since the PAT has a layered structure of the delicate network of thin collagen fibers, it is possible to harvest supple and thin tissue.[3] In clinical cases, the PAT is often harvested from the inguinal region or the outside of the thigh in consideration of the cosmetic result and invasiveness.[4] In this model, the PAT was harvested from the back of the rat. It was considered that the back skin of the rat has good mobility with bones and muscles and that the back has a human-like lubricant adipofascial system. In the histologic results of our study, the backs of the rats had a loose connective tissue layer between the latissimus dorsi muscle and the panniculus carnosus muscle. This layer consisted mainly of reticulated collagen fibers. This connective tissue could be harvested in a sheet-like form, in a similar fashion to that in human surgeries.

The harvested PAT was transplanted to an exposed bone wound lacking periosteum. Exposed bone wounds, 10×15 mm in size, lacking periosteum, created on the rat head have been reported to take 82 days to heal untreated.[2] In our study, the total wound area was significantly reduced in the PAT(+)group when compared with that in the PAT(−) group. All the wounds transplanted with the PAT were healed 28 days after surgery. This result, similar to the result obtained in clinical cases, is considered to indicate that PAT transplant promotes the healing of intractable wounds.

The reason why PAT transplant is effective for wound healing remains unknown. The exact mechanism by which PAT engrafts on ischemic tissue is also unknown. There is an opinion that revascularization easily occurs because the PAT itself has abundant blood vessels.[5] We suspect that a bridging phenomenon[10] similar to full-thickness skin grafting is involved in PAT engraftment, and Hayashi et al and Abe et al have stated a similar possibility.[6,7] There is also an opinion that the abundant stem cells contained in the PAT are involved in promotion of healing.[8] Elucidation of the mechanism of PAT engraftment and healing promotion is considered to be an issue for future studies.
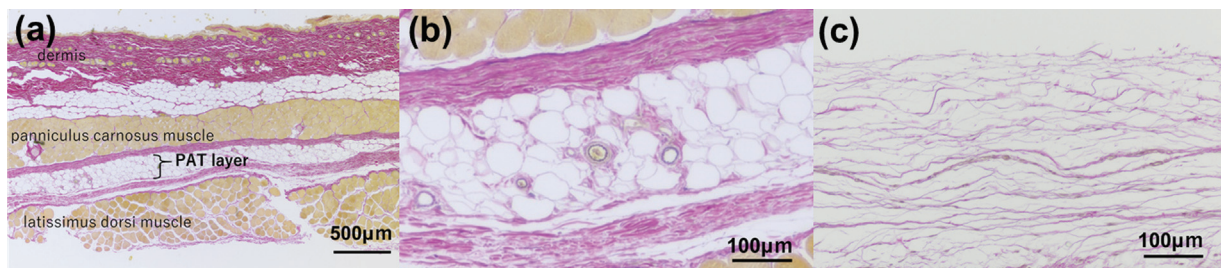

Fig. 4 (a) Histological findings on the back of the rat. Before PAT harvesting, the PAT exists as a layered structure between the stained latissimus dorsi and the panniculus carnosus muscles. (b) Histologic findings at high magnification in the PAT layer. The PAT layer consists of reticulated collagen fibers and blood vessels. (c) Histologic findings of the PAT after harvesting and before transplantation. The PAT retains a multilayered structure of connective tissue.

In this study, the PAT size and bone exposure areas were the same. Therefore, the only part in contact with the well-vascularized tissue was the PAT margin. We consider that the PAT engrafted because the wound was small, but it is possible that the PAT would not have engrafted had the size of the wound and the type of exposed tissue been different. To establish a reliable surgical method, future studies using different types of exposed tissues (tendons or artifacts), as well as different overlap areas with well-vascularized tissue, are needed.

Conclusion

In this study, we harvested connective tissue from the backs of Wistar rats in a sheet form to simulate human PAT transplant, and significant promotion of healing was observed in the bone-exposed rats. This clinically relevant rat model is, thus, useful for elucidating the mechanism of PAT transplant and establishing a reliable surgical method.

Research Ethics and Patient Consent

All the study procedures were performed in accordance with the guidelines of our institutional animal care and use committee (approval number 2019-401).

Conflict of Interest
None declared.

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