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Original Article

Effect of Basic Fibroblast Growth Factor in Perifascial Areolar Tissue Transplant

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Abstract

Background: Perifascial areolar tissue (PAT) transplant is a technique in which a sheet of connective tissue on the fascia is harvested and transplanted to the wound bed. PAT engraftment fails when the exposed area of tendons, bones, or artificial materials is large. On the other hand, combination of tissue transplant and basic fibroblast growth factor (bFGF) improves the survival rate of the transplanted tissue.

Methods: A wound model was created in which the artificial material was exposed on rats' backs. All the rats underwent PAT transplant, but the rats were divided into two groups according to the PAT processing method beforehand. In one group, the PAT was immersed in water for injection before transplant (bFGF[−] group), and in the other group, the PAT was immersed in bFGF product (bFGF[+] group). Specimens were collected 7 days after surgery to assess the histologic thickness of the PAT and the gene expression in the PAT.

Results: The thickness of the PAT in the tissue slices was significantly higher in the bFGF(+) group than in the bFGF(−) group. Expressions of CD34 and COL3A1 were significantly higher in the bFGF(+) group than in the bFGF(−) group.

Conclusion: The results of this study indicate that adding bFGF to the PAT transplant may promote PAT engraftment and wound healing by increasing angiogenesis and may increase granulation formation, which may result in a stronger covering that prevents the prosthesis from being exposed.

Keywords

- perifascial areolar tissue
- loose connective tissue
- basic fibroblast growth factor
- ulcer

Introduction

Perifascial areolar tissue (PAT) transplant is a technique in which a sheet of connective tissue on the fascia is harvested and transplanted to the wound bed. This technique allows rapid wound bed preparation to be achieved in intractable wounds. The advantages of this technique include minimally invasive graft extraction, technical ease, and short operation time.[1] However, basic research and verification of PAT transplant effectiveness are scarce, and the limits of PAT engraftment are unknown. In particular, in many cases PAT engraftment fails when the exposed area of tendons, bones, or artificial materials is large. On the other hand, basic fibroblast growth factor (bFGF) is widely used to promote healing of wounds such as burns, chronic wounds, and pressure ulcers owing to its angiogenic activity.[2] In addition, it has been reported that the combination of tissue graft (bone, cartilage, etc.) and bFGF improves the survival rate of the transplanted tissue.[3,4] The purpose of this study was to examine the effect of combining bFGF with the PAT graft.

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Materials and Methods

PAT Transplant Model to Wounds in Which the Artificial Materials Are Exposed

Six female Wistar rats (CLEA Japan, Tokyo, Japan), each aged 11 weeks and weighing between 180 and 200 g, were used to create the model. The rats were kept in cages with one rat per cage in a pathogen-free environment with a 12-hour light/dark cycle and free access to food and drink. The entire process was carried out in accordance with the recommendations made by our institution's animal care and use committee (permission number 2021-101). To provide general anesthesia during surgery, inhalation of 4% isoflurane was administered by use of a rodent ventilator (TK-5, Biomachinery, Chiba, Japan) inside an induction box. Isoflurane was vaporized in clean 96 to 100% air and inhaled at a rate of 500 mL/min by use of a homemade facemask to maintain anesthesia. With visual respiratory monitoring, which included looking for symptoms of chest and abdomen motions, the isoflurane gas concentration was adjusted to 2 to 4%.

The wound bed to which the PAT was grafted was created by modifying the silicon sheet exposure model reported in the past, and the blood flow from the wound bed was partially restricted.[5,6] A circular skin and panniculus carnosus muscle defect was created on the back with a diameter of 20 mm. The PAT on the latissimus dorsi muscle present in the same wound was harvested with a diameter of 20 mm (►**Fig. 1a–c**).[7] A silicon sheet (Wakomu Seisakusyo, Co., Ltd., Saitama, Japan) with a diameter of 10 mm and a thickness of 0.5 mm was inserted on the fascia after the PAT had been harvested (►**Fig. 1d**), and sutured with 6–0 monofilament needle thread to the fascia at two locations and fixed (►**Fig. 1e**). The PAT was implanted on the artificial material-exposed wound created in this way and then covered with a wound dressing (SI-Aid, ALCARE, Co., Ltd., Tokyo, Japan), and the margin was sutured with a 5–0 monofilament needle thread (Keisei Medical Industrial Co., Ltd, Tokyo, Japan) at six locations (►**Fig. 1f, g**). All the rats underwent PAT transplant, but the rats were divided into two groups according to the PAT processing method before transplant. In

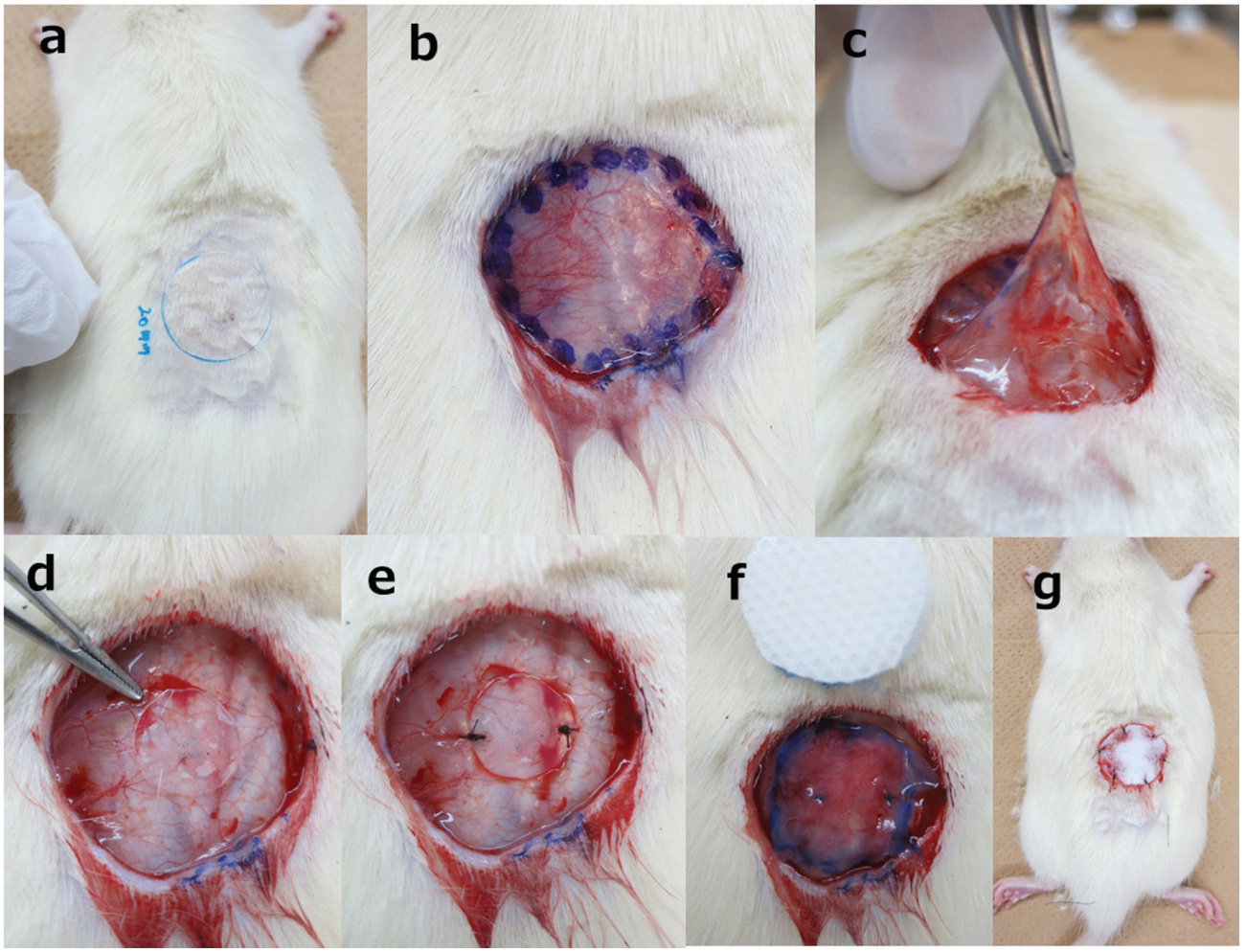

Fig. 1 (**a**) A 20-mm-diameter skin was designed on the rat's back. (**b**) After the skin and panniculus carnosus muscle were removed, the perifascial areolar tissue (PAT) was marked over the latissimus dorsi muscle with a diameter of 20 mm. (**c**) The PAT to be harvested was pinched with forceps. (**d**) A silicon sheet with a diameter of 10 mm and a thickness of 0.5 mm was inserted on the fascia after the PAT had been harvested. (**e**) The silicon sheet was fixed by suturing it to the fascia at two places with 6–0 monofilament needle thread. (**f**) The harvested PAT was covered over the wound with a silicon sheet fixed. (**g**) A wound dressing was placed over the PAT and fixed with 5–0 monofilament needle thread around the perimeter.

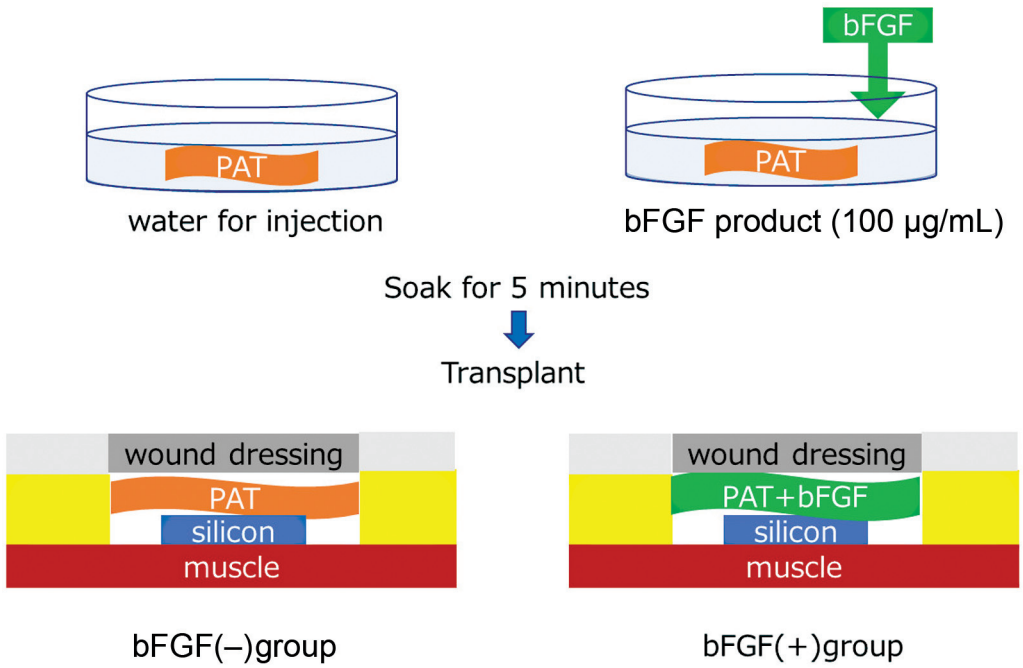

Fig. 2 Perifascial areolar tissue (PAT) transplant model and schema of each group. In one group, the PAT was immersed in water for injection before transplant (bFGF[–] group, $n = 3$), and in the other group, the PAT was immersed in bFGF product for 5 minutes (bFGF[+] group, $n = 3$).

one group, the PAT was immersed in water for injection (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) for 5 minutes before transplant (bFGF[–] group, $n = 3$), and in the other group, the PAT was immersed in a bFGF product at a concentration of 100 μg/mL (trafermin: Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) for 5 minutes (bFGF[+] group, $n = 3$; ►**Fig. 2**). Rats were randomly numbered and alternately assigned to the bFGF(–) and bFGF(+) groups. After surgery, each rat was housed in one cage and managed. Seven days after surgery, the rats were euthanized under carbon dioxide inhalation and the wound dressing was removed.

Histologic Examination

The specimen was excised as a whole, including the skin around the wound and the deep muscle body, and the left section was prepared by dividing it at the center of the sagittal section (►**Fig. 3a**). Four-micrometer-thick sagittal slices were stained with the Masson trichrome stain. The thickness of the PAT after transplant was measured using this slice preparation. The thickness of the PAT was measured as the average value of the three set sites. The thickness of the three-set site was the thickness of the tissue at the vertical lines drawn from the center and the left and right 1/4 points of the reference line drawn horizontally on the existing silicon portion (►**Fig. 3b, c**).

Analysis of Gene Expression

Gene expression in the transplanted PAT was evaluated. On the seventh day after the operation after the wound dressing had been removed, the part directly above the silicon was collected as a sample from the right PAT divided at the center of the sagittal section (►**Fig. 3a**) and stored frozen at –80°C. From this sample, total ribonucleic acid (RNA) was purified using an RNeasy Fibrous Tissue Mini Kit (Qiagen, Venlo, Netherlands), and complementary DNA (cDNA) was synthesized using a SuperScript VILO cDNA Synthesis Kit (Thermo Fisher Scientific K.K., Tokyo, Japan). Gene expression analysis was performed by means of quantitative real-time polymerase chain reaction (qPCR) (QuantStudio 5, Block Format: 96-wells, Thermo Fisher Scientific K.K.). All the steps were performed according to the protocol recommended by the manufacturer. Regarding the selection of primers, the target genes were *CD34* (Rn03416140-ma), collagen III (*COL3A1*) (Rn01437681-m1), and collagen I (*COL1A1*) (Rn01463848-m1), and the internal standard gene was *GAPDH* (Rn01775763-g1; Thermo Fisher Scientific K.K.).

Statistical Analysis

The *t*-test was used for statistical analysis. Data were expressed as means ± standard deviations (means ± SDs). Differences with $p \leq 0.05$ were considered significant. *Z* scores were used in this work to identify outliers in the statistical analysis of the qPCR results, which had scores with absolute values of 1.25 or higher for each cycle threshold (cycle threshold [Ct]) value.[8]

Results

When the wound dressing was removed on the seventh day after surgery, PAT perforation or tear had not occurred in any of the cases, and the artificial material was covered. Macroscopically, in the bFGF(–) group, the PAT had not collapsed, but silicon was visible in the deep layer of the thin PAT (►**Fig. 4a, c**). In contrast, in the bFGF(+) group, silicon was not visible and was covered with thick tissue (►**Fig. 4b, d**). The thickness of the PAT in the tissue sections was

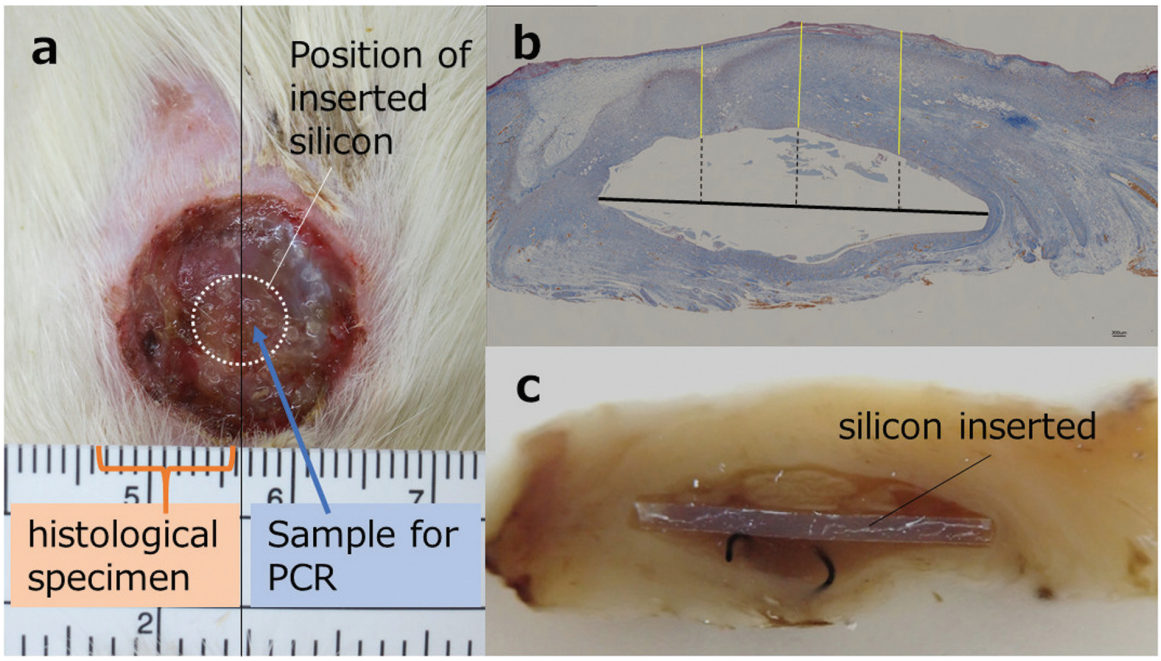

Fig. 3 (**a**) Seven days after surgery, the rats were euthanized under carbon dioxide inhalation and the wound dressing was removed. The left-sided tissue divided at the midsagittal section was used for histologic examination, and the right-sided tissue immediately above the perifascial areolar tissue (PAT) was used for gene expression analysis. (**b**) The thickness of the PAT was measured as the average value of the three set sites. The thickness of the three-set site was the thickness of the tissue at the vertical lines drawn from the center and the left and right 1/4 points of the reference line drawn horizontally on the existing silicon portion. The length of the *straight yellow line* is the thickness of the PAT. PCR, polymerase chain reaction. (**c**) Macroscopic findings of tissue after formalin fixation. The location of silicon insertion is indicated.

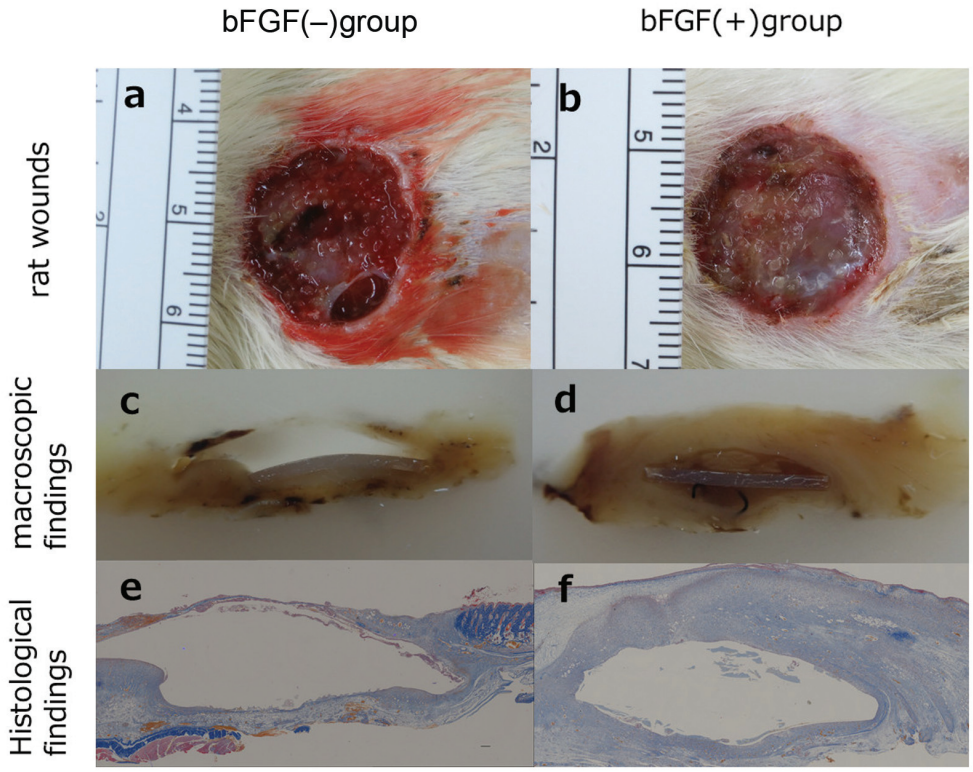

Fig. 4 (**a**) Wounds of rats in the bFGF(−) group. Silicon was visible in the deep layer of the thin perifascial areolar tissue. (**b**) Wounds of rats in the bFGF(+) group. Firm granulation has formed. (**c**) Macroscopic finding of the bFGF(−) group. The tissue on the silicon is extremely thin. (**d**) Macroscopic finding of the bFGF(+) group. The silicon is covered with a thick tissue. (**e**) Histologic finding of the bFGF(−) group. Connective tissue that stained blue with Masson trichrome staining is observed. (**f**) Histologic finding of the bFGF(+) group. A thick layer of connective tissue is observed over the space of the silicon sheet.

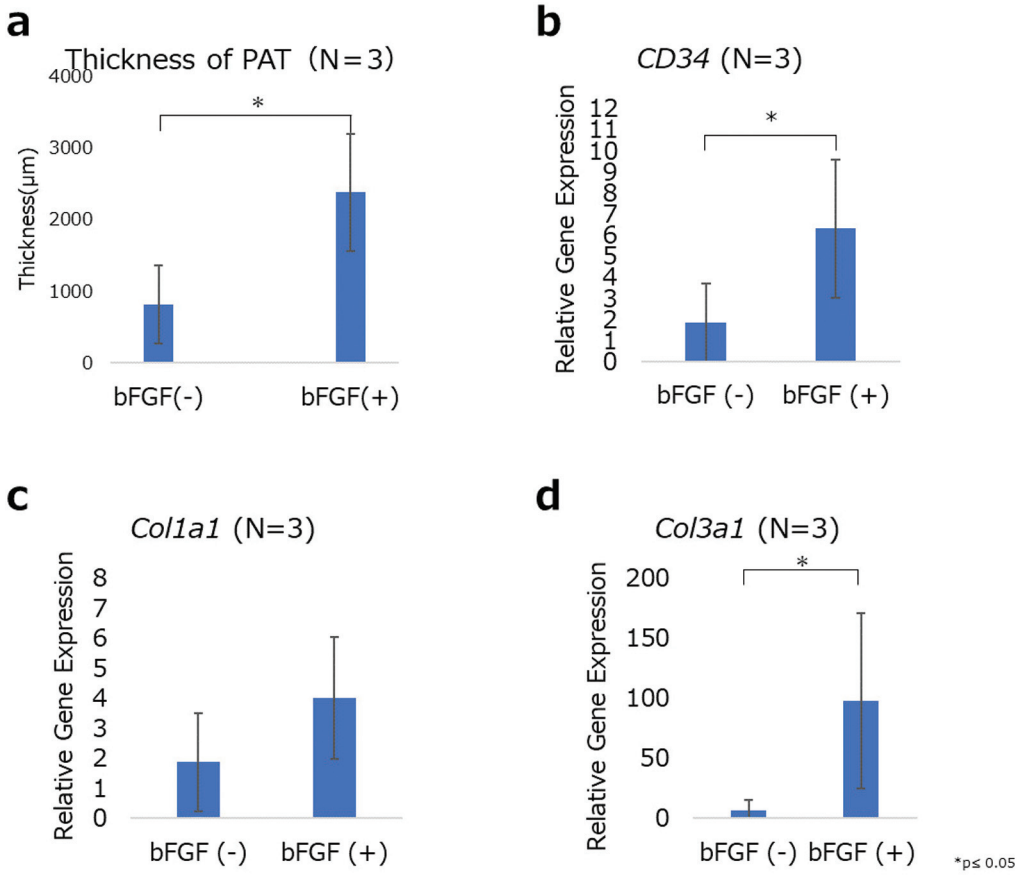

Fig. 5 (**a**) Evaluation of perifascial areolar tissue (PAT) thickness. The thickness of the PAT in tissue sections was significantly higher in the bFGF (+) group (2.37 ± 0.81 mm) than in the bFGF(−) group (0.81 ± 0.54 mm; $p = 0.05$). (**b**) *CD34* expression was significantly higher in the bFGF(+) group (GE: 6.28 ± 3.29) than in the bFGF(−) group (GE: 1.81 ± 1.85; $p = 0.05$). (**c**) Expression of *COL3A1* was also significantly higher in the bFGF(+) group (GE: 97.2 ± 73.1) than in the bFGF(−) group (GE: 6.28 ± 3.29; $p = 0.04$). (**d**) Regarding *COL1A1*, the expression was higher in the bFGF(+) group (GE: 4.00 ± 2.03) than in the bFGF(−) group (GE: 1.85 ± 1.63), but not significantly so ($p = 0.11$).

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Regarding gene expression (GE), *CD34* expression was significantly higher in the bFGF(+) group (GE: 6.28 ± 3.29) than in the bFGF(−) group (GE: 1.81 ± 1.85; $p = 0.05$).

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Discussion

Since the first report of PAT transplant by Kouraba in 2013,[9] some clinical reports of PAT transplant have been published. However, these were retrospective case reports about clinical patients, and background factors such as wound size, location, and exposed tissue were not standardized. In actual clinical practice, there are many cases in which PAT transplant fails, but few reports on such cases exist. On the other hand, attempts to combine PAT and bFGF to increase the success rate of PAT have also been reported,[10] but basic verification has not been performed. In 2022, we reported on basic research carried out for the first time on PAT transplant. In that report, PAT transplant in a rat bone defect model significantly shortened the healing period when compared with the untreated group. We also reported on a PAT harvesting method and histologic evaluation in rats.[7] The current research is the second basic research study conducted on PAT transplant and focuses on the expansion of indications for PAT transplant.

This time, we created a wound where the artificial material was exposed and we implanted the PAT there. Angiogenesis between the wound and the PAT is blocked in the central part in contact with silicon, so the PAT takes blood flow from the surroundings and the wound margin and survives.[5,6] The expected effect of adding a bFGF preparation to the PAT transplant is thought to be the promotion of angiogenesis and granulation formation. Tanaka et al reported the proliferation effect of bFGF on vascular endothelial cells in vitro,[11] and Okumura et al reported significant blood vessel elongation in the rabbit cornea.[12] In this study, the addition of bFGF to the PAT also showed a predominant increase in *CD34* expression in the PAT after transplant in the bFGF(+) group when compared with the control group. This predominant increase suggests that angiogenesis is enhanced in PAT. In

addition, Okumura et al reported that inserting a paper disk impregnated with bFGF into the backs of rats significantly increased the dry weight of the granulation tissue, including the disk, after 7 days.[12] In the evaluation of PAT thickness in this study, the bFGF(+) group was significantly thicker, which we considered to be due to the ability of bFGF to promote granulation formation. Regarding PAT gene expression, the expression of *COL3A1*, which is involved in the formation of type III collagen, was predominantly elevated in the bFGF(+) group, suggesting that the increase in PAT thickness was the result of an increase in type III collagen. No significant difference between the two groups was found in the expression of *COL1A1*. In the early stages of general wound healing, a large amount of type III collagen appears, and as the wound matures, type I collagen gradually increases over a period of 1 to 2 weeks after surgery.[13,14] On the seventh day after the operation, which was the time of sample collection in this experiment, we considered that the effect of bFGF on type I collagen was small because it was in the early stages of the wound healing process.

In this experiment, the PAT did not break down in any of the rats on the seventh day after surgery, but thin connective tissue such as that in the bFGF(−) group is thought to break down easily during the course of the experiment. This finding indicates that adding bFGF to the PAT transplant may promote PAT engraftment and wound healing by increasing angiogenesis and granulation formation, which may result in a stronger covering that prevents the prosthesis from being exposed. However, it is unclear whether the administered bFGF is a result of its acting on cells in the recipient wound or in the cells within the PAT. Considering the results of a previous report in which the weight of granulation was increased simply by impregnation of a paper disk with bFGF,[12] we speculate that the bFGF administered in this study also had an effect on the recipient wound. On the other hand, there is also the opinion that PAT contains an abundance of stem cells,[15] and it is assumed that the administered bFGF also acts to some extent on cells within the PAT.

In clinical practice, bFGF quickly disappears after being sprayed on a wound, so frequent administration or continuous administration using artificial dermis as a carrier is recommended.[16,17] In this study, we chose a method of administering bFGF by soaking it in PAT, referring to the paper disk method.[12] We speculate that PAT also plays the role of a carrier that stores bFGF for a certain period of time. Future tasks will be to evaluate the behavior and distribution of bFGF and to examine which cells bFGF acts on. It is also necessary to consider the optimal bFGF administration method and dosage.

Adding bFGF to the PAT transplant promote PAT engraftment and provides a strong coverage that prevents the prosthesis from being exposed. This method makes it possible to cover large artificial materials, which have failed in clinical practice, and suggests the possibility of greatly expanding the indications for PAT transplantation. However, because our model is in a limited situation and the number of individuals is small, the limitations and success rate of this surgery are unknown. In order to establish a more reliable method, we have positioned this study as a pilot study, and we plan to evaluate the behavior and distribution of bFGF and to study the use of artificial materials of different sizes and types.

Conclusion

Adding bFGF to the PAT transplant may promote PAT engraftment and wound healing by increasing angiogenesis and granulation formation, which may result in a stronger covering that prevents the prosthesis from being exposed.

Ethical Approval

All the study procedures were performed in accordance with the guidelines of our institutional animal care and use committee (approval number 2021-101).

Authors' Contributions

J.O. conceived the idea of the study. Y.S. developed the statistical analysis plan and conducted the statistical analyses. J.O. drafted the original manuscript. K.S. and M.S. supervised the conduct of the study. All the authors reviewed the manuscript draft and revised it critically for intellectual content. All the authors approved the final version of the manuscript.

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Conflict of Interest

J.O. reports grant from JSPS Kakenhi.

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