



Original Article

Basic fibroblast growth factor-impregnated collagen gelatin sponge completes formation of dermis-like tissue within 2 weeks: A prospective cohort study

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ABSTRACT

Introduction: This study included patients who underwent full-thickness skin reconstruction using a two-stage procedure comprising basic fibroblast growth factor-impregnated collagen gelatin sponge (bFGF-CGS) implantation and autologous skin grafting, and the take rate of skin grafts was compared between groups of patients who underwent autologous skin grafting after a waiting period of <2 weeks or ≥ 2 weeks.

Methods: An acute, full-thickness skin defect was treated with thorough debridement of contaminated/necrotic tissue, followed by washing with saline and hemostasis with electrocautery. Then, an FGF-CGS was fixed to the skin defect wound using non-absorbable sutures, and after confirming regeneration of sufficient dermis-like tissue, the second-stage autologous skin grafting was performed for wound closure. Patients were divided into two groups according to the waiting period before the second operation, namely, <2 weeks (early group) and ≥ 2 weeks (late group), and the take rate of skin grafts was compared.

Results: We enrolled and treated 25 cases (18 men, 7 women; mean age: 49 [range 2–86] years). The mean take rate of skin grafts was 93% (range 80%–100%) in the early group and 92% (range 65%–100%) in the late group, with no significant difference between the two groups. There was a significant difference between the groups in mean time to complete healing: 25.2 ± 9.7 days in the early group vs 44.7 ± 27 days in the late group ($p < 0.05$).

Conclusion: Our data suggest that bFGF-CGF can form dermis-like granulation tissue with sufficient quality as a graft bed for skin transplantation within 2 weeks.

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1. Introduction

In wound surgery, various wound dressings, medical devices, and regenerative medicine products are used, and the use of artificial dermis as a scaffold for regeneration of dermal components is a standard treatment for larger full-thickness skin defects.

Abbreviations: bFGF, basic fibroblast growth factor; bFGF-CGS, basic fibroblast growth factor-impregnated collagen gelatin sponge.

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Artificial dermis has been well-documented as a very useful biomaterial for the reconstruction of full-thickness skin defects in extensive burns [1], acute traumatic skin defects [2], and reconstructive surgery [3,4].

The advantages of using artificial dermis for full-thickness skin defects include that (1) dermal components can be supplied to the wound, so that a thin split-thickness skin graft is sufficient for epidermalization and wound closure, minimizing the skin graft donor area, and (2) it a vascular bridge can be provided even if there is a small area of exposed tendon or bone at the base of the wound, enabling wound closure with the artificial dermis and a very thin split-thickness skin graft for wounds that cannot be closed with a skin graft alone.

However, to achieve complete wound closure and epithelialization using this material, it is necessary to perform second-stage autologous skin grafting about 3 weeks after the initial artificial dermis grafting, over the dermis-like tissue that is formed by sufficient vascularization of the artificial dermis [5]. This 2–3 week waiting period is the cause of several problems, including (1) prolonged suffering of the patient, (2) increased incidence of wound infection, and (3) reduced take rates (50%–80%) [6]. It is therefore clear that shortening this waiting period will expand the usefulness of artificial dermis in the clinical setting. In light of these issues, in 2013, Morimoto et al. developed a new hybrid artificial dermis containing a basic fibroblast growth factor (bFGF) drug delivery system and conducted a clinical trial in chronic ulcers [7]. This bFGF-impregnated collagen gelatin sponge (bFGF-CGS) contains 10% alkali-treated gelatin in a standard artificial dermis. The alkali-treated gelatin binds to positively charged bFGF and gradually releases bFGF at the wound surface over a period of 3 weeks as the gelatin is absorbed by the body [8]. In Japan, bFGF preparations have been widely used in clinical practice as a highly effective treatment for skin ulcers due to their fibroblast proliferative effect [9] and angiogenesis-promoting effect [10]. The indications for bFGF-CGS have also expanded to include skin avulsion injuries of the extremities [11] and second-degree burn injuries [12]. Thus, the application of bFGF-CGS to a full-thickness skin defect, through its function as a bFGF drug delivery system, may contribute to reducing the time needed for regeneration of dermis-like tissue, consequently reducing the risk of infection and shortening the duration of illness compared with artificial dermis alone. In this study, we enrolled patients who underwent full-thickness skin reconstruction using a two-stage procedure comprising bFGF-CGS implantation and autologous skin grafting, and compared the take rate of skin grafts between groups of patients who waited <2 weeks or ≥2 weeks before autologous skin grafting over the dermis-like tissue, with the aim of determine whether bFGF-CGS would allow secondary skin grafting to be performed within 2 weeks.

2. Material and methods

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and set by our Institution. All patients provided their written informed consent for the procedures described in this study and for the publication of the photographs included in this manuscript. This study was approved by the Ethics Committee of Tokyo Women's Medical University.

2.1. Study design

This was a single-center prospective cohort study. Inclusion criteria for the study were the presence of an acute skin injury requiring debridement and full-thickness skin reconstruction, and an American Society of Anesthesiologists score ≥2. The exclusion criteria were as follows: (1) history of diabetes that may be associated with prolonged wound healing, (2) ongoing long-term steroid therapy for collagen disease or other diseases, (3) cancer-bearing patients receiving chemotherapy, and (4) severe skin and soft tissue damage with extensive bone/tendon exposure.

2.2. Wound bed preparation and implantation of bFGF-CGS

An acute, full-thickness skin defect was treated with thorough debridement of contaminated/necrotic tissue, followed by washing with saline and hemostasis with electrocautery. After debridement, the contour of the skin defect was traced using a surgical drape. The tracing was scanned and the obtained image data were used to

accurately measure the skin defect size using ImageJ software version 1.80 (National Institutes of Health, Bethesda, MD). We used a CGS (PELNAC Gplus®, Gunze, Kyoto, Japan), a commercially available artificial dermis in Japan. This artificial dermis is a modified version of the conventional bilayered artificial dermis and consists of an upper silicone sheet with thickness of 0.12 mm and a lower collagen sponge with alkali-treated gelatin with thickness of 3 mm. The operator prepared the bFGF-CGS with human recombinant bFGF liquid (Fiblast® spray; Kaken Pharmaceutical, Tokyo, Japan) at 7–14 µg/cm² in the operating room about 10 min just before application. This dose is equivalent to the dose of bFGF used in a clinical trial of the bFGF-CGS for chronic wounds, with demonstrated safety and efficacy [7]. It was ensured that the daily dose of bFGF should not exceed 1000 µg, in accordance with its handling rules. The bFGF-CGS was fixed to the skin defect wound using non-absorbable sutures and then covered with a wound dressing, such as ointment gauze, or a negative pressure wound treatment system (RENASYS™, Smith & Nephew Wound Management, London, UK) for wound management.

2.3. Second-stage autologous skin grafting

Regeneration of sufficient dermis-like tissue at the graft site was confirmed by 3 plastic surgeons were not involved in this study and not directly involved in the patients' medical care. Then, second-stage autologous skin grafting was planned for wound closure. The date for secondary skin grafting was scheduled at the earliest possible date, taking into account the social background of each patient. Patients were divided into two groups according to the waiting period for the secondary operation, namely, <2 weeks (early group) and ≥2 weeks (late group), and the take rate of skin grafts was compared. In both groups, autologous tissue grafts were either full-thickness skin grafts (FTSG) or split-thickness (0.012 inch) skin grafts (STSG), depending on the recipient site. A 0.012-inch STSG was applied to the dermis-like tissue after bFGF-CGS implantation. The skin graft was applied either as a sheet graft with drainage holes of a few millimeters or as a 1.5- to 3-fold expanded mesh graft. The donor site was in the head, thigh, or buttock, depending on the patient's age and the location of the graft site. The skin graft was fixed with a conventional tie-over dressing or a negative pressure wound treatment system for a total of 7 days in all cases.

2.4. Follow-up and outcome measures

The extent of secondary skin graft take was assessed by 3 plastic surgeons 7 days after secondary skin grafting. The take area of skin graft was traced using a surgical drape and accurately measured postoperatively using ImageJ software, as in the measurement of the skin defect size. The ratio of the calculated take area of a skin graft (cm²) to the total skin defect size (cm²) was calculated as the take rate of the skin graft (%). Areas where skin grafts did not take were treated conservatively with ointment. The time from the initial operation to complete healing was calculated in days. Wound follow-up was continued until complete healing was achieved in all cases. No additional application of bFGF products to the wounds was performed after the initial operation.

2.5. Statistical analysis

Results are expressed as mean ± SD, and *p* < 0.05 was considered significant. The skin defect size, the take rate of skin graft, the take area of skin graft, and period of complete healing in both groups were analyzed using unpaired Student's *t*-test in GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA).

Table 1
Clinical cases.

	Early group (range or %)	Late group (range or %)	P
No.	13	12	
Age (yr)	42.3 ± 25.8	59.3 ± 26	0.123
Skin defect size (cm ²)	46.6 ± 40.7	20.6 ± 16.5	0.052
Take rate of skin grafts (%)	93 (80–100)	92 (65–100)	0.77
Take area of skin graft (cm ²)	39.6 ± 9.7	18.0 ± 4.1	0.057
Period for complete healing (days)	25.2 ± 9.7	44.7 ± 27	<0.05
Pathology			
Burn	6 (46)	7 (58)	
Trauma	3 (23)	3 (25)	
Other	4 (31)	2 (17)	
Wound location			
Head and neck	0 (0)	3 (25)	
Upper extremity	3 (23)	2 (17)	
Lower extremity	10 (77)	7 (58)	
Secondary operation procedure			
STSG	10 (83)	8 (67)	
FTSG	2 (17)	4 (33)	

STSG, Split thickness skin graft; FTSG, Full thickness skin graft.

3. Result

3.1. Patient and injury characteristics

We enrolled and treated 25 cases (18 male, 7 female; mean age: 49 [range 2–86] years). Injury types were deep burns in 13 patients, traumatic skin defects in 6 patients, and other injuries in 7 patients. The locations of wounds requiring reconstruction were the head

and neck in 3 patients, the upper extremities in 4 patients, and lower extremities in 17 patients. The mean time to secondary skin grafting in all patients was 15.7 ± 8 days, and the mean skin defect size was 32.7 [range 2–142] cm² (Table 1).

3.2. Comparison of the early and late groups

The mean time to secondary skin grafting was 9.7 ± 1.7 days in the early group (n = 13) and 22.2 ± 7 days in the late group (n = 12), with a significant difference between the two groups. The reasons for this delay in the late group were (1) the patient's domestic, work, and economic circumstances, (2) restrictions on surgery due to the COVID-19 pandemic, or (3) treatment of other injuries in patients with polytrauma.

The mean skin defect size was 46.6 ± 40.7 cm² in the early group and 20.6 ± 16.5 cm² in the late group, with no significant difference between the groups. The mean take rate of skin grafts did not significantly differ between the groups: 93% (range 80%–100%) in the early group and 92% (range 65%–100%) in the late group. The mean take area of skin graft was 39.6 ± 9.7 cm² in the early group and 18.0 ± 4.1 cm² in the late group, and this difference was not significant. The mean time to complete healing was 25.2 ± 9.7 days in the early group and 44.7 ± 27 days in the late group, with a significant difference between the groups (p < 0.05; Table 1).

4. Selected cases in the early group

4.1. Patient 1

A 3-year-old boy sustained a deep burn injury to his right upper extremity due to exposure to deep-frying oil (Fig. 1A). On the second day of injury, the patient was placed under general anesthesia



Fig. 1. Findings during the course of treatment in Case 1. (A) A 3-year-old boy sustained a deep burn injury to the right upper extremity (B) On the second day of injury, debridement of necrotic tissue was performed (C) A bFGF-CGS was applied to the resulting skin defect wound (D) On postoperative day 13, the skin defect wound was completely covered with firm dermis-like tissue (E) A second operation was performed for split-thickness skin grafting using the scalp and buttocks as donor sites (F) On postoperative day 7, the take rate of the skin graft was 95%.

and debridement of necrotic tissue was performed down to the layer where good bleeding points were obtained in the right hand and fingers, forearm, and elbow (Fig. 1B). After thorough hemostasis and washing, a bFGF-CGS was applied to the resulting skin defect wound and fixed with a negative pressure wound treatment system at a suction pressure of -60 mmHg (Fig. 1C). A second operation was performed on postoperative day 13, when the skin defect was completely covered with firm dermis-like tissue (Fig. 1D). After the dermis-like tissue was gently debrided with a curette, split-thickness skin grafting was performed using the scalp and buttocks as donor sites (Fig. 1E). Fixation of the skin graft was performed, and the negative pressure wound treatment system was applied again at a suction pressure of -60 mmHg. On postoperative day 7, the negative pressure wound treatment system was removed, and 95% take of the graft was confirmed (Fig. 1F). With subsequent conservative treatment with ointment, wound closure was completed 23 days after the initial operation.

4.2. Patient 2

A 29-year-old woman sustained a deep burn injury to the dorsum of her left foot due to exposure to hot *miso* (fermented soybean paste) at work (Fig. 2A). The injury was treated conservatively, but did not heal. On day 19 of injury, the patient was placed under general anesthesia and debridement of necrotic tissue was performed down to the layer where good bleeding points were obtained. After thorough hemostasis and washing (Fig. 2B), a bFGF-CGS was applied to the resulting skin defect wound with exposure of the extensor digitorum longus and was fixed with a negative pressure wound treatment system at a suction pressure of -60 mmHg (Fig. 2C). A second operation was performed on postoperative day 10, when the skin defect was completely covered

with firm dermis-like tissue (Fig. 1D). The extensor digitorum longus was completely covered with the dermis-like tissue (Fig. 2D). After the dermis-like tissue was gently debrided with a curette, split-thickness skin grafting was performed using the left thigh as the donor site (Fig. 2E). The skin graft was fixed, and the negative pressure wound treatment system was applied again at a suction pressure of -60 mmHg. On postoperative day 7, the negative pressure wound treatment system was removed, and 100% take of the graft was confirmed (Fig. 2F).

4.3. Patient 3

An 80-year-old woman was bitten by a large dog while walking, resulting in a skin defect on the right elbow. On the day of injury, under general anesthesia, debridement of the contaminated tissue down to the muscle layer was performed in the right elbow and surrounding area, followed by thorough hemostasis and washing. During this procedure, a depressed area formed in the graft bed due to partial loss of the brachioradialis muscle (Fig. 3A). A bFGF-CGS was applied to the resulting skin defect wound and fixed with a negative pressure wound treatment system at a suction pressure of -60 mmHg (Fig. 3B). A second operation was performed on postoperative day 7. The bFGF-CGS silicone sheet was removed, revealing that the skin defect wound was completely covered with well-vascularized dermis-like tissue (Fig. 3C). After the dermis-like tissue was gently debrided with a curette, split-thickness skin grafting was performed using the thigh as the donor site (Fig. 3D), and the graft fixed again with the RENASYS negative pressure wound treatment system at a suction pressure of -60 mmHg. On postoperative day 7, the negative pressure wound treatment system was removed, revealing that the skin graft did not take at the site corresponding to the aforementioned depression at the site of



Fig. 2. Findings during the course of treatment in Case 2. (A) A 29-year-old woman sustained a deep burn injury to the dorsum of the left foot (B) On day 19 of injury, under general anesthesia, debridement of necrotic tissue was performed down to the layer where good bleeding points were obtained (C) A bFGF-CGS was applied to the resulting skin defect wound with exposure of the extensor digitorum longus (D) On postoperative day 10, the skin defect wound was completely covered with firm dermis-like tissue (E) A second operation was performed for split-thickness skin grafting using the left thigh as the donor site (F) On postoperative day 7, the take rate of the skin graft was 100%.

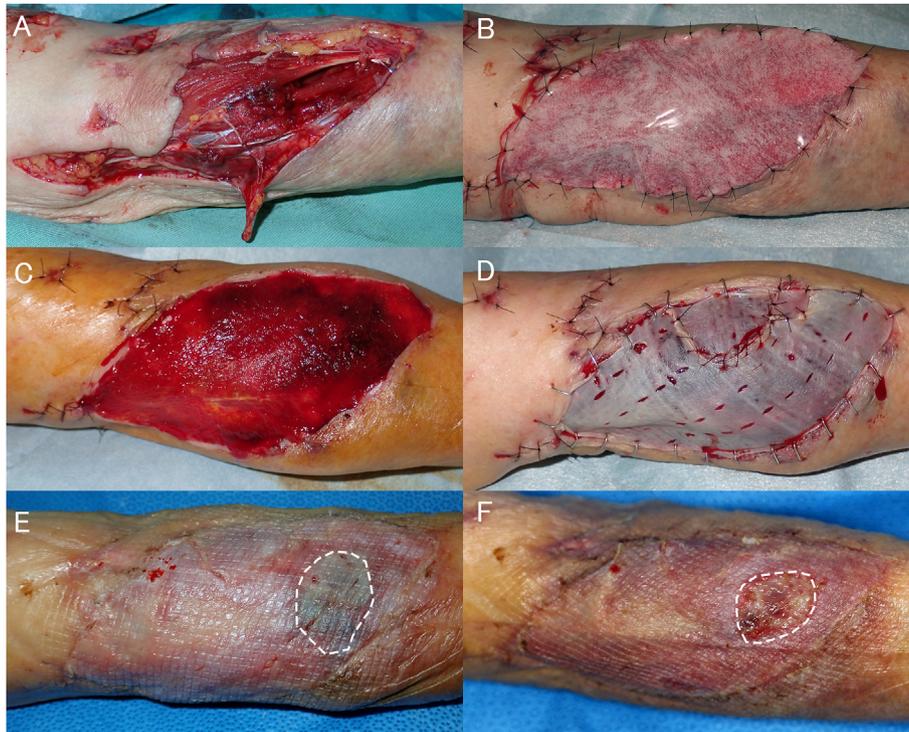


Fig. 3. Findings during the course of treatment in Case 3. (A) An 80-year-old woman sustained a skin defect on the right elbow (B) On the day of injury, debridement of the contaminated tissue down to the muscle layer was performed, followed by application of a bFGF-CGS to the resulting skin defect wound (C) On postoperative day 7, the skin defect wound was completely covered with firm dermis-like tissue (D) A second operation was performed for split-thickness skin grafting using the thigh as the donor site (E) On postoperative day 7, there was an area where the skin graft did not take (white dotted line), resulting in a skin graft take rate of 80% (F) Subsequently, ulceration was observed in the area where the skin graft did not take (white dotted line).

brachioradialis muscle defect. This resulted in a skin graft take rate of 80% (Fig. 3E). Subsequently, ulceration was observed in the area where the skin graft did not take (Fig. 3F). With conservative treatment with ointment, wound closure was completed 26 days after the initial operation.

5. Discussion

The results of this study showed that in the reconstruction of full-thickness skin defects using bFGF-CGS, the take rate of skin grafts did not significantly differ between groups of patients who waited <2 weeks or ≥ 2 weeks for dermis-like-tissue construction, suggesting that the use of bFGF-CGS reduces the waiting period to less than 2 weeks. We speculate that this effect was due to two main factors.

The first possibility is that slow release of bFGF in the artificial dermis may have promoted the synthesis of basement membrane proteins and thereby improved the graft take rate with a shorter waiting period. Fibroblasts stimulate the expression of basement membrane components, leading to the formation of a basement membrane zone, suggesting that fibroblasts produce laminin and type IV and VII collagen or influence the effects of keratinocytes on basement membrane formation through a keratinocyte–fibroblast interaction [13]. Our previous study demonstrated that keratinocyte sheets prepared in temperature-responsive culture dishes have significantly enhanced survival because the basement membrane and intercellular adhesion proteins are preserved [14]. Hasegawa et al. conducted a two-stage wound repair experiment in nude rats, in which a human cultured epidermal sheet graft was transplanted onto bFGF-CGS-generated dermis-like tissue. They reported that the bFGF-CGS group showed a 2-fold increase in the take rate of epidermal sheet grafts compared with the conventional

artificial dermis group, speculating that this effect was due to the proliferation of epidermal basal cells and the production of basement membrane proteins such as type IV collagen [15]. These findings suggest that the sustained release of bFGF from bFGF-CGS improves the take rate of skin grafts by stimulating the production of basement membrane proteins.

The second possibility is that the sustained release of bFGF in the artificial dermis promotes neovascularization in the dermis-like tissue. bFGF is known to exert its action via its specific receptor, the FGF receptor, which is expressed in fibroblasts, smooth muscle cells, macrophages, endothelial cells, neurons, and astrocytes, and its most potent effect is thought to be angiogenic [16]. bFGF potentially induce angiogenesis via their powerful proliferative effects on endothelial cells and fibroblasts and facilitate endothelial cell migration by regulating proteolysis and adhesion molecules [17]. We have previously reported that transplantation of adipose-derived aldehyde dehydrogenase-expressing cells, a population of cells with a high expression ratio of FGF2 mRNA in rat stromal-vascular fractions, along with artificial dermis resulted in a significant increase in the number of neovessels in the dermis-like tissue in comparison with animals transplanted with other mesenchymal cells [18]. These results suggest that bFGF-CGF improved the take rate of skin grafts by promoting angiogenesis through continuous release of bFGF in the artificial dermis for 3 weeks and thereby accelerating vascularization of the autologous skin graft applied in the second operation.

In the present study, the mean waiting period for the group of patients who underwent skin grafting in less than 2 weeks was 9.7 days. A task for future research is to investigate the extent to which the waiting period can be further shortened by using bFGF-CGF. Recently, attempts have been made to achieve full-thickness skin reconstruction by simultaneous transplantation of a single-layer

artificial dermis and an autologous skin graft [19,20]. We are also interested in examining whether similar simultaneous transplantation can be performed using bFGF-CGF.

Limitations of this study include the small sample size and heterogeneity of the study population in terms of age and the location and type of injury. Further studies are needed in patients with the same type of injury (e.g., burns), the same location of injury, as well as surgical treatment (e.g., STSG and FTSG) in a larger study population.

6. Conclusion

In two-stage reconstruction of acute full-thickness skin defects using bFGF-CGF, there was no difference in the take rate of skin grafts between groups of patients who waited <14 days or ≥14 days before secondary skin grafting. Our data suggest that bFGF-CGF can form dermis-like granulation tissue with sufficient quality as a graft bed for skin transplantation within 2 weeks.

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Declaration of competing interest

The authors declare no conflicts of interest.

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