The Japanese Experience with Basic Fibroblast Growth Factor in Cutaneous Wound Management and Scar Prevention: A Systematic Review of Clinical and Biological Aspects

Mohamed Abdelhakim ⓘ · Xunxun Lin · Rei Ogawa

ABSTRACT

Introduction: Basic fibroblast growth factor (bFGF) plays several key roles in wound healing. Over the last 2 decades, clinical and basic research on bFGF has been actively conducted in Japan with reports on its potent efficacy in accelerating the healing of chronic ulcers and burn wounds by stimulating key cellular players in the skin. However, its efficacy remains unrecognized internationally. Thus, this study reviews current knowledge about the therapeutic value of bFGF in wound management and scar prevention accumulated in Japan over the last 2 decades.
Methods: We review the Japanese literature that demonstrates the anti-scarring effects of bFGF and exhaustively assess how these effects are exerted. Using the search terms "bFGF OR growth factors AND wound healing in Japan" and "bFGF AND scar prevention in Japan," we conducted a search of the PubMed database for publications on the role of bFGF in wound and scar management in Japan. All eligible papers published between 1988 and December 2019 were retrieved and reviewed.
Results: Our search yielded 208 articles; 82 were related to the application of bFGF for dermal wound healing in Japan. Of these, 27 fulfilled all inclusion criteria; 11 were laboratory studies, 7 were case reports, 4 were clinical studies, and 5 were randomized controlled trials.
Conclusion: Further research, with recognition of the therapeutic value of bFGF in wound and scar management and its clinical applications, is needed to provide additional clinical advantages while improving wound healing and reducing the risk of post-surgical scar formation.

Keywords: Basic fibroblast growth factor; Burn injuries; Chronic ulcer; Wound healing; Scar prevention

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Key Summary Points

This article reviews current knowledge about the therapeutic efficacy of bFGF in wound management and scar prevention, which has been accumulated in Japan over the last 2 decades, including Japanese animal model studies, case reports, clinical studies, and randomized controlled trials.

The application of bFGF immediately after debridement and before grafting can improve the healing of chronic ulcers or second-degree burns and prevent pathological scar development.

bFGF signaling promotes both fibroblast proliferation and migration in wound healing by activating the PI3K/Rac1/JNK pathway and may prevent pathological scar formation by inhibiting TGFβ1/SMAD signaling.

Several bFGF delivery systems are being developed, including controlled-release bFGF formulations such as bFGF gelatin sheets, which provide better healing outcomes. However, further research on these alternative formulations may provide optimal clinical advantages.

INTRODUCTION

Wound healing normally is a finely orchestrated multi-phase process that involves a variety of cell types. Studies on wound healing have revealed that dysfunction in some growth factors plays a role in non-healing wounds. These findings have directed advanced bioengineering technology to consider the use of exogenously prepared growth factors and cytokines.

Several growth factors have demonstrated potent efficacy for surgical use such as platelet-derived growth factor (PDGF) [1], vascular endothelial growth factor (VEGF) [2], fibroblast growth factor (FGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF),

transforming growth factor beta (TGF-β), and granulocyte–macrophage colony-stimulating factor (GM-CSF) [3, 4].

Preparations that contain recombinant growth factors are available for external use in various formulations, for example, solution, spray, ointment, and gel. However, as summarized in Table 1, the use of these preparations is associated with certain limitations regarding safety and drug delivery systems, which require high doses and/or repeated application, resulting in dire side effects including oncogenesis [5–7].

In this article, we focus on basic fibroblast growth factor (bFGF), a member of the FGF family of growth factors and signaling proteins. In 1988, Kaken Pharmaceutical Co., Ltd., Tokyo, Japan, obtained exclusive licensing rights to recombinant human bFGF (rhbFGF) Trafermin. This drug was originally developed for patients with decubitus ulcers and skin ulcers.

Following several clinical trials and extensive research and development focusing on rhbFGF for tissue regeneration, in 2001, Kaken Pharmaceutical successfully launched Fiblast®, the world's first rhbFGF preparation marketed in Japan as a topical spray for accelerating healing of burn wounds and diabetic leg ulcers. During the ensuing 8 years, it was observed that Fiblast® spray potently and safely promoted wound healing and reduced scar formation in the clinical setting throughout Japan. Based on these findings, along with additional clinical research and good safety data, the Clinical Practice Guidelines for Burn Injuries published by the Japanese Society for Burn Injuries in 2009 recommended bFGF as treatment for second-degree burns. Since then, bFGF has become widely used as a treatment modality for wound healing and scar reduction in the clinical setting in Japan.

However, the excellent efficacy of bFGF in wound healing remains largely unrecognized internationally. This may reflect the fact that while other countries have also conducted research on the ability of growth factors to improve wound healing, clinical and basic research in Japan has proactively investigated the role of bFGF in wound healing over the last

Table 1 Specific growth factors and their roles in clinical applications for promoting wound healing

Growth factors	Platelet-derived growth factor PDGF (Regranex®)	Vascular endothelial growth factor (VEGF 165)	EGF Epidermal growth factor (Heberprot-P®)
Administration	Topical	Topical	Topical or intralesional Injection
Type of wound	Diabetic foot ulcer	Diabetic foot ulcer	Burns, non-healing ulcer, and diabetic foot ulcer
Action	Maintains cell growth and division, chemo-attractant for mesenchymal cells, angiogenesis	Enhances angiogenesis; stimulating proliferation and migration of endothelial cells	Promotes proliferation and migration of keratinocytes and tensile strength of new skin; induces fibronectin production
Limitations	Increased cancer risk reported with higher doses	Few attempts of using VEGF as an adjunctive treatment in wound healing	Healing is hindered by matrix metalloproteinases rapidly degrading growth factors or cytokines.
	Minimal efficacy in pressure ulcers and venous ulcers	Most VEGF therapies are focused on anticancer treatments by inhibiting proliferation of tumor blood vessels	Lack of sophisticated delivery systems for providing sustained levels of EGF and inhibiting its degradation.
			Pain at the injection site is common
Reference(s)	[73–75]	[76–79]	[80, 81]

Recombinant growth factors are available in various formulations (e.g., solution, spray, ointment, and gel). However, some limitations regarding safety, cost, and drug delivery systems accompany these medications as listed above

20 years and are thus at the forefront of clinical experience and research leading this application.

In North America and Europe, significant attention has been paid and massive efforts have been focused on FGFs to generate FGFR inhibitors for application in the field of oncology [8, 9]. In China, recent advances in bioengineering have led to the production of FGF ligands, revealing their role in enhancing wound repair and regeneration of injured tissue [10–13].

Simultaneously, the clinical application of FGF ligands in the field of oncology as well as in wound healing has been reported in Japan. Nevertheless, the Japanese clinical experience with FGF ligand products, especially bFGF, has demonstrated a remarkable positive impact in accelerating the process of healing and in the management of chronic wounds such as diabetic foot ulcers. Thus, improving the recognition of the potential clinical application of bFGF in wound healing and tissue repair in the West is necessary to overcome the financial burden of wound management, such as diabetic foot ulcers, and to provide sufficiently improved wound healing.

Therefore, in this article we review current knowledge about the therapeutic value of bFGF in wound management and scar prevention, which has been accumulated in Japan over the last 2 decades. In particular, we review animal studies, case reports, clinical studies, and randomized controlled trials (RCTs) that demonstrate the anti-scarring effects of bFGF in Japan. We also exhaustively assess the mechanism by which bFGF exerts these effects.

METHODS

Literature Search and Data Selection

Using the search terms "bFGF OR growth factors AND wound healing in Japan" and "bFGF AND scar prevention in Japan," all original articles related to bFGF treatment in wound and scar management in Japan were identified by a PubMed search. Eligible papers published between 1988 and December 2019 were retrieved and reviewed. In addition, important reference articles from the included articles were reviewed. Three independent researchers performed the literature search to identify all relevant studies.

Inclusion Criteria
This study included articles that (1) reported on the use of topical bFGF in wound healing management and scar prevention; (2) described the effect of bFGF on these wounds. There were no restrictions with regard to the number of patients in the clinical study or the follow-up duration. The systematic review also aimed to include all in vivo studies that employed topical bFGF-treated models of wound healing and scarring along with all in vitro studies that involved bFGF-treated wounds or pathological scar-derived tissues/cells. The language of the article was not restricted to English.

Exclusion Criteria
The following articles were excluded: (1) articles on the use of bFGF for non-cutaneous wound healing; (2) imperfect literature reviews; (3) conference papers that reported data with unresponsive associated authors; (4) repeated publications.

Statistical Analysis

No formal statistical analysis was performed because the extensive methodological heterogeneity of the articles limited this study to a qualitative analysis. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

RESULTS

A total of 208 articles were identified from the search, as shown in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart for literature attrition (Fig. 1). After screening by title, 82 were relevant to the application of bFGF for dermal wound healing in Japan. Subsequently, 60 were screened by abstract for adherence to the inclusion criteria.

Finally, a total of 27 full-text articles satisfied all the eligibility criteria and were reviewed in their entirety including: 11 laboratory studies, 7 case reports, 4 clinical studies, and 5 RCTs. The included articles are summarized (Tables 2, 3).
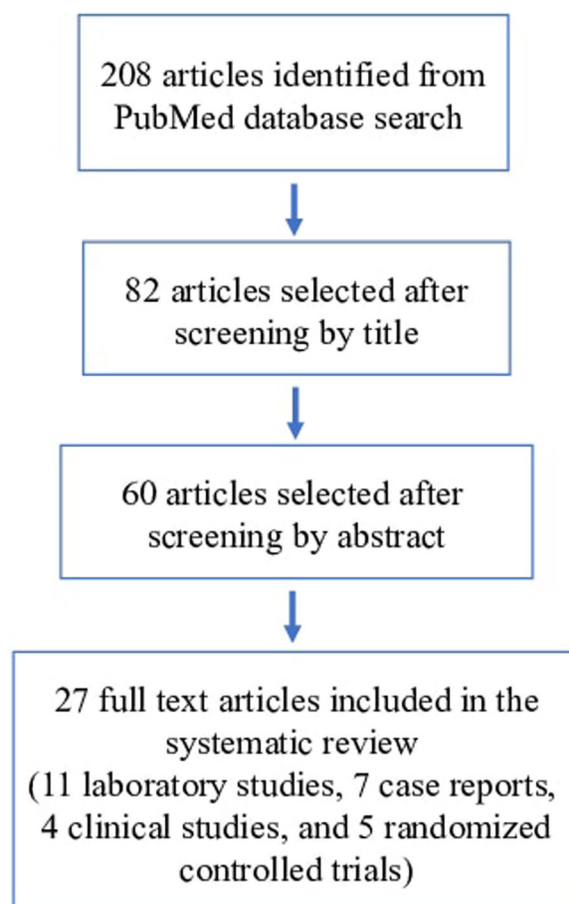

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(11 laboratory studies, 7 case reports, 4 clinical studies, and 5 randomized controlled trials)

Fig. 1 PRISMA flowchart showing literature attrition

Table 2 Laboratory studies on the role of bFGF in cutaneous wound management and scar prevention

Title	Type of study	Method	Number of subjects	Results
Eto et al. [33]	In vitro In vivo	Evaluation of the therapeutic remodeling effects of basic fibroblast growth factor (bFGF) treatment in an animal model using human hypertrophic scar tissue implanted into nude mice	6	Significant decrease in scar tissue weight and collagen quantity
Funato et al. [34]	In vitro	Examination of the effect of bFGF on apoptosis in normal rat palatal fibroblasts and rat palatal scar fibroblasts using the TUNEL assay	3	bFGF induced apoptosis in myofibroblasts during palatal scar formation
Akasaka et al. [50]	In vitro	Investigation of the mechanisms underlying pro-apoptotic effects of bFGF on granulation tissue fibroblasts during wound healing after pretreatment with transforming growth factor (TGF)-beta1	5–7	bFGF promoted apoptosis of injured tissue-derived fibroblasts pre-treated with TGF-β1
Kanazawa et al. [53]	In vitro	Examination of bFGF-induced fibroblast migration in wound healing with concurrent blockade of the effect of bFGF on fibroblast proliferation by using mitomycin-C	5	bFGF promoted dermal fibroblast migration during the wound healing process by activating the PI3K-Rac1-JNK pathway
Kawai et al. [65]	In vivo	Evaluation of the effect of artificial dermis with bFGF-impregnated gelatin microspheres or bFGF in solution when implanted into full-thickness skin defects on the back of guinea pigs	4	Incorporation of bFGF into the artificial dermis demonstrated effectiveness by accelerating fibroblast proliferation and capillary formation in a dose-dependent manner
Kanda et al. [66]	In vitro In vivo	Application of collagen-gelatin sponge (CGS) impregnated with 7 μg/cm^2 or 14 μg/cm^2 of bFGF to full-thickness skin defects of normal mice and decubitus ulcers created in diabetic mice (length of the neoepithelium, and total area of newly formed capillaries in CGS were evaluated)	36	Artificial dermis, CGS, impregnated with 7-μg/cm^2 bFGF accelerated dermis-like tissue formation 2 or 3 times earlier than artificial dermis alone

Table 2 continued

Title	Type of study	Method	Number of subjects	Results
Kanda et al. [67]	In vitro	Evaluation of the ability of a scaffold, CGS, for sustained release of bFGF, using a pressure-induced decubitus ulcer model in genetically diabetic mice by assessment of the wound area and histological assessment of neo-epithelization	40	CGSs impregnated with 7–14 μg/cm^2 bFGF accelerated wound healing
Tabata, et al. [68]	In vitro In vivo	Evaluation of the biological activity of controlled release of bFGF incorporated into gelatin hydrogel after subcutaneous implantation into the back of mice	6	Controlled release of biologically active bFGF caused by biodegradation of the acidic gelatin hydrogel induced a prolonged vascularization effect
Tabata et al. [69]	In vivo	In vivo release of bFGF from a biodegradable gelatin hydrogel carrier was compared with in vivo degradation of hydrogel in a diffusion chamber, and implanted in the mouse subcutis for certain periods of time	6	Biologically-active bFGF was released as a result of in vivo degradation of the hydrogel and induced significant neovascularization
Mizuno et al. [70]	In vitro In vivo	Examination of the stability of bFGF in a chitosan film and the therapeutic effect on wound healing in genetically diabetic mice (db/db mice)	5	The rate of healing was accelerated by promotion of fibroblast proliferation and granulation tissue formation
Matsumoto et al. [72]	Ex vivo	Histological analyses of effectiveness of bFGF-impregnated gelatin sheet in a murine model	4	The findings suggested that controlled release of bFGF using gelatin sheet is effective for promoting wound healing

DISCUSSION

Clinical and Experimental Significance of bFGF

Local bFGF Treatment Accelerates Wound Healing

Wound healing normally begins with hemostasis and then progresses through three overlapping phases driven by biomechanical responses, namely, inflammation, proliferation, and maturation. bFGF, along with other growth factors, plays key roles in wound healing as shown by the significantly slower healing of full-thickness skin wounds in bFGF-knockout mice than in wild-type mice [14]. Moreover, many studies show that external application of bFGF promotes wound healing. For example, a study found greatly improved skin hardness with local bFGF injections following lower limb reconstructive surgery with tissue engineering using a tissue scaffold and secondary split-thickness skin graft [15]. Similarly, increased granulation and accelerated epithelialization were observed after treating rat full-thickness acute incisions with bFGF fused to a fibrin-binding peptide (Kringle1) compared with

Table 3 Clinical studies on the role of bFGF in cutaneous wound management and scar prevention

Title	Type of study	Method	Number of subjects	Results
Akita et al. [19]	Randomized controlled trial (RCT)	Clinical assessment of postoperative color uniformity in split-thickness skin grafting	40	bFGF treatment contributed to a better color match with skin grafting postoperatively
Morimoto et al. [20]	RCT	Clinical evaluation of the safety and efficacy of collagen/gelatin scaffold impregnated with bFGF (7 or 14 µg/cm^2) in the treatment of chronic skin ulcers after debridement	17	In 16 out of 17 patients, the wound bed improved 14 days after application without serious adverse reactions
Uchi et al. [21]	RCT	Clinical assessment of topical bFGF treatment of non-ischemic diabetic ulcers for 8 weeks, in a dose-dependent manner	150	bFGF accelerated wound healing in diabetic ulcers with 75% or greater reduction in the area of the wound
Akita et al. [22]	RCT	Clinical assessment of patients receiving topical bFGF or no bFGF; outcomes were compared for clinical scar extent, passive scar hardness, elasticity, and moisture analysis of the stratum corneum at 1 year after complete wound healing	153	Combined use of bFGF and artificial skin substitute led to improved wound quality (scars) and facilitated wound healing
Hayashida et al. [23]	RCT	Clinical evaluation of the effect of bFGF treatment in pediatric patients with deep second-degree burn wounds	20	Accelerated healing, reduced scarring, and improved color matching with normal skin compared with controls up to half a year postoperatively
Akita et al. [15]	Clinical study	Clinical assessment of sequential lower extremity reconstruction using an artificial dermis with or without bFGF administration, and secondary split-thickness skin grafting by measuring hardness using a durometer, and moisture parameters for at least 6 months after the final procedure and comparison with normal skin controls	12	bFGF-treated sequential artificial dermis and skin grafting demonstrated better scarring and well-organized stratum corneum after healing

Table 3 continued

Title	Type of study	Method	Number of subjects	Results
Ono et al. [31]	Prospective clinical study	Investigation on bFGF injected into the dermis of wound margins in full-thickness wounds in patients postoperatively after resection of skin tumors	230	Significant reduction of hypertrophic scarring and widening of remnant scars without any serious side effects
Sugamata et al. [62]	Clinical study	Clinical assessment of combined artificial dermis and application of Fiblast® spray in 6 cases of traumatic fingertip amputation	6	The shapes of the fingertips were satisfactorily reconstructed in all patients
Matsumoto et al. [72]	Clinical study	Examination of the safety of bFGF-impregnated gelatin application for patients	4	The findings suggested that controlled-release bFGF using gelatin sheet is effective for promoting wound healing
Saijo et al. [82]	Case reports	Application of combined treatment of artificial dermis and bFGF to treat cranial bone-exposing wounds followed by free skin grafting	2	The formation of granulation tissue was promoted and acted as a wound bed for the subsequent skin grafting
Kurokawa et al. [83]	Case report	Topical application of bFGF to chronic leg ulcers at a dose of 30 μg/day for 3 months	1	The ulcer area was reduced by promoted angiogenesis, re-epithelization, granulation and scar formation
Asai et al. [84]	Case report	Topical application of a mixture of peripheral blood mononuclear cells (PBMC) and bFGF to diabetic foot ulcer	1	The ulcer was completely closed and no new ulceration recurred after 6 months follow-up
Ito et al. [85]	Case report	Combined application of bilayer artificial dermis graft and bFGF without secondary skin grafts or flaps to large soft tissue defect of the heel after a degloving injury	1	bFGF induced wound closure and successful reconstruction without sacrificing skin from donor sites
Akita et al. [86]	Case report	Simultaneous application of bFGF with regular surgical debridement and skin grafting was investigated for skin hardness by clinical examination and instrumental measurement	1	Wounds treated with bFGF produced scars that were significantly less hard 1 year after final wound closure

Table 3 continued

Title	Type of study	Method	Number of subjects	Results
Muneuchi et al. [87]	Case reports	0.1 mL of bFGF (Fiblast®) was injected into the sutured collagen layer of artificial dermis followed by daily injection of bFGF for 2 weeks, topical ointment with recombinant bFGF was then continued	2	The wound was completely epithelialized 10 weeks after injury with good sensory recovery
Yamaka et al. [88]	Case report	Topical application of rhbFGF 30 μg/daily in combination with prostaglandin E1 ointment applied directly to therapy-resistant chronic leg ulcers in scleroderma	1	The lesions were completely re-epithelialized within 20 days and the ulcers did not recur during the 20-month follow-up period

positive controls. The authors also noted that after subcutaneous implantation, a fibrin scaffold suffused with the Kringle1-bFGF fusion protein efficiently induced local angiogenesis, indicating that this approach was also useful for wounds deficient in plasma clot formation [16].

In addition, bFGF treatment of incisional wounds in diabetic rats was found to improve epithelialization, granulation, and wound-breaking strength to levels observed in non-diabetic mice [17].

Several RCTs have been conducted in Japan to examine the efficacy and safety of bFGF in treating chronic skin ulcers, diabetic ulcers, and venous ulcers. An RCT on the utility of Fiblast® spray to improve healing of ulcers or second-degree burns after debridement and split thickness skin grafting revealed that applying bFGF immediately after debridement and before grafting significantly improved scar quality and graft color match [18, 19].

Furthermore, a first-in-human RCT evaluated the utility of an artificial dermis with sustained-release bFGF to improve healing of chronic skin ulcers. The artificial dermis comprising a collagen/gelatin sponge was designed to release bFGF for > 10 days. The patients were randomized to application of either a low-dose (7 mg/cm^2 bFGF impregnation) or high-dose (14 mg/cm^2 bFGF impregnation) preparation after

wound debridement. The degree of wound improvement was assessed 14 days after the sponge was applied. Of the 17 patients divided into the two low- and high-dose groups, 16 demonstrated significant wound improvement, as indicated by the granulation and epithelialization that reduced the wound area by ≥ 50%. No serious adverse effects were reported [20]. Moreover, a placebo-controlled RCT assessed the ability of topical 0.001% (50 mg) or 0.01% (500 mg) bFGF application for up to 8 weeks to improve healing of non-ischemic diabetic ulcers. The low- and high-dose treatments reduced the wound area by > 75% in 72.3% and 82.2% of the patients, respectively. In contrast, this reduction was only seen in 57.5% of the placebo-treated patients. Thus, bFGF treatment accelerated diabetic ulcer healing [21]. In addition, bFGF treatment was found to improve healing of partial thickness skin burns in two RCTs. The first trial was in adult patients; 119 wounds were treated with topical 1 μg/cm^2 bFGF, while 122 wounds received standard care. The bFGF-treated wounds closed on average 3 days earlier than the control wounds. The second trial was in pediatric patients who were treated with the same regimen used in the adults; the 15 treated wounds closed on average 3.7 days before the control wounds [22, 23].

Local bFGF Treatment Prevents Pathological Scar Formation

Scarring is influenced by multiple factors, and this accounts for the wide variations of the different clinical types of scars. These range from typical flat white scars, sunken atrophic scars, and aggressively growing fibroproliferative pathological scars, namely, keloids and hypertrophic scars [24].

These pathological scars often develop following trauma and burn injuries and are quite common; in the US alone about 40 million cases of pathological scars are reported every year [25]. Over the past decade, findings from clinical and animal studies have greatly improved our understanding of the pathogenesis of keloids and hypertrophic scars, particularly that their development and progression is driven by chronic inflammation [26]. This knowledge has facilitated the development of therapies that prevent or reduce pathological scarring and act at least in part by blocking or ameliorating wound/scar inflammation. These include silicone tape, compression garment therapy, steroid injections, laser therapy, cryosurgery, radiotherapy, and surgical excision [26–30]. However, because pathological scars remain difficult to treat, there are continued ongoing research efforts to develop new therapies to prevent their formation. In Japan, studies showing that bFGF accelerates wound healing and smooth epithelialization have led to considerations that local application of bFGF could also prevent the formation of pathological scars in trauma and burn injuries. This hypothesis was supported by a report where immediate rinsing with or injection of bFGF into sutured incisional wounds following resection of skin tumors significantly reduced the likelihood of developing hypertrophic scars or scar widening after healing versus untreated control wounds [31]. Also, repeated bFGF treatment started soon after wounding for formed hypertrophic scars in full-thickness excisional wounds in rabbit ears greatly decreased pathological scarring as shown by significant reductions in scar elevation and epidermal thickness indices [32].

Local bFGF Treatment May Reduce Pre-existing Pathological Scars

Some evidence suggests that bFGF treatment could also reduce pre-existing pathological scars. Eto et al. showed that implanting human hypertrophic scars onto nude mice and treating the scars with a controlled-release bFGF formulation decreased the size of the scars. The hydroxyproline content also decreased, and collagen fiber degradation was observed. Thus, bFGF induced collagen degradation in hypertrophic scars. This observation was supported by the fact that bFGF treatment upregulated matrix metalloproteinase-1 expression in fibroblasts from hypertrophic scars, but not in fibroblasts from normal dermis [33]. Also, there is some evidence that bFGF could induce myofibroblast apoptosis. Funato et al. reported that converting palatal fibroblasts into myofibroblasts followed by treatment with bFGF resulted in apoptosis. Interestingly, they showed that compared to normal palatal fibroblasts, myofibroblasts from palatal scars are more sensitive to bFGF, as shown by the greater tyrosine autophosphorylation of their bFGF receptors [34].

Cellular Mechanism and Signaling Pathways of bFGF

Local bFGF Treatment Accelerates Wound Healing by Several Cellular Mechanisms

The mechanisms underlying these wound healing effects of bFGF reflect the multiple properties of this glycoprotein. Specifically, due to its strong mitogenic properties, bFGF accelerates the division and proliferation of cells in the wound bed, namely, endothelial cells, dermal fibroblasts, and keratinocytes. Moreover, its chemoattractant properties influence the migration of these cells during wound healing, thereby promoting neovascularization and epithelialization. In addition, bFGF attracts leukocytes to the wound, thus helping to initiate the inflammatory phase of wound healing. It also strongly stimulates the production and metabolism of major extracellular macromolecules such as collagen, tropoelastin, and

hyaluronic acid by fibroblasts, thereby shaping scar tissue maturation [35].

bFGF Signaling Pathways that Participate in Wound Healing

bFGF is known to efficiently promote fibroblast proliferation and migration [36, 37]. The effects of bFGF on signaling pathways that drive these and other important fibroblast functions in normal and abnormal wound healing remain incompletely understood. However, there are several lines of evidence suggesting that FGFs, and in particular bFGF, activate other signaling pathways in fibroblasts, as follows.

In relation to the signaling initiated in fibroblasts by bFGF exclusively, in vitro and in vivo studies show that bFGF accelerates human fibroblast migration by independently activating the PI3K/Rac1/JNK pathway [38]. This is supported by several studies on cultured human dermal fibroblasts, which show that bFGF treatment induces fibroblast migration by significantly increasing Akt, Rac1, and JNK activity [39, 40]. Moreover, Shi et al. showed that reactive oxygen species (ROS) are a key component in this cascade of events [41]. These findings led to proposing the notion that bFGF promotes the migration of human dermal fibroblasts by binding to and activating FGFR, which stimulates Akt. This drives the phosphorylation of Rac1, which activates both JNK and NOX [41]. Both events then lead to the production of ROS, which activates FAK and paxillin, in turn inducing the cytoskeletal rearrangement needed for fibroblast migration (Fig. 2) [41, 42].

bFGF Could Prevent Pathological Scarring by Various Mechanisms

Regulating ECM Synthesis and Degradation bFGF may prevent excessive dermal deposition of collagen, which plays a crucial role in the development and progression of keloids and hypertrophic scars. This deposition is the result of an imbalance between collagen synthesis and degradation that leads to a disorganized and voluminous fiber structure in the dermis [43, 44]. While collagen deposition is a necessary part of normal wound healing, excessive deposition causes pathological scarring [45, 46]. Because bFGF treatment accelerates the appropriate deposition and distribution of collagen in wounds [47–49], the application of bFGF to the injured area soon after wounding
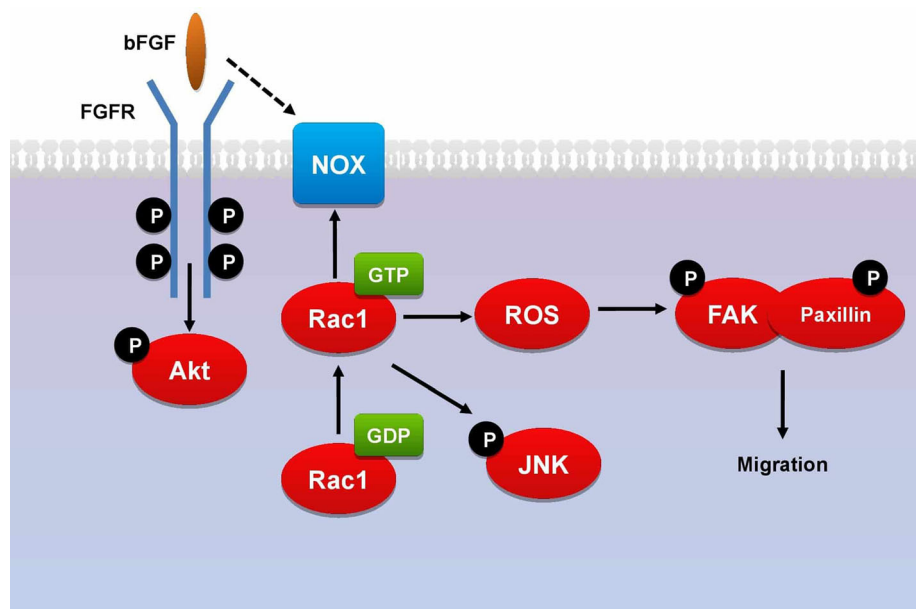

Fig. 2 Proposed signaling pathway of bFGF in promoting the migration of human dermal fibroblasts by activating PI3K/Akt-Rac1-FAK-JNK signaling, which induces the cytoskeletal rearrangement needed for fibroblast migration

may hasten the appropriate development of the ECM in the wound, and this effect of local bFGF may help to prevent the onset of the chronic inflammation that drives pathological scarring.

Inducing Apoptosis of Granulation-Tissue Fibroblasts bFGF treatment was reported to reduce collagen density, decrease expression of fibronectin, tissue inhibitor of metallopro- teinase-1, collagen I, and collagen III, and increase expression of matrix metallopro- teinase-1 in ear wounds. bFGF also increased apoptosis of fibroblasts in granulation tissue [32]. This is consistent with a report where bFGF treatment induced apoptosis in granulation- tissue fibroblasts. In contrast, bFGF had no effect on fibroblasts from uninjured dermis, suggesting that bFGF might inhibit scar forma- tion by promoting programmed cell death of fibroblasts in granulation tissue throughout the remodeling stage of wound healing [50].

Interfering with Myofibroblast Forma- tion bFGF may also prevent pathological scarring by interfering with the formation of myofibroblasts, which is considered the most important mechanism underlying the develop- ment of keloids and hypertrophic scars. Myofi- broblasts are activated collagen-hypersecreting cells that differentiate terminally from the nor- mally quiescent dermis-derived cells (particu- larly dermal fibroblasts) into activated collagen- hypersecreting myofibroblasts. This event occurs by a process called endothelial/epithelial to mesenchymal transition (EMT). Formation of myofibroblasts is the most important mecha- nism underlying the development of keloids and hypertrophic scars [36]. Indeed, Tiede et al. showed that multipotent human dermis- derived progenitor cells tend to differentiate into myofibroblasts in the presence of serum and that co-culture with bFGF inhibits this mesodermal differentiation; bFGF-cocultured cells expressed much lower levels of the myofi- broblast marker alpha-smooth muscle actin (α- SMA) than control cells [36]. It is likely that this effect of bFGF is also mediated by the ability of this cytokine to inhibit the TGFβ1/SMAD-de- pendent pathway, and the study of Shi et al. in hypertrophic rabbit ear showed that topical application of bFGF downregulated α-SMA expression as well as TGFβ1/SMAD signaling [32]. This is believed to be initiated through the expression of miR-20a, which in turn represses the expression of the TGFβ receptor complex (ALK5 and TGFBR2) and SARA, thus inhibiting Smad2 and Smad3 activation, which plays an important role in the development of fibropro- liferative disorders (Fig. 3) [51].

Finally, bFGF may impede pathological scarring by promoting fibroblast proliferation [37, 52]. It may also act by enhancing fibroblast migration. Kanazawa et al. showed that bFGF promoted the migration of fibroblasts when the proliferation of these cells was blocked by mit- omycin C treatment [53].

Effect of bFGF on Non-fibroblast Cell Types

The findings described above show that bFGF promotes fibroblast migration and perhaps proliferation, differentiation, and survival of these cells also. It should be noted that the name fibroblast growth factor (FGF) reflects the fact that these molecules were initially known for these effects on fibroblasts. However, it was later shown that FGFs were identical to endothelial cell growth factors [54, 55]. Thus, FGFs do not act specifically on fibroblasts. Moreover, several animal studies showed that bFGF affects not only fibroblast and endothelial cell functions [56, 57], but also influences ker- atinocyte proliferation and migration [58, 59], nerve regeneration [60], and vascular smooth muscle cell proliferation [61].

Methods of bFGF Delivery for Improving Wound Healing

In Japan, hrbFGF preparation Fiblast® spray has been used as a topical spray to improve wound healing by directly spraying it onto the wound area. This is then followed by applying a stan- dard dressing. Its efficacy in this formulation is demonstrated in a study by Sugamata et al. where the healing period was shortened and good reconstruction of the fingertip shapes was achieved in amputated human fingertips
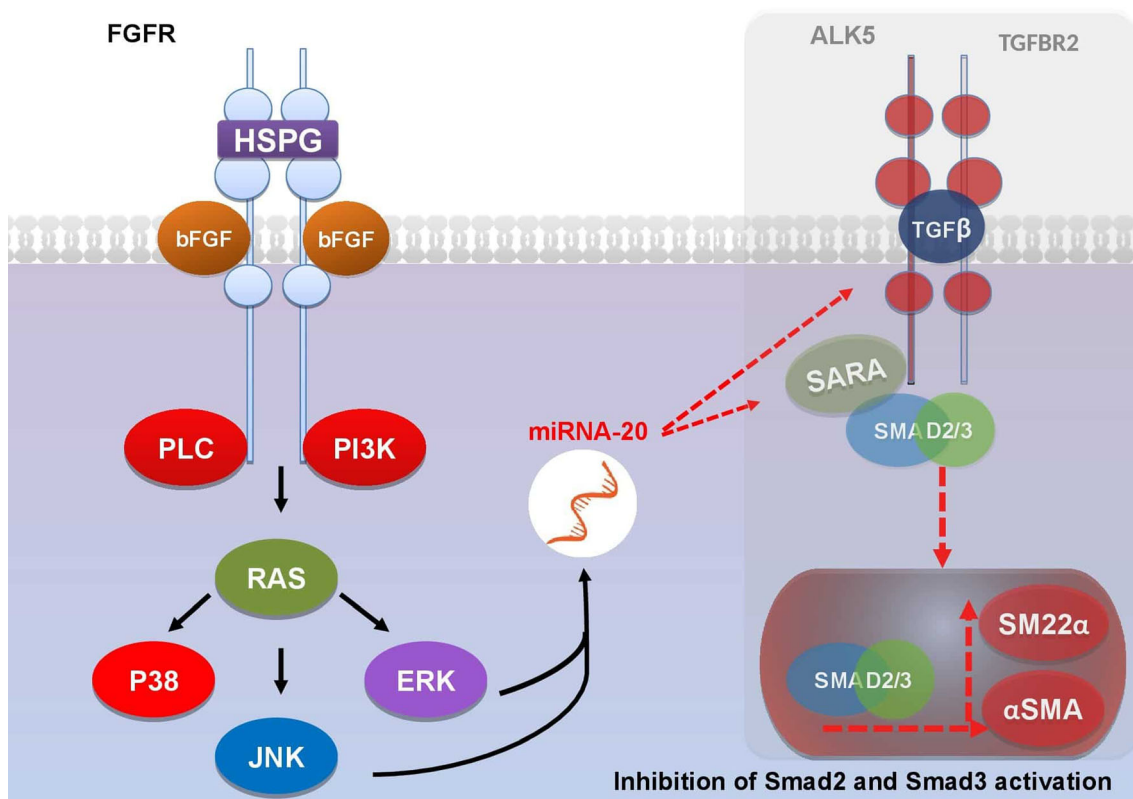
Fig. 3 bFGF downstream mediators Ras and PI3K activate miR-20a expression, which in turn inhibits the expression of the TGFβ receptor complex (ALK5 and TGFBR2) and SARA, thus blocking the Smad2 and Smad3 activation, which plays an important role in the development of fibroproliferative disorders

reconstructed with artificial dermis and then treated with Fiblast® spray [62].

Currently, Fiblast® spray is routinely used throughout Japan for negative pressure wound therapy. This combination therapy became available after the Japanese national health insurance system coverage of the use of the V.A.C. ATS® Negative Pressure Wound Therapy System (Kinetic Concepts, Inc., Tokyo, Japan). This widespread use of the Fiblast® spray is emblematic of its considerable effectiveness in wound healing.

Controlled Release of bFGF Is Associated with Increased Efficacy

Free bFGF is water soluble and is rapidly degraded in vivo. Thus, it has a short half-life [63]. Therefore, various formulations have been developed to prevent this degradation. Xiang et al. showed that bFGF-encapsulated liposomes (bFGF-lip) improved healing of deep second-degree burns in rats relative to treatment with blank liposomes [64]. Similarly, another study by the Department of Plastic and Reconstructive Surgery in the Graduate School of Medicine of Kyoto University showed that artificial dermis with incorporated biodegradable gelatin bFGF-containing microspheres when implanted into full-thickness skin defects in guinea pigs accelerated fibroblast proliferation and wound angiogenesis [65]. Further experiments by this group showed that implantation of murine full-thickness skin wounds with bFGF-impregnated collagen/gel sponges greatly accelerated the formation of dermis-like tissue [66]. This group also found that similar treatment of debrided decubitus ulcers in diabetic mice accelerated wound closure, epithelialization, and angiogenesis [67]. Thus, this group began an investigator-initiated first-in-human RCT in 2010 to examine the effects of this approach in patients with persistent ulcers. The findings were
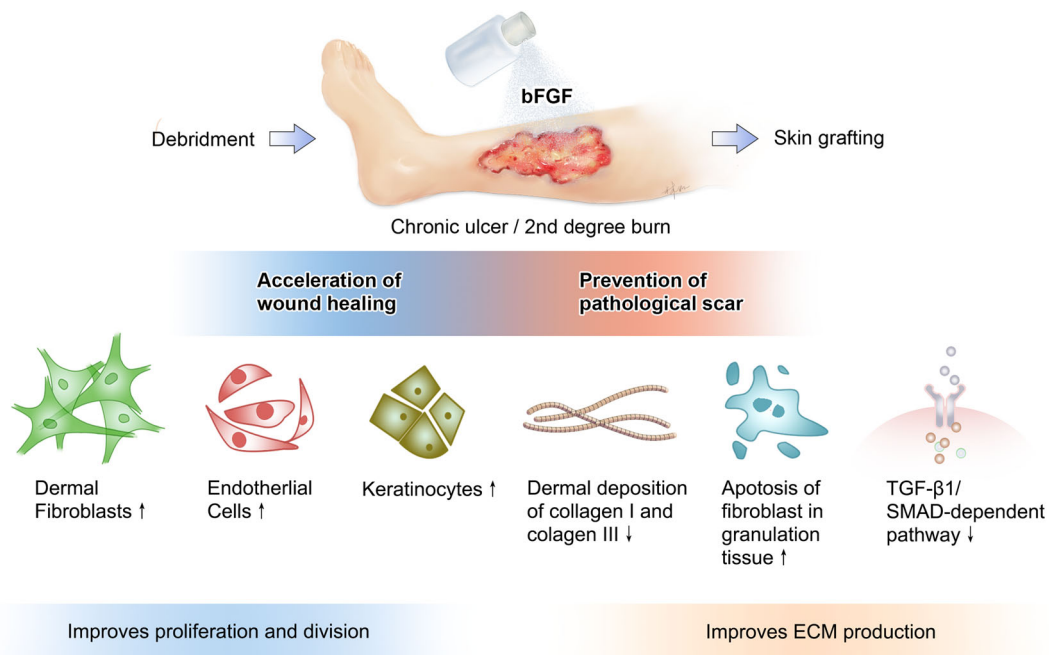
Fig. 4 Illustrative overview of the biological actions of bFGF application in chronic ulcer and burn injury involved in accelerating wound healing and preventing pathological scar development

reported in 2013 as described previously. Briefly, an artificial dermis consisting of a collagen/gelatin sponge with sustained release of bFGF for > 10 days significantly improved chronic ulcer healing [20].

Another group examined the effect of implanting a controlled-release bFGF-impregnated gelatin hydrogel in mouse incisions. This innovative approach significantly increased neovascularization when subcutaneously implanted in the back of the mice [68, 69]. This early study showed that a gelatin scaffold could release a single growth factor or drug in a controlled fashion, thereby increasing the efficacy of the active agent in wound healing.

Fiblast® Spray vs. bFGF-impregnated Gelatin Sheet

In contrast with studies reporting the effectiveness of bFGF in accelerating wound healing, a study has shown no significant difference between bFGF-chitosan film compared with chitosan film only when applied to surgically created full-thickness wounds in diabetic mice [70]. Additionally, a pilot, randomized, double-blind study compared the topical application of

bFGF with placebo in 17 diabetic patients with neurotrophic foot ulcers and reported similar outcomes of bFGF and placebo for healing chronic diabetic ulcer of the foot. Thus, it was hypothesized that using a single growth factor such as bFGF alone might be insufficient for healing of such severe wounds [71].

The effectiveness of the bFGF-impregnated gelatin sheet was compared with conventional spray administered in a murine model for wound healing. Compared with the bFGF spray group, the bFGF-gelatin group demonstrated significantly better wound healing outcomes including faster wound closure, greater area of mature collagen at the early stage of wound healing, and higher vascular density. This suggests that bFGF-impregnated gelatin sheet yields a better acceleration effect on wound healing compared with bFGF spray [72].

There is now considerable evidence showing that bFGF accelerates healing of a wide range of wounds, including pressure ulcers, second degree burns, leg ulcers, and diabetic ulcers, in addition to prevention of scar formation. A large body of research also indicates that bFGF effectively accelerates the process of wound

healing and can prevent the development of pathological scars as illustrated in Fig. 4. This is as a result of stimulating the proliferation, differentiation, and migration of fibroblasts and endothelial cells and keratinocytes in the skin, in addition to regulating ECM production and metabolism as previously discussed. These potent effects on key cellular players in wound healing account for the widespread routine use of the commercially available rhbFGF preparation Fiblast® spray to promote wound healing and prevent fibroproliferative scarring in the clinical setting throughout Japan.

This study has some limitations. First, the review was restricted to the Japanese literature and as such may not reflect research findings abroad. Second, the list of keywords used for retrieving articles in the PubMed database search was incomplete and thus may not represent the full range of such studies. Third, the majority of the studies were animal studies and so are not representative of clinical applicability. Hence, further translational research is needed.

CONCLUSION

In the 2 decades since Fiblast® spray was launched in Japan, several other bFGF delivery systems have been developed, including controlled-release bFGF formulations such as bFGF gelatin sheets. Further research on these alternative formulations is needed to clarify additional clinical advantages, including greater convenience and lower treatment costs, while simultaneously efficiently improving wound healing and reducing the risk of post-surgical scar formation.

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Data Availability. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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