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(2010年4月1日～2011年3月31日迄)

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ

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研究成果の刊行物・印刷物

Note

MESENCHYMAL STEM CELL THERAPY FOR CUTANEOUS RADIATION SYNDROME

Sadanori Akita,* Kozo Akino,† Akiyoshi Hirano,* Akira Ohtsuru,‡ and Shunichi Yamashita‡§**

Abstract—**Systemic and local radiation injuries caused by nuclear power reactor accidents, therapeutic irradiation, or nuclear terrorism should be prevented or properly treated in order to improve wound management and save lives. Currently, regenerative surgical modalities should be attempted with temporal artificial dermis impregnated and sprayed with a local angiogenic factor such as basic fibroblast growth factor, and secondary reconstruction can be a candidate for demarcation and saving the donor morbidity. Human mesenchymal stem cells and adipose-derived stem cells, together with angiogenic and mitogenic factor of basic fibroblast growth factor and an artificial dermis, were applied over the excised irradiated skin defect and were tested for differentiation and local stimulation effects in the radiation-exposed wounds. The perforator flap and artificial dermal template with growth factor were successful for reconstruction in patients who were suffering from complex underlying disease. Patients were uneventfully treated with minimal morbidities. In the experiments, the hMSCs are strongly proliferative even after 20 Gy irradiation in vitro. In vivo, 4 Gy rat whole body irradiation demonstrated that sustained marrow stromal (mesenchymal stem) cells survived in the bone marrow. Immediate artificial dermis application impregnated with cells and the cytokine over the 20 Gy irradiated skin and soft tissues demonstrated the significantly improved fat angiogenesis, architected dermal reconstitution, and less inflammatory epidermal recovery. Detailed understanding of underlying diseases and rational reconstructive procedures brings about good outcomes for difficult irradiated wound healing. Adipose-derived stem cells are also implicated in the limited local injuries for short cell harvesting and processing time in the same subject.**
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* Department of Plastic and Reconstructive Surgery, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan; † Department of Neuroanatomy, Nagasaki University, Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan; ‡ Takashi Nagai Memorial International Hibakusha Medical Center, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan; § Atomic Bomb Disease Institute, Nagasaki University, Nagasaki University, Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan; ** World Health Organization, Avenue Appia 20, 1221 Geneva 27, Switzerland.

For correspondence contact: Sadanori Akita, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan, or email at akitas@hf.rim.or.jp.

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Key words: World Health Organization; exposure, radiation; radiation damage; radiotherapy

INTRODUCTION

THERE IS increasing worry regarding both systemic and local radiation injuries caused by nuclear power plant (NPP) reactor accidents, therapeutic irradiation for malignancy, interventional radiology (IVR) of unexpectedly prolonged fluoroscopic procedures for cardiovascular diseases such as arrhythmia or ischemic heart diseases, or nuclear medicine over-dose intakes of the radioactive material for internal radiation therapy. These conditions should be properly treated and prevented in order to save lives and improve local wound healing (Francois et al. 2007). However, total clinical analysis and experimental evidence-based data were not available. Nagasaki University authors' group was selected as the global strategic center for radiation health risk control by Japan's Ministry of Education, Culture, Sports and Technology and is now working to establish therapeutic regimens, guidelines for prevention of radiation injuries, and possible regeneration medical and surgical therapy for radiation injuries using patients' own adipose tissue-derived stem cells.

Often seen chronic radiation injuries are well handled by sufficient blood supply to the radiated tissues, especially in the cartilage, bare bone, and hardened scar tissues. For this purpose, local-, distant- and microsurgical vascularized flaps are applied. Recent development of micro-vasculature of the skin and soft tissues including the connective tissues plays a major role in acceleration of local wound healing. Also, externally administered angiogenic growth factors such as basic fibroblast growth factor (bFGF) together with temporal wound coverage of artificial skin substitute is very effective for those patients with severe injuries and patients with co-morbidities who are intolerant to extensive surgeries (Akita et al. 2006). In contrast, acute phase radiation injury often results in a fluctuated response to medication and surgery. Also, systemic exposure of radiation often

ameliorates the body immune response, cellular proliferation, and differentiation capacity in total body; thus early administration of cells, preferably radiation-resistant, cell-renewing, and of high differentiation capacity (in this context, stem cells from the bone marrow or adipose cells), are recommended. In order to elucidate efficacy of these stem cells, both in vitro and in vivo experiments are undertaken.

MATERIALS AND METHODS

Chronic local radiation injuries

Often experienced in radiation therapy for malignancy, cardiovascular modalities should be categorized as difficult wounding with poor vasculature.

From January 1990 to April 2007, 10 (8 females and 2 male) patients who demonstrated radiation injuries such as telangiectasia, xerosis, epidermal atrophy karatoses, and fibrosis, as well as deep ulcers in the costal ribs and sternum due to adjuvant radiation therapy post-mastectomy or prolonged fluoroscopic procedures for cardiovascular diseases, were surgically treated and included in this investigation.

Other selective clinical cases used angiogenic growth factor, namely human recombinant basic fibroblast growth factor (rh-bFGF), which is clinically approved and widely used in Japan for clinical wounds with skin substitutes, which are also clinically available not only in Japan but many other nations including the U.S., the majority of EU nations, and several Asian counties, and the effectiveness of using the artificial skin substitutes in the chronic radiation injuries is temporal coverage and sustainability of both internal and external cells and growth factors. Therefore, combined use of bFGF and artificial skin substitute leads to improved quality of wounds (scars) as well as facilitated wound healing (Akita et al. 2008). Additionally, one case was treated with autogenous adipose-derived stem cells (ADSCs) for a sacral radiation ulcer for the first time in the world; the injury was caused 40 y previously by a therapeutic radiation at fractionate 50 Gy.

Acute local radiation injuries

When the radiation does not affect harvesting donor-sites such as abdomen, thighs, buttock and arms, adipose-derived regenerative cells (ADRCs) are often the first choice for immediate regeneration for radiation-exposed wounds since the lipoaspirated fat cells are easily processed within a few hours in a closed circuit of the processing machine used for each specific patient.

The Internal Review Board (IRB) of the ethics committee of Nagasaki University approved this modality for radiation injured wound healing (No. 08070296).

Acute systemic radiation injuries

Extensive in vitro and in vivo studies are explored using human mesenchymal stem cells since these cells are readily available in frozen cell stockpiles, and thus offer potential therapeutic regimens for unscheduled radiation injuries. Also, the ADSCs are a prime candidate for stem cell banking and stockpiling.

An in vivo model, and whole body irradiation by an x-ray generator

Animals 10 wk old and weighing 300–350 g were used. Animals were obtained from CLEA JAPAN (Tokyo, Japan) and housed in the laboratory animal center for biomedical research, Nagasaki University School of Medicine (Nagasaki, Japan). The protocol of the animal experiment was approved by the Institutional Animal Care and Use Committee of Nagasaki University, No. 0204080111. They were handled according to the guidelines established for animal care at the center. Each rat had free access to both sterile water and standard rodent soft chow ad libitum.

4 Gy or 20 Gy whole body irradiation to 10 nude rats (F344/NJCl-rnu) that had deleted T-cell function (and thus acute immune rejection to human derived cells was minimized) were performed at Atomic bomb Disease Institute, Nagasaki University, by an x-ray radiation generator (EXS-300-5, 200kV, 15 mA, 0.405 Gy min^{-1}; Toshiba Medical Systems Corporation, 1385 Shimoishigami, Otawara-shi, Tochigi-ken, Japan). Animals were divided into 2 groups of 5 each: control group and hMSCs with bFGF-treated group. Surgical procedures were performed immediately after irradiation.

Angiogenic growth factor, basic fibroblast growth factor (bFGF)

Genetically recombinant human bFGF (Fiblast®, Trafermin) was purchased from Kaken Pharmaceutical Co., Inc (Bunkyo, Tokyo, Japan). The freeze-dried samples were dissolved in phosphate buffered saline (PBS) at a concentration of 1 mg mL^{-1} and dissolved in culture medium 30 min before experimental use.

RESULTS

Chronic local radiation injuries

All surgeries were uneventfully performed, the mean post-operative follow-up was 11 y and 3 mo (3 y to 16 y), and the average age was 67 y (53 to 78 y). Above all, in the cases of the compromised hosts such as the aged with systemic conditions, there has already been successful treatment with the patient's own ADSCs for the intractable local radiation injury in our institute. The patient was very old and was first not under consideration for this new modality; however, considering the patient's

other condition, this clinical trial was applied, and 81 days after regenerative surgery the chronic radiation injury completely healed, and demonstrated to be durable to external stimuli 3 weeks later at 103 days post-op (Fig. 1).

Acute local radiation injuries

Autologous ADSCs (or "regenerative" cells), which are distant from radiation sites, are most favorable for regeneration and conditioning for the pre-reconstruction procedures. When the radiation is safely distant from the adipose cell donor sites such as lower abdomen, thighs, buttocks, and arms, then liposuction of the adipose cells is started. Approval for treatment of the radiation injuries is obtained through IRB of Nagasaki University, and the adipose cell processing in the operation room within 2 h is underway using a Celution™ system with a collaboration of Cytori Therapeutics, Inc. (San Diego, CA). Preliminary studies of the cell characteristics of the ADSCs or ADRCs are very comparable to those of the hMSCs, and thus experimental data with hMSCs may be applicable to the ADSC/ADRC in regeneration and wound healing. Among notable characteristics, multilineage differentiation mechanisms promote the complex difficult wound healing in regards to epithelialization, neo-vascularization, and matrix deposition by fibroblast production (Zuk et al. 2002).

Acute systemic radiation injuries

An in vitro stem cell biology and analysis. In order to investigate human mesenchymal stem cell proliferation, sub-confluent cultured hMSCs were used and irradiated by an x-ray radiation generator. The cells were immediately transferred to the incubators after irradiation. For control cells, different species of origins were used. Both human neuroblastoma cells (NG1087-15) and

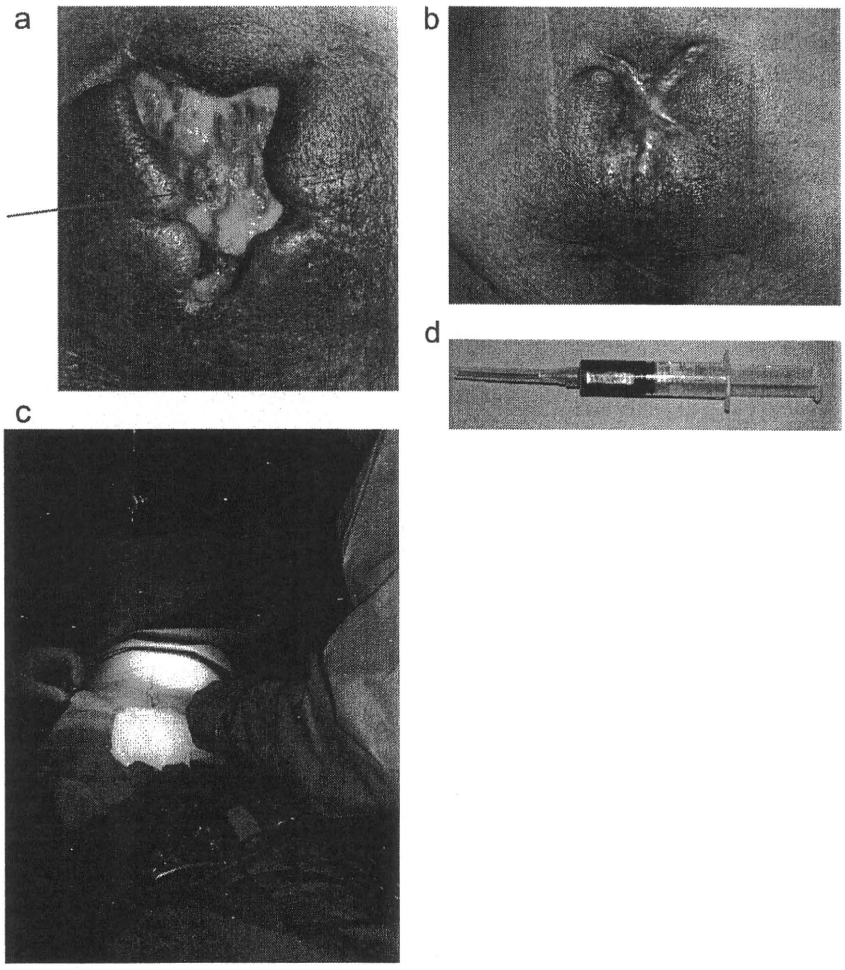

Fig. 1. World first adipose-derived stem cell therapy for radiation injury: a: Pre-op. The arrow indicates the necrotized exposed sacral bone; b: At 103 d after the ADSC transplantation. Skin, subcutaneous tissue, bone and muscle were regenerated and healed; c: Harvesting the fat tissue through 5-mm incision by cannulization; d: ADSC in the syringe. 3.8×10^7 cells were obtained.

rat pheochromocytoma cells (PC-12) were used. Cell proliferation was consistent in three cell groups in the normal condition (no radiation and normal medium); however, 20 Gy irradiation caused cell death in groups of NG1087-15 and PC-12 in 48 h. In contrast, the hMSCs survived up to 96 h.

In electron microscopy, irradiated hMSCs demonstrated surface microvilli all over the cells; however, the hMSCs still survived after 60 Gy irradiation, which is considered a medium dose and induces significant intestinal bleeding.

An in vivo analysis after whole body irradiation. After 20 Gy whole body irradiation, the seemingly radiation-affected surfaces were removed surgically including the panniculus carnosus. Immediate resurfacing with skin substitutes impregnated with hMSCs and bFGF facilitated wound healing. At day 10, the histology demonstrated vascular rich subcutaneous tissues with more interstitial cellularity.

Lower dose (4 Gy) irradiation to the whole rat body and bone marrow histology demonstrated loss of the hematopoietic lineage cell, but marrow stromal cells survived (Fig. 2).

DISCUSSION

Management of radiation injuries composes two major parts (Fig. 3): localized injuries and systemic injuries. Among localized radiation injuries, chronic injuries are more common in the medical field after cancer radiation therapy. Usually management of these chronic wounds is well-handled by well-vascularized tissue transfers as various plastic surgical procedures have proved. The choice of therapeutic treatment should take into consideration each patient's general condition and preference. On the other hand, when the local radiation injuries are encountered in an acute phase, there are high chances for innovative procedures using autogenous stem cells. Since hMSCs are resistant to radiation as demonstrated by the in vitro cell proliferation curve, they are able to produce protein avoiding cell apoptosis (Chen et al. 2006). Also, increasing evidence demonstrates that ADSCs are similar to hMSCs in cell properties and characteristics both in vitro and in vivo (Zuk et al. 2002). When localized radiation was distant enough from the donor sites' adipose tissues, immediate debridement and regeneration using ADSCs [which are available for processing within 1 hour simultaneously in a same operation theater without cell culture, since adipose tissues (fat tissues) are abundant in adult humans compared to other stem cell sources] were performed. In the limited clinical circumstances of high risk patients such as the elderly or those with chronic local infection, there is still an opportunity of harvesting and processing the patient's own fat-derived stem cells successfully as seen in our case.

For treatment for systemic radiation injuries, stockpiled stem cells should be globally available through a

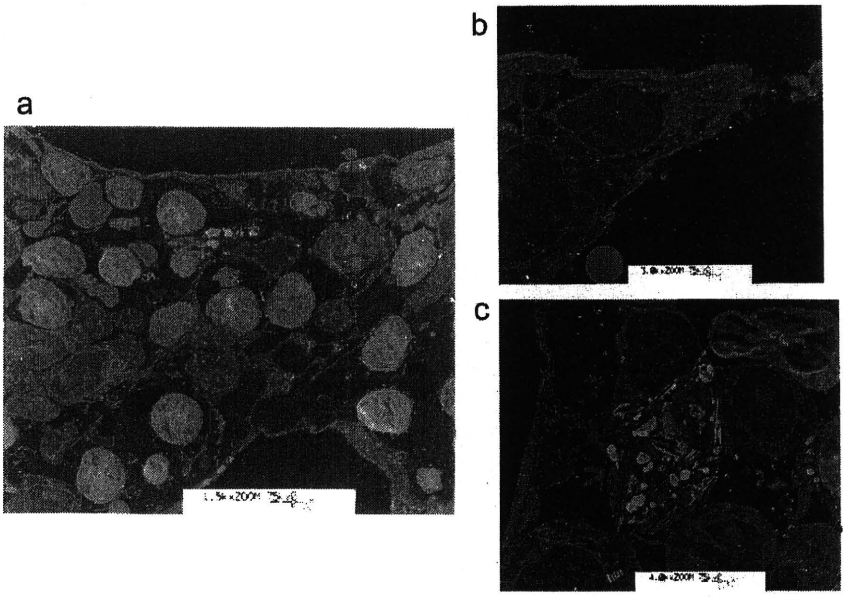

Fig. 2. Electron microscopy of 4 Gy rat whole body irradiated bone marrow: a: Lower magnitude. The hematopoietic cells turned round as seen in white. There are some empty cell shelves demonstrated as black round morphology (×1,500); b: There are predominantly euchromatin marrow stromal cells. These cells represent the marrow stromal (or mesenchymal stem) cells (×3,000); c: There are some phagocytic macrophages observed (×3,000).

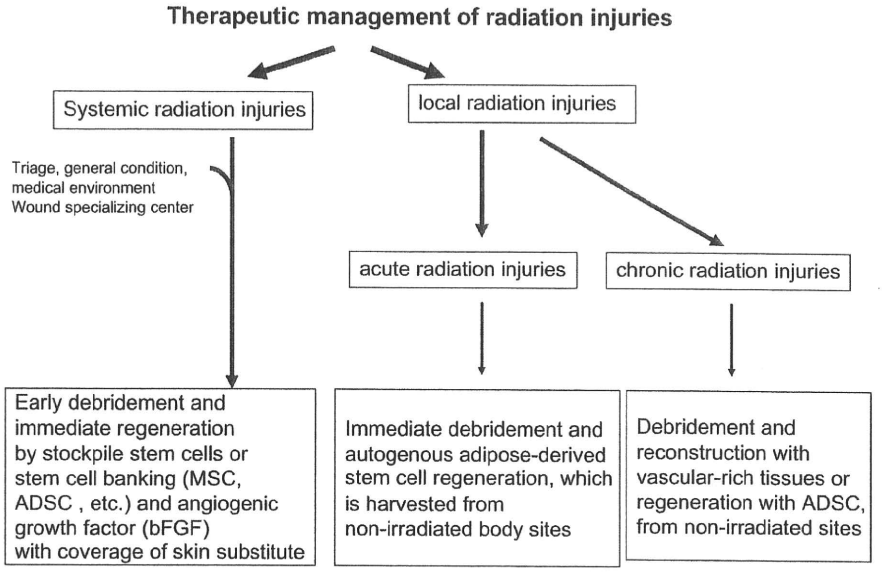

Fig. 3. Flow chart of therapeutic management of radiation injuries. Each patient condition should be carefully monitored first. In case of systemic radiation injuries, first the patients' general conditions and their medical environment, considering the triage, should be considered. Stem cell therapy, supplied from the "stockpile" or "stem cell banking," which is augmented by cytokine, should be the first line of therapy; on the other hand, local injures are sub-divided into "acute" and "chronic" cases. For less invasive therapeutic modality, adipose-derived stem cells are highly recommended even for "severely-injured" or "host-compromised" patients. Each sub-divided group can be handled by experts.

medical assistance network system under the World Health Organization Radiation Emergency Medical Preparedness and Assistance Network (WHO-REMPAN), in which Nagasaki University is highly involved, or other international frameworks. Early resurfacing of the damaged skin and subcutaneous tissues is as important as hematological and intestinal system resuscitation (Weinstock et al. 2008).

Also, therapeutic guidelines for systemic radiation injuries are anticipated from practical and regulatory view points. Highlighting innovative technology and devices such as currently existing medicines and devices are expected on behalf of preparing to treat "systemic" radiation injuries most effectively.

Therapeutic regimens of radiation injuries used to be dependent on each sub-specialty in the medical field such as internal medicine, radiology, and surgery.

Recent establishment of wound care specialty, mostly led by plastic surgeons but also by other supporting specialists such as nurses, dermatologists, gastrointestinal physicians, and surgeons, may practically handle these rare but significant "radiation injuries" with interdisciplinary approaches (Gottrup 2004). Disciplines that are more specialized for "radiation injuries" may be required.

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ORIGINAL RESEARCH – CLINICAL SCIENCE

Basic fibroblast growth factor is beneficial for postoperative color uniformity in split-thickness skin grafting

Sadanori Akita, MD, PhD; Kozo Akino, MD, PhD; Aya Yakabe, MD; Katsumi Tanaka, MD; Kuniaki Anraku, MD; Hiroki Yano, MD, PhD; Akiyoshi Hirano, MD

Graduate School of Biomedical and Sciences, Department of Developmental and Reconstructive Medicine, Division of Plastic and Reconstructive Surgery, Nagasaki University, Nagasaki, Japan

Reprint requests:
Sadanori Akita, MD, PhD, Department of Plastic and Reconstructive Surgery, School of Medicine, Nagasaki University, 1-7-1 Sakamoto machi, Nagasaki 8528501, Japan.
Tel: +81 95 819 7327;
Fax: +81 95 819 7330;
Email: akitas@hf.rim.or.jp

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ABSTRACT

Color changes of visible and exposed body surfaces, such as the face and extremities, after burn injury or surgery, such as skin grafting, flap, or sclerotherapy for vascular malformations, are sometimes a concern. The consequences reduce the satisfaction of both patients and physicians. An easy and reproducible method has not yet been established for an objective analysis of color changes; therefore, we tested a hand-held color analyzer (NF-333; Nippon Denshoku Co. Ltd) with data transport to a computer database and analysis software for posttreatment skin color change. The parameters included L, a, and b, which measure clarity, red, and yellow, respectively. Two groups were prospectively divided with 20 (11 females and nine males) patients per group. One group received skin grafting plus basic fibroblast growth factor (bFGF) spray daily and the other group received only skin grafting. The patients were randomized by the date of their first visit to our hospital. Patients were treated with bFGF on odd days, while patients who came on even days were included in the non-bFGF-treated group. The donor site for skin grafting was the lateral thighs and the thickness was similar in both groups. The results were compared at 1-year post-treatment follow-up. Clinical and objective assessments of the scars were performed 1 to 1 $\frac{1}{2}$ years after complete healing. Color change differentials in comparison with the surrounding skin were lower with bFGF treatment in all parameters ($p < 0.01$), along with clinical assessment with the Vancouver Scar Scale; therefore, the treatment contribute to a better color match with skin grafting postoperatively.

Color changes are often major problems after traumatic injuries or reconstructions by flaps or skin grafting. Split-thickness skin grafting is most widely used as a reconstruction modality because it is considered very effective for immediate wound coverage over the majority of the wound bed, except bones or tendons, although there are several limitations of split-thickness skin grafting, such as scar contracture, less hair growth, and less durable and prominent color differences from the surrounding recipient. Abnormal pigmentation more frequently occurs in split-thickness skin grafting than in full-thickness skin grafting.[1] Also, differences in the selection of the flap donor site may affect the color match, affecting patients' final satisfaction.[2] The pathophysiology of pigmentation changes after traumatic or surgical insults is not fully understood but scar tissue, once secondarily healed, is a barrier to the translocation of melanin to keratinocytes and melanocyte migration.[3] The relationship of melanosomes and lysosomes should be taken in account to understand the mechanism of pigmentation, although both are derived from the endosomal compartment by being modulated gene products.[4] Higher melanosome content represents hyperpigmentation, while more melanosomes attaching to lysosomes may lead to a lighter skin color. In more detail, the functional characterization of melanosomes is a complex of approximately 1500 proteins in all stages and about 600 proteins in any given stage.[5] Hemoglobin deposition also influences erythematous skin color, as is often seen in patients with anemia or polycythemia or during the course of wound healing after skin grafting within 1 month, when revascularization to the graft is complete.[6] Previously, early administration of a growth factor, basic fibroblast growth factor (bFGF), resulted in better scar quality as well as accelerating wound healing in second-degree burns in infants[7] and adults.[8] Subjective pigmentation and vascularity as well as other subjective and objective parameters were significantly improved in wounds treated with bFGF. Also, less hard scar tissue developed when bFGF was used in surgery with skin grafting for burn ulcers.[9]

We therefore tested if early administration of bFGF contributes to a better color match adjacent to the recipient site by objective measurement with a color meter.

PATIENTS AND METHODS

Patients and surgeries

We enrolled 40 subjects (18–81 years old; average 43.2 ± 16.7 years of age, 20 with bFGF treatment and 20 with non-bFGF treatment) in this investigation from April 2004 to March 2006 after approval from the internal review board of Nagasaki University Hospital. The bFGF treatment group included 11 female and nine male patients

Table 1. Patient profiles

	bFGF (n=20)	Non-bFGF (n=20)
Sex (F:M)	11 : 9	11 : 9
Age (years)	42.9 ± 17.1	43.4 ± 16.8
TBSA (%)	8.9 ± 4.3	9.5 ± 5.0
Surgery type	Burn=12	Burn=11
	Scar=5	Scar=6
	Tumor=3	Tumor=3
Location	Buttock=3	Buttock=2
	Face=7	Face=9
	Extremity=3	Extremity=2
	Trunk=7	Trunk=7
Healing time (days)	17.9 ± 2.3	19.6 ± 2.1**

**$p < 0.02$.
bFGF, basic fibroblast growth factor; F, female; M, male; TBSA, total body surface area.

with an average age of 42.9 ± 17.1 years (18–78 years old), Fitzpatrick skin type III (n=12) and IV (n=8), with various reconstruction locations, such as the buttocks (n=3), face (n=7), extremities (n=3), and trunk (n=7). The non-bFGF treatment group (control group) included 11 female and nine male patients with an average age of 43.4 ± 16.8 years (18–81 years old), Fitzpatrick skin type III (n=13) and IV (n=7) with reconstruction locations such as the buttocks (n=2), face (n=9), extremities (n=2), and trunk (n=7). The patients were randomized by the date of their first visit to our hospital. Patients were treated with bFGF on odd days, while patients who came on even days were included in the non-bFGF-treated group. There was no statistically significant difference between bFGF and non-bFGF groups in terms of age or reconstruction location. Other than bFGF use, all therapeutic regimens were the exactly the same between groups. For instance, the timing of dressing changes and the use of ointment-impregnated gauzes from initial treatment until wound healing were identical.[10]

There were 12 burn resurfacings, five scar revisions, and three posttumor resections in the bFGF-treatment group, with 11 burn resurfacings, six scar revisions, and three posttumor resections in the control group. All debridement was performed in the same manner with resection depth to the subcutaneous tissue. Donor sites of split-thickness skin grafts were the patients' lateral thighs with a thickness of 0.01 in. using an electric dermatome (Table 1). There were no remarkable setbacks during and after surgery in any cases.

In the bFGF-treated group, immediately after debridement and complete hemostasis, a bFGF spray was used according to the manufacturer's recommendations. There were no other factor differences between groups other than the use of the bFGF spray on the debrided wounds. Clinical and objective assessments of the scars were performed 1 to 1 $\frac{1}{2}$ years after complete healing.

bFGF (Trafermin, Fiblast Spray®) and non-bFGF treatment

Genetically recombinant human bFGF was used as the spray. The bFGF was initially used by spraying immediately after thorough debridement and hemostasis.

The concentration of bFGF was 30 μg bFGF per 30 cm^2 area or less as 100 μg of freeze-dried bFGF dissolved in 1 mL of 0.01 w/w benzalkonium chloride-containing solution, with 300 μL sprayed over a 30 cm^2 area from 5 cm distance, and 0.3 mL of this concentration of solution was applied using this method. Ointment-impregnated gauze was applied to wounds treated with bFGF after waiting for 30 seconds.

The non-bFGF-treatment groups received only ointment-impregnated gauze without bFGF spraying. Standard procedures for stabilizing burn wounds were applied for all cases.[10]

Scar scaling

Scars were evaluated by the senior authors (S.A., A.Y., and K.A.), who evaluated each others' patients in a blind fashion 1 year after complete wound healing. Scar scaling was determined using the Vancouver Scar Scale, which included pigmentation (0=normal, 1=hypopigmented, 2=mixed, and 3=hyperpigmented), pliability (0=normal, 1=supple, 2=yielding, 3=firm, 4=ropes, and 5=contracture), height (0=flat, 1= < 2 mm, 2=2–5 mm, and 3=5 mm), and vascularity (0=normal, 1=pink, 2=red, and 3=purple).[11] Evaluation was confirmed by two more authors independently, who are also wound specialists; therefore, each wound was assessed by five different evaluators. Parameters of each scar were obtained by averaging the individual score by five evaluators.

Color meter

A color meter was used to assess scar clarity (L), red (a), and yellow (b), respectively, with a hand-held color meter weighing 420 g, including batteries, for the main body of the system and 110 g for the hand-piece probe, the color analyzer (NF-333; Nippon Denshoku Co. Ltd., Osaka, Japan). The light source was a multicolored LED. All data were easily transferred to Microsoft Excel 2003 files on a laptop computer via a data connector and the differentials of each polarized color criterion parameter (L, a, and b) were standardized with the surrounding intact skin. The delta ratio of each parameter was then compared and statistically analyzed. The measurement of each point was always perpendicular to the scar and was repeated five times immediately after touching the scar surface, and the mean value of three adjacent points at least 8 mm apart and 12 mm from the edge of intact skin was assessed at 25 °C room temperature and 50% humidity with air conditioning under the same lighting conditions in a single room. The accuracy of this system is traceable to the standard of the National Institute of Standards and Technology (NIST), USA.

The accuracy of this function is determined by the choice of optical filters, which is determined by an optimization criterion by combing methodologies from differential geometry with statistical error analysis. It is shown that the magnitude of errors associated with the optimal filters is typically half of that for typical RGB filters in a three-parameter model of human skin coloration.[12] Recently, a relatively easier skin chromatometer was used for temporal changes of postskin grafting evaluation with relevant multiple factors such as age, type of skin grafting,

anatomical differences of the donor site or recipient site, and the Fitzpatrick skin type.[13]

Effect of benzalkonium chloride on the color meter

Because there was a concern about using benzalkonium chloride for color analysis, the same percentage of benzalkonium chloride solution was applied to the lower half of each patient's lateral thigh in 20 patients. The control was the upper half of each patient's donor site and other wound management was identical. The comparison used exactly the same skin graft method. The accurate concentration of the benzalkonium chloride in the bFGF medium is unknown due to the company's confidentiality; however, 0.01 w/w% concentration of the preservative for the regular eye drops is used for this investigation.

Histology

After completion of the skin grafting in 1 to $1\frac{1}{2}$ years, anatomically comparable tissues were harvested for resection of the small ingrown tissue arrangement with obtaining both patients and the patients' family's informed consent. The identical tissue sample from one patient was subject to formalin-fixed and paraffin-embedded 5 μm section for hematoxylin and eosin staining for histological analysis and for Masson's trichrome staining for collagen bundles and cytoplasms.

Statistics

The results are expressed as the mean ± standard deviation. Data between groups were evaluated by one-way analysis of variance (ANOVA) with the Bonferroni multiple comparison procedure, and p-values < 0.05 were considered significant.

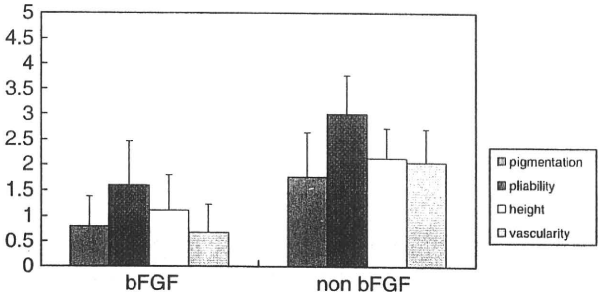
RESULTS

Wound healing and healing rate

Wound healing after debridement and skin grafting was uneventful in all patients in both bFGF and control groups examined. The average wound healing rate in bFGF-treated wounds of 17.9 ± 2.3 days was significantly shorter than that of control wounds of 19.6 ± 2.1 days ($p < 0.02$). Debridement procedures in all patients included in this investigation were performed at the subcutaneous tissue level regardless of the primary cause in both groups.

Clinical scar assessment

Clinical evaluation of pigmentation, pliability, height, and vascularity showed significant differences between bFGF-treated and non-bFGF-treated scars (0.8 ± 0.6 vs. 1.8 ± 0.9, 1.6 ± 0.9 vs. 3.0 ± 0.8, 1.1 ± 0.7 vs. 2.1 ± 0.6, 0.7 ± 0.6 vs. 2.0 ± 0.7; bFGF vs. non-bFGF-treated [control], pigmentation, pliability, height, vascularity, respectively, $p < 0.001$) (Figure 1). Detailed analysis of the cause of surgery in the two groups did not show significant differences on the Vancouver scale parameter.



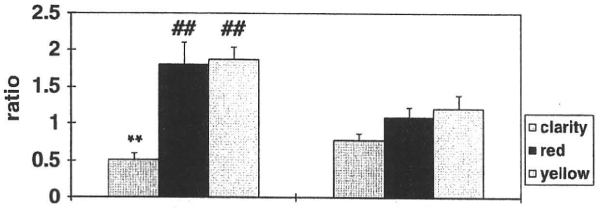
**; P<0.01, compared to each parameter of bFGF group

Figure 1. Vancouver Scar Scale. Four independent specialists evaluated 1 year after complete wound healing. The results were 0.8 ± 0.6 vs. 1.8 ± 0.9, 1.6 ± 0.9 vs. 3.0 ± 0.8, 1.1 ± 0.7 vs. 2.1 ± 0.6, 0.7 ± 0.6 vs. 2.0 ± 0.7 for basic fibroblast growth factor (bFGF)-treated vs. non-bFGF-treated, pigmentation, pliability, height, vascularity, respectively ($p < 0.01$). Most importantly, both pigmentation and vascularity showed significantly greater values in the non-bFGF group.

Also, there was a significant correlation between the values of vascularity (y) and pigmentation (x) in all patient data ($y=0.393x+0.857$, $r=0.376$, $p < 0.01$).

Color meter analysis

The clarity of bFGF-treated scars was significantly higher and closer to 1 than that of control scars when the values were normalized by the adjacent skin clarity (0.78 ± 0.10 vs. 0.51 ± 0.10; bFGF-treated scar, control scar, $p < 0.001$). The value, represented as red when the number was positive and high, was significantly higher in control scars than bFGF-treated scars (1.80 ± 0.31 vs. 1.10 ± 0.13; control scar, bFGF-treated scar, $p < 0.0001$).



**; P<0.001, compared to clarity parameter of bFGF group
##; P<0.01, compared to each parameter of bFGF group

Figure 2. Color meter analysis. Color meter data showed objective scar colors in three dimensions (clarity, redness, and yellowness) 1 year after complete wound healing. The clarity of basic fibroblast growth factor (bFGF)-treated scars was significantly higher and closer to 1 than control scars when values were normalized by the adjacent skin clarity (0.78 ± 0.10 vs. 0.51 ± 0.10 for bFGF-treated scar, control scar, respectively, $p < 0.001$). When the number was positive and high, red was significantly higher in control scars than bFGF-treated scars (1.80 ± 0.31 vs. 1.10 ± 0.13 for control scar vs. bFGF-treated scar, respectively, $p < 0.0001$). When the number was positive and high, yellow was significantly higher in control scars than bFGF-treated scars (1.87 ± 0.18 vs. 1.21 ± 0.19 for control scar vs. bFGF-treated scar, respectively, $p < 0.0001$).

The *b* value, represented as yellow when the number was positive and high, was significantly higher in control scars than bFGF-treated scars (1.87 ± 0.18 vs. 1.21 ± 0.19; control scar, bFGF-treated scar, $p < 0.0001$) (Figure 2).

Torso split-thickness skin grafting treatment with or without bFGF showed the remarkable improvement of the postoperative color match in the bFGF treatment (Figure 3).

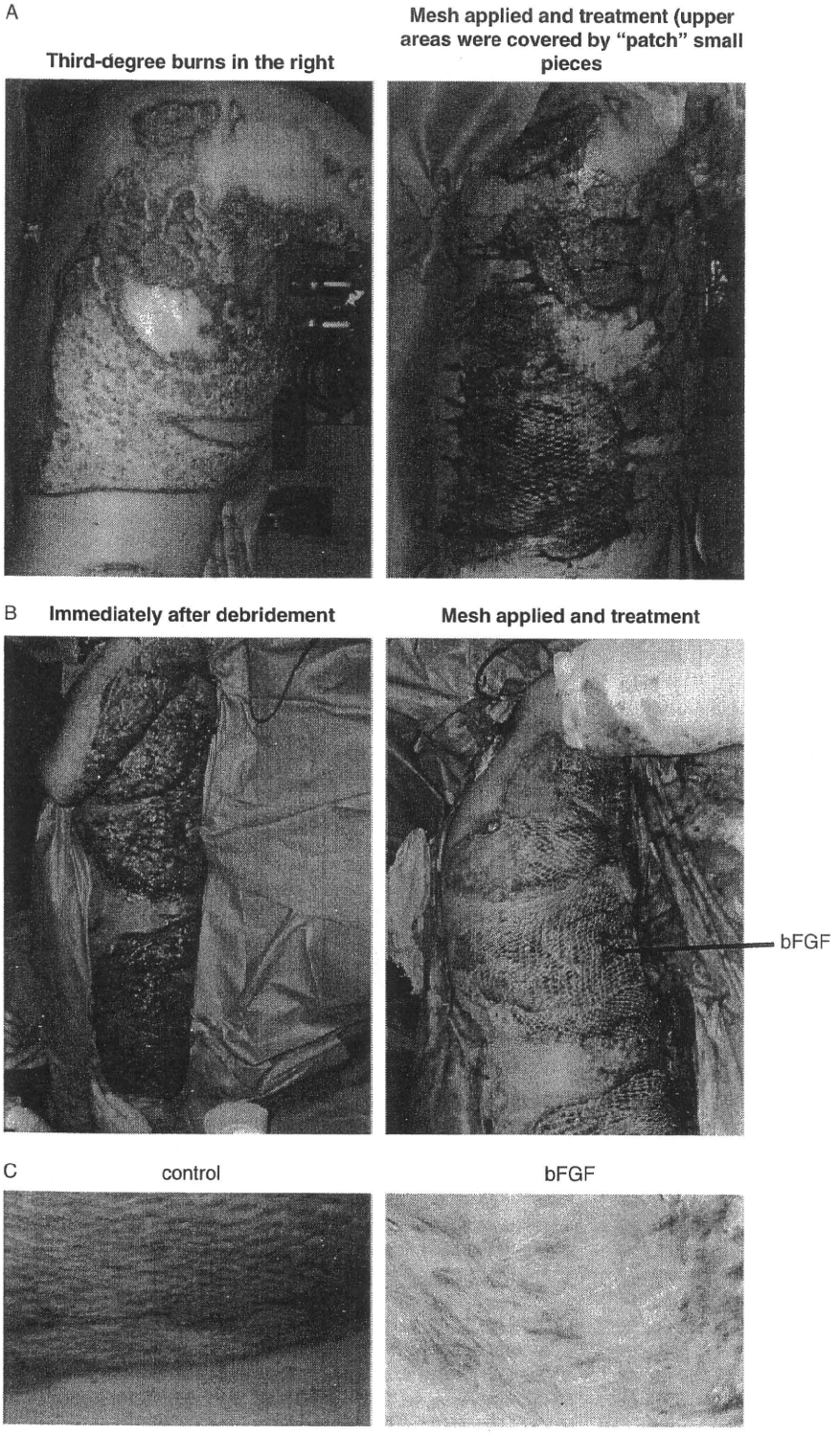


Figure 3. Burn torso cases. (A) A 55-year-old female with right torso third-degree burns treated with only 0.01 in. mesh skin grafting from the lateral thigh. The right panel shows the mesh skin grafting after the complete eschar debridement over the fat tissue. (B) A 56-year-old female with left torso third-degree burns treated with basic fibroblast growth factor (bFGF) treatment and 0.01 in. split-thickness mesh skin grafting from the lateral thigh. The third-degree burn areas were debrided to the fat layer and bFGF treatment with coverage by 0.01 in. split-thickness mesh skin grafting. The bFGF spraying over the mesh skin grafting continued to complete wound healing. (C) Eighteen-months postoperatively, the close-up views of the grafted wound color match. The control skin color compared with the intact skin in the lower half showed more reddish and darker in the left, while the color of the bFGF-treated group matched well with the intact skin and showed clear in the left lower quadrant of the view.

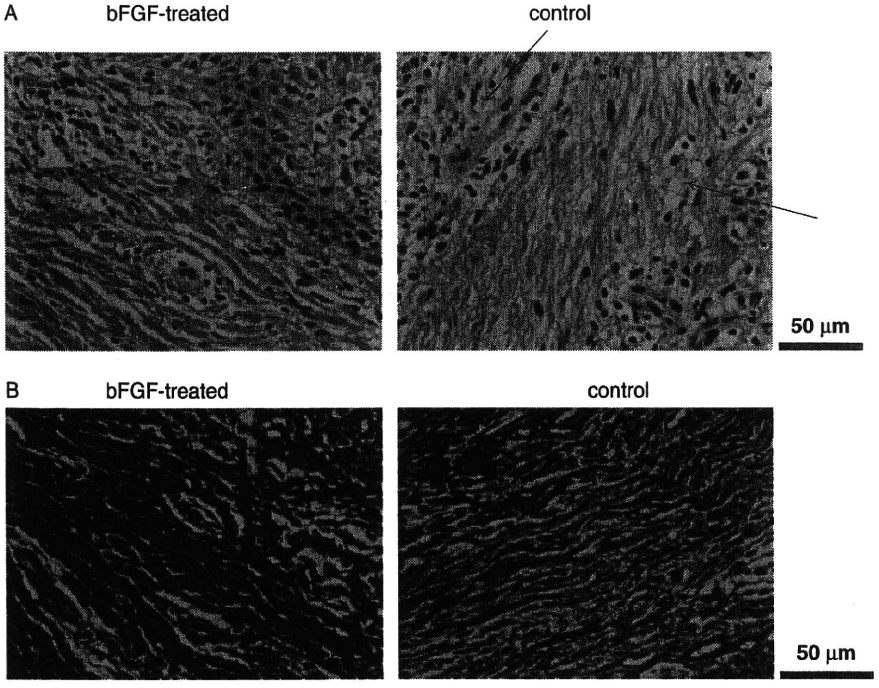


Figure 4. Histological comparison of the same patient in the identical anatomical location. (A) Hematoxylin and eosin staining showed that the basic fibroblast growth factor (bFGF)-treated tissue showing an organized dermal arrangement with a thick rete ridge and the intact basal layers in the epidermis, whereas control skin showed more flat and thin and disorganized dermal component and the epidermal layers were surrounded by the majority of scars and little normal structured dermis and more melanin-positive cells as indicated by arrows. (B) Masson's trichrome staining showed that the bFGF-treated tissue showing more cytoplasmic areas and arrayed collagen bundles compared with the control.

Effect of benzalkonium chloride in color meter

With a 0.01 w/w% benzalkonium chloride solution at the donor site of split-thickness skin grafting, there is no difference between nonbenzalkonium chloride and 0.01 w/w% benzalkonium chloride solution, 0.53 ± 0.09 vs. 0.58 ± 0.10, 1.83 ± 0.29 vs. 1.77 ± 0.28, 1.94 ± 0.14 vs. 1.85 ± 0.15 for parameters of clarity, red, yellow, control, and benzalkonium chloride solution, respectively. There were no statistically significant differences between groups in all parameters.

Histology

Anatomically identical tissue samples from the buttocks of the patient, the bFGF-treated skin, showed an organized dermal arrangement with a thick rete ridge epidermis, whereas control skin showed more flat and thin and disorganized dermal component and the epidermal layers were surrounded by the majority of scars and little normal-structured dermis and more melani-positive cells are observed in the control tissue. In the Masson's trichrome staining, the bGF-treated scar showed greater number of cytoplasms and more organized collagen bundles compared with the control (Figure 4).

DISCUSSION

Skin grafting is one of the most useful reconstructive modalities for skin and subcutaneous tissue defects; however, postoperative scars are sometimes a determining factor of patient satisfaction; in particular, the color match between the grafted skin and surrounding recipient skin should be carefully considered and evaluated. Split-thickness skin grafting is widely used for primary wound coverage after skin cancer resection or relatively extensive skin defect coverage, such as extensive burns, because donor-site morbidity is lower and wider skin grafts are available.[14] Dark-skinned patients sometimes show remarkable pigment mismatch because there are profound differences in the degree and extent of melanization between donor and recipient sites, because the disorder of melanization causes either hyperpigmentation or hypopigmentation in the melanocyte–melanosome complex.[15]

In order to solve color mismatch, deepithelialized split-thickness skin grafts are applied to relatively small defects and epithelization is successfully induced from the adjacent epidermis.[16] Skin color and functional analyses of keratinocytes were attempted using a human skin substitute with cells from different skin pigmentation types. In a clinical study of temporal color change in skin grafts, there was a tendency for the color to become lighter, with less redness and increased yellow after 15 days to 3 years of follow-up,[13] while melanocyte levels in scars are considered to reach those of the adjacent epidermis after 10 years.[15] In this investigation, there were correlations of greater vascularity and pigmentation in clinical assessment and this was confirmed with higher values of red and yellow in color and blacker clarity as determined by a color meter. This may explain the accelerated wound healing that correlates with the better color quality of skin grafting in the maturation phase of wound healing. Also, there was a significantly increased amount of melanin content and matured melanosomes in darker skin-derived keratinocytes.[17] Our study category of the skin type was almost identical in bFGF treatment and non-bFGF treatment in the Fitzpatrick skin type.

In attempting to improve coloration after flap reconstruction in the face of Caucasians, the thin, depilated split scalp overgrafting led to the better relative value of

brightness, contrast, cyan, magenta, and yellow in the Fitzpatrick skin types I–III, of which method was evaluated objectively by digital input of photographing.[18] In our study, the direct measurement of the patient skin colors without any technical indirect intervention was performed.

We performed clinical and objective assessment 1 year after the completion of wound healing because clinically scars are stabilized and matured in burns and in combination with an artificial dermis when bFGF is used for wounds.[7–9,19] This time point was reasoned from the relevant factors influencing color changes of skin grafting stabilized over months[13] as showed here in between 12 and 18 months.

Previously, bFGF showed improved scar quality in terms of clinical scar hardness and objective durometer values as well as accelerating wound healing. Skin barrier function, shown by transepidermal water loss and scar softness by a duromter or a Cutometer, was significantly recovered by early administration of bFGF for burns and traumatized wounds, showing better scarring and a well-organized stratum corneum after healing. Another approach for a better esthetic outcome for large and deep facial burns uses an artificial dermis of collagen/glycosaminoglycan with tangential excision of the eschars.[20]

In contrast, there are reports that bFGF and other growth factors augmented gene expressions and protein secretions in collagen remodeling and pigmentation[21] or an increased expression of $p125^{FAK}$ on melanocytes by bFGF[22]; however, these results are derived from in vitro experiments with cell culture and thus more complicated mechanisms may exist for clinical relevance. We assume that accelerated wound healing, maintenance of the complex system of melanization, and diminishing activity of erythema by bFGF will lead to a better clinical color match.

ACKNOWLEDGMENTS

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. bFGF and non-bFGF were compared in a 72-year-old female for wound color change. **A:** Third-degree flame burns of bilateral lower extremities were demonstrated on day 5 after the event. **B:** At 18 months after healing, comparison of the two treatments demonstrated significant differences in color as darker, reddish, and more yellowish with non-bFGF treatment.

Figure S2. Ankle cases. **A:** A 66-year-old male with a third-degree burn of the foot treated with 0.01-inch split thickness patch skin grafting in the distal of the lateral malleolus of the right ankle. The color was much darker and less clear in comparison to the surrounding tissue. **B:** A 53-year-old female with a diabetic foot ulcer treated with bFGF and 0.01-inch split thickness patch skin grafting in the distal of the lateral malleolus of the right ankle, where the bone is superficially removed. Twelve months postoperatively, the color matched with the surrounding tissue in the dorsum of the foot.

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