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Research Article

Noncultured Autologous Adipose-Derived Stem Cells Therapy for Chronic Radiation Injury

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Increasing concern on chronic radiation injuries should be treated properly for life-saving improvement of wound management and quality of life. Recently, regenerative surgical modalities should be attempted with the use of noncultured autologous adipose-derived stem cells (ADSCs) with temporal artificial dermis impregnated and sprayed with local angiogenic factor such as basic fibroblast growth factor, and secondary reconstruction can be a candidate for demarcation and saving the donor morbidity. Autologous 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 tested for Patients who were uneventfully healed with minimal donor-site morbidity, which lasts more than 1.5 years.

1. Introduction

There is an increasing worry on radiation injuries probably caused by nuclear power plant (NPP) reactor accidents, therapeutic irradiation for malignancy, and interventional radiology (IRV) of unexpectedly prolonged fluoroscopic procedures for cardiovascular diseases such as arrhythmia, ischemic heart diseases, or nuclear medicine of overdose intake of the radioactive for nuclear medicine of internal radiation therapy. The problems are concerning chronic radiation injury as well as how to heal such local and systemic injures acutely. Local chronic radiation injury is resistant to conventional therapeutic modalities such as flap coverage or skin grafting because the deteriorated margins are sometimes indistinguishable from normal intact tissue, and thus sufficient enough debridements are not obtained with surgeons' naked eyes.

These conditions should be treated properly for the sake of life saving and improvement of local wound healing [1]. However, data of total evidence-based clinical analysis were not established yet. Authors' institute, Nagasaki University, is selected as a global strategic center for radiation health risk control by the Japan's Ministry of Education, Culture, Sports and Technology from FY 2007 to 2011 and exploring to establish such therapeutic regimens, to prevent the radiation injuries, and possibly to regenerate medical and surgical therapy for radiation injuries by using patients' own adipose tissue-derived stem cell therapy.

Often seen chronic radiation injuries are well handled by sufficient enough 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 microvasculature of the skin and soft tissues including the connective tissues plays major roles in attributing to accelerate local wound healing. Also, externally administered angiogenic growth factor 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, patients with comorbidities, who are

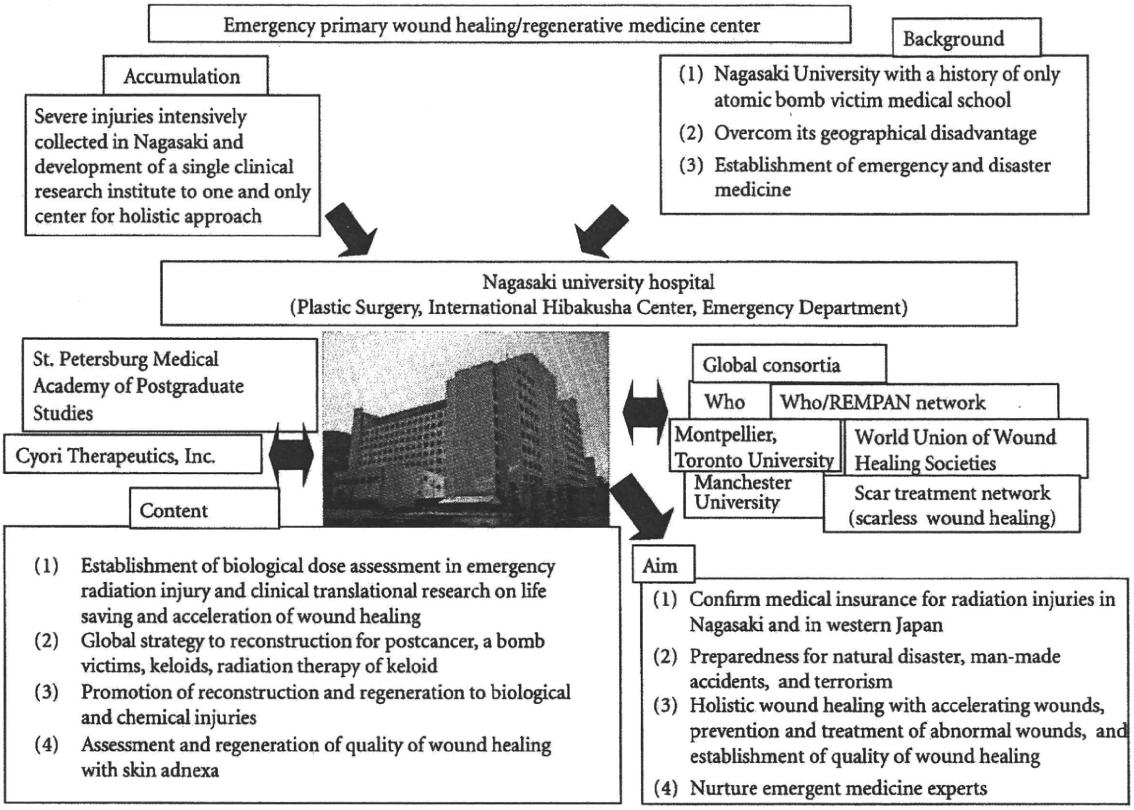

FIGURE 1: Strategy of emergency radiation injury. Collaborative work with highly established international centers and organ is proposed.

intolerant to the extensive and long surgeries [2]. Here, chronic radiation-injured wounds are tested with non-cultured autologous adipose-derived stem cells and clinical implications are discussed.

2. Materials and Methods

2.1. Treatment of Chronic Local Radiation Injury with Conventional Methods and Stem Cells. Often experienced in radiation therapy for malignancy, cardiovascular modalities should be categorized as difficult wounding with poor vasculature or less healing potentials.

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

Other selective clinical cases used angiogenic growth factor namely human recombinant basic fibroblast growth factor (rh-bFGF), which is clinically approved and widely used for clinical wounds in Japan with skin substitutes, which are also clinically available not only in Japan but also in many other nations including USA, 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 (scar tissue) as well as facilitated wound healing [3].

One case was treated with non-cultured autologous adipose-derived stem cell (ADSC) for chronic sacro-coccygeal radiation ulcer in 2008, which was caused by a therapeutic radiation at fractionate 50 Gy at 40 years previously.

2.2. Methods. This study was approved by the Ethics Committee of the Nagasaki University Hospital, and written informed consent was obtained from all patients (approved no. 08070296) and partly supported by the Global COE (Center of Excellence) Program E08, Global Strategic Center for Radiation Health Risk Control, and it was funded by the Japan Society for the Promotion of Science. This national research grant enables us to investigate 3 main themes related to radiation health risk: (1) atomic-bomb disease followup cohort research with over 60-year continuous research history, (2) radiation basic science, and (3) international radiation health research. Especially, this radiation regeneration research was involved in further international collaboration framework under international organizations such as WHO (World Health Organization) and IAEA (International Atomic Energy Agency) (Figure 1).

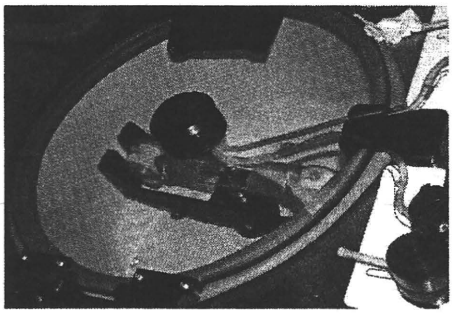

FIGURE 2: The Adipose-derived stem cells are processed in a closed-circuit machine within 1.5 hours.

2.3. Harvesting of Adipose Tissue by Liposuction and Isolation of ADSCs. 3–5 mm incisions, two incisions in the abdomen, four incisions in the thigh, and two incisions in the gluteal region, were made on the abdominal region, the thigh, and the gluteal region. The subcutaneous layer was infiltrated with a lactated Ringer's solution with addition of 0.5 mL of epinephrine and 25 mL of 1% lidocaine per 500 mL. Adipose tissue was suctioned using an 18-G Becker cannula with a 50 mL syringe. Total 250 gram-fat tissues, 120 grams from the abdominal region, 80 grams from the gluteal regions, and 50 grams from the thighs were harvested.

ADSCs were isolated from the suctioned adipose tissue by using the Celution system. (Cytori Therapeutics, Inc., USA). Briefly, the suctioned adipose tissue was introduced into the Celution cell-processing device, which automatically and aseptically extracts and concentrates the mononuclear fraction of adipose tissue and removes unwanted or deleterious cells, cell and matrix fragments such as lipids. By using the Celution system, a 5 mL solution is added to isolated ADSCs in about one and a half hour (Figure 2). The whole procedure is in a closed circuit and this reduces the chance of the contamination.

The small portion of processed ADSCs was used for the ex vivo cell culture and confirmed the proliferation and differentiation potential. The ADSCs-rich fraction was then plated onto collagen type-I-coated plastic culture flasks in a serum-free medium for primate embryonic stem cells (Primate ES medium, RiproCELL, Tokyo), and the cells, clonally expanded, were collected and stored in Liquid Nitrogen as the primary ADSCs. ADSCs were subcultured when they reached to 80% confluence. Cells were treated with trypsin/EDTA solution, neutralized with tripsin-neutralizing solution, and collected by centrifugation for 5 minutes at 1,200 rpm. The pellects were resuspended in a fresh medium; the number of cells was counted, and 3×10^5 cells were plated into T25 flasks (25 cm^2) for subculture while the rest of the cells were stored in liquid nitrogen.

2.4. Adipose-Derived Stem Cell Grafting and Postoperative Management. For the scaffold purpose, we used the artificial dermis (Terudermis, Olympus-Terumo Biomaterials Co., Ltd., Japan) (Figure 3). The Terudermis is composed of two layers: a lower layer of bovine atelocollagen and an upper layer comprising a silicone sheet which protects against infection and dryness from the outside. After minimum debridement, the Terdermis was multilayered and stacked over freshly debrided wounds. The silicone sheets were removed except top Terudermis. The two-thirds of isolated ADSCs alone were injected; around the debrided wounds, at the base of the wounds, and into Terudermis. Another one-third of ADSCs was mixed with the autologous adipose which was rinsed with a lactated Ringer's solution. In the Celution system, after isolating ADSCs, the disposable cell collection plastic case one was again used to mix the suctioned fat, which is rinsed separately in the 50-cc syringe and repeated until the oil droplets are removed. After being mixed, it was injected into a zone of hard fibrotic tissue around the debrided wounds in 2-cm width in all directions.

2.5. Angiogenic Growth Factor and Basic Fibroblast Growth Factor (bFGF). Genetically recombinant human bFGF (Fiblast, Trafermin) was purchased from Kaken Pharmaceutical Co., Inc (Tokyo, Japan). The Freeze-dried bFGF was dissolved in 5 mL of benzalkonium chloride containing solution right before the first use and stored at 4°C for one day, with 300 μL sprayed over 30 cm^2 area from 5 cm distance, and 0.3 mL per day of this solution was applied over the wound. One week after removing the silicone layer, human recombinant fibroblast growth factor (bFGF: Fiblast, Kaken Co., Ltd., Japan) (Figure 4) was sprayed. The wound was covered with nonadherent occlusive foam dressing.

3. Results

3.1. Treatment of Chronic Local Radiation Injury with Conventional Method. All wounds were healed after several surgical modalities. None of the cases was healed with single procedure (2 to 6 surgeries, mean 4.3).

Of our cases, one breast-cancer patient was treated by a standardized Halsted method with major and minor pectoralis muscle, radical neck, and axillary and internal mammary lymph node dissections. This patient has undergone 50-Gy fractionate radiation therapy postoperatively. The radiated area showed chest fistula deep to the pleura with surrounding unhealthy hardened scar tissue and chronic inflammation.

The whole affected area was sequentially excised in 3 reconstructive surgeries, starting with rectus abdominis musculocutaneous flap, then latissimus dorsi musculocutanous flap, and finally with groin-free flap. In the course after each surgery, the margin of the flap was partially dehiscent and necrotized, which required further touchups? The total number of the reconstructive surgery was 6 (Figure 5).

3.2. Treatment of Chronic Local Radiation Injury with Adipose-Derived Stem Cells. Regeneration method with patient's own non-cultured ADSCs was planned for a patient underwent 50-Gy fractionate radiation therapy for uterine cancer 40 years ago. The pigmented sacrococcygeal region appeared with central intractable wound. Necrotized bone and fascia muscle along with malodour were observed. The ADSCs-treated chronic radiation wounds underwent

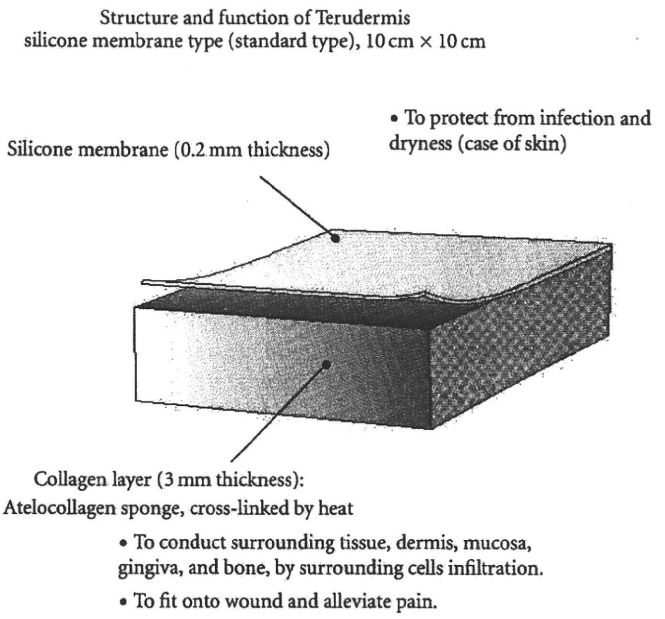

FIGURE 3: Freeze-dried bilayer artificial dermis made of bovine dermis. The outer membrane of silicone layer is easily removed and easily soaked with cell-containing solution.

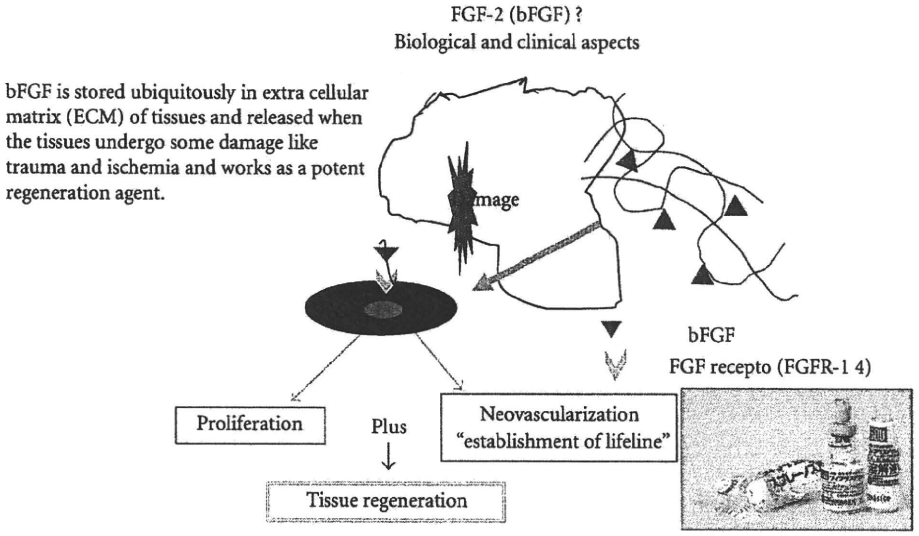

FIGURE 4: Commercially available growth factor and basic fibroblast growth factor (bFGF). Mode of action is explained and the mechanism is proposed.

debridement to remove unhealthy superficial necrotized bone, fascia, periosteum, and muscle. 3.8×10^7 cells in 5-mL of final volume from 250 mL of subcutaneous aspirated fat obtained from nonradiated area were used. Some ADSCs were directly injected in wound bed and margins; others were soaked with the artificial dermis. In a few days postoperatively, the silicone upper layer of the artificial dermis (Terdermis) was removed, and bFGF was sprayed over the regenerated wound for three weeks. There was no significant adverse effect neither in donor site or treated wound. The wound was healed uneventfully by day 82 and no sign of recurrence appeared, but the regenerated tissue developed mature in 1.5 years (Figure 6).

4. Discussion

Local radiation injuries caused during medical therapy for malignant tumors [4] and heart disease [5] may be accompanied with systemic symptoms of hematologic, neurologic, and gastrointestinal symptoms such as neutropenia, thrombopenia, fatigability, nausea, and diarrhea by contact to the

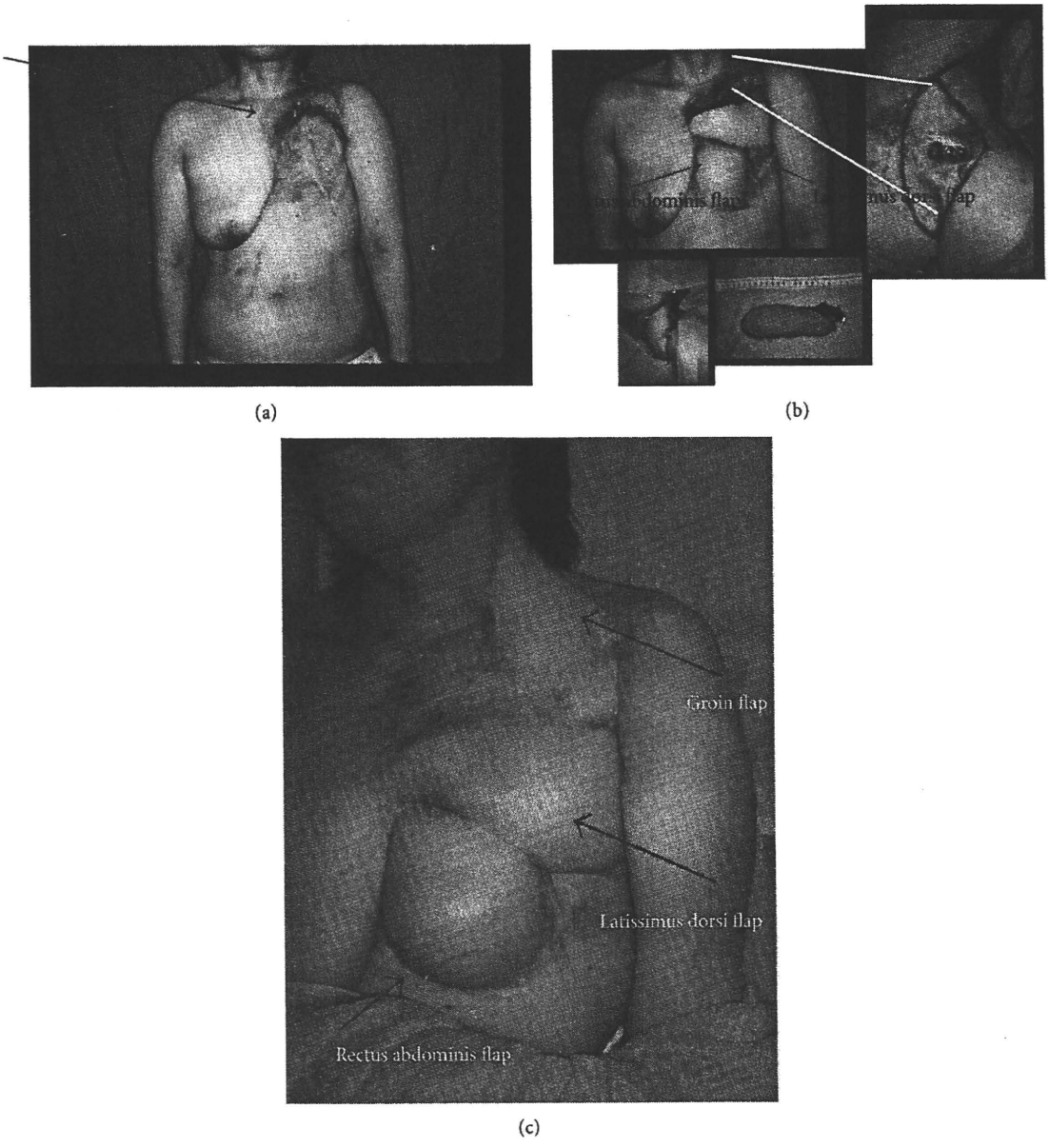

FIGURE 5: 55-year-old woman underwent a left breast cancer surgery by a standardized Halsted methods, followed by 50-Gy fractionate radiation therapy 15 years previously. (a) The chest demonstrates fistula to the costal rib and adjacent to the pleura as the arrow depicts, and the surrounding tissues were firm and various-degree inflammation existed. (b) Sequential three major flaps (rectus abdominis, latissimus dorsi, and free groin flap) are used for total coverage. (c) In 7 years postoperative view. There is irregularity of the scar margins.

scrap yard radioactive wastes without notice [6] or exposure to the radiation accidents [7] by touching gammagraphy radioactive source by mistake [8]. Since locally radiated tissues show decreased or insufficient vascularity and tissue damage, demonstrating erythema, teleangiectasia, pigmentation, or dermal atrophy, once wound is developed, it is often intractable and further leading to tissue necrosis, infection, and later fibrosis in demonstrating chronic radiation injury syndrome [9]. Therefore, radiation-injured wounds tend to persist for a long time, show impaired healing, and be prone to recurrence even by minor trauma. Radiated wounds are treated by adequate debridement both in the depth and in the width and covered with well-vascularized tissues or by cultured bone-derived mesenchymal stem cells [8]; however, the long-term outcome is not warranted, and donor-site morbidity and the duration for treatment are sometimes concerned, especially for the aged patients or patients who somehow have problems in harvesting the donors or being limited due to the coexisting diseases. As seen in our reconstructive cases, the surgical modalities constantly required multiple surgeries partly due to the definitive damage-free margins of the affected tissue.

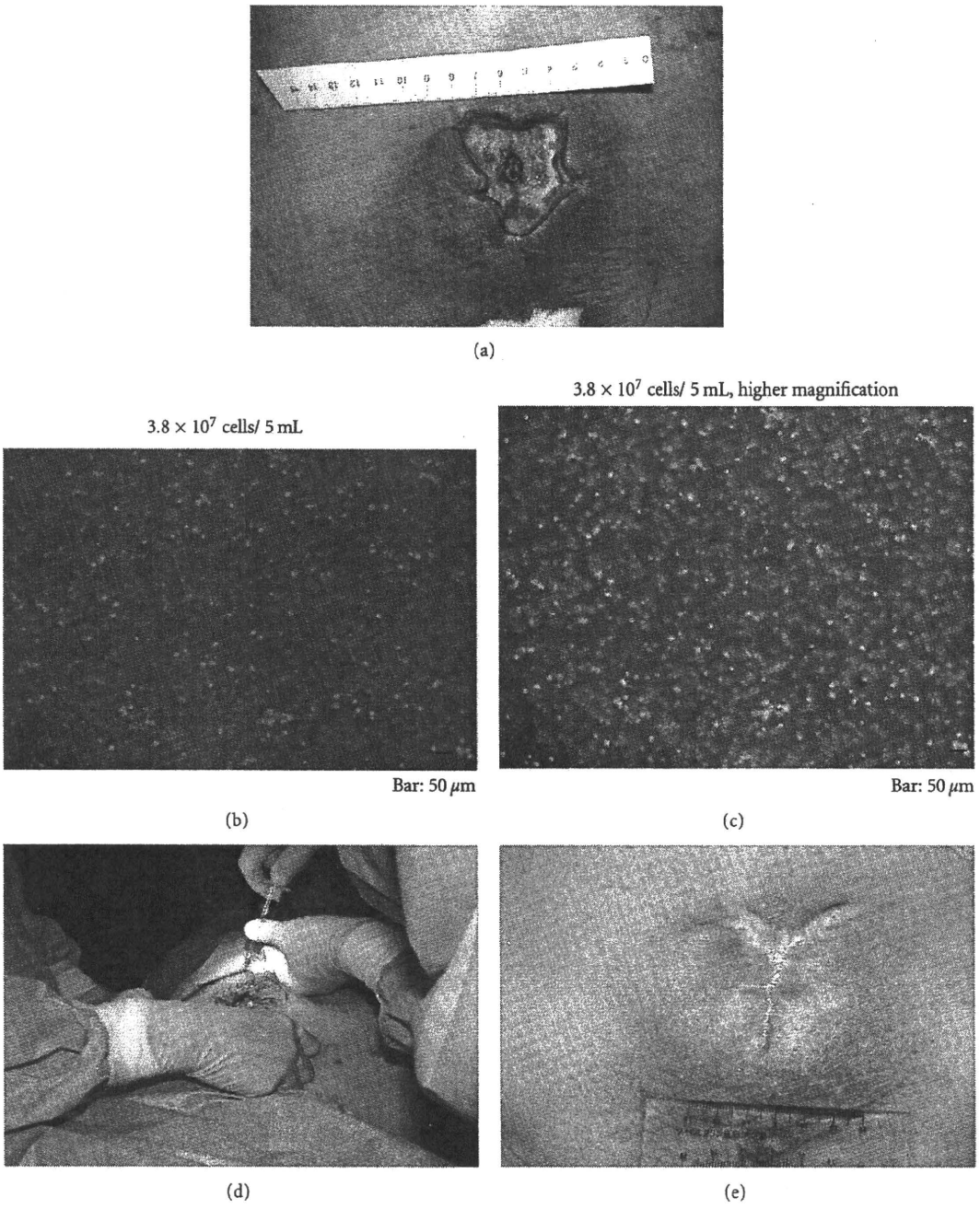

FIGURE 6: 89-year-old woman underwent a uterine cancer surgery followed by 50-Gy fractionate radiation therapy 40 years previously. (a) In 10 × 10 cm area of radiation, 5 × 10 cm area was exposed. Bone, fascia, and muscle as well as skin and fat were affected. (b, c, d) After careful debridement, 3.8×10^7 cells/5 mL were applied over the wound bed and margins and soaked with artificial dermis. In a few days postoperatively, bFGF was sprayed over the peeled-off inner regenerated tissue for 21 days. (e) In 1.5 years postoperative view. The regenerated tissue remained durable, soft, and pliable.

Application of Stem cell therapies for repair and regeneration has recently been investigated at a clinical level in variously defected or injured tissues, among which stem cells and adipose-derived stem cells (ADSCs) can be harvested with a minimally invasive procedure by liposuction procedure through a small incision. Similar to our method but in detail very different, Clinically purified autologous lipoaspirates were used as treatment for radiotherapy tissue damage of consecutive 20 patients. Indirectly, induced ADRCs have potential in cell therapy for radiation injury due to increasing neovascularization and retention of the fat property [10].

This enables us to adopt this regeneration method for patients with severe comorbidity such as elderly systemic disease and physical wasting state (data not shown). The

ADSCs contain several types of stem and regenerative cells, including endothelial and smooth muscle cells and their progenitors and preadipocytes [11]. The ADSCs have the capacity to differentiate into multiple lineages and cell types including mesodermal tissues such as fat, bone, cartilage, endothelial cells of endodermal origin, and neurons and epidermis of ectodermal origin as seen in the mesenchymal stem cells [12].

Management of radiation injuries composes two major parts. One is localized injuries and the other is of systemic injuries. Among localized radiation injuries, chronic injuries are more common in the medical field after caner radiation therapy. Usually management of these chronic wounds is well handled by well-vascularized tissue transfers as various plastic surgical procedures have proved. In consideration of each patient general condition and preference, the choice of therapeutic selections should be performed. On the other hand, when the local radiation injuries are encountered in an acute phase, there are high chances for innovative procedures using autologous stem cells. The hMSCs are resistant to radiation. We have previously demonstrated *in vitro* cell proliferation curve and are also able to produce protein avoiding cell apoptosis [13]. And the application of cultured bone-derived mesenchymal stem cells successfully healed severe local radiation wounds. However, the cultured stem cell therapy takes longer period as long as 16 days before cell therapy and required multiple (5 times) cell injections as well as 2 skin grafting, 2 flaps, and 1 artificial dermis coverage [14]. Also, increasing evidences demonstrate that ADSCs are similar to hMSCs in cell properties and characteristics both *in vitro* and *in vivo* [11]. ADSCs are highly yielding and less invasive for donor sites. The acute myocardial infarction porcine models by improving left ventricular function, perfusion, and remodeling [15]. When localized radiation was distant enough from the donor sites adipose tissues, immediate debridement and regeneration happens using adipose-derived stem cells, which are available for processing within 1.5 hours simultaneously in the same operation theater without cell culture since adipose tissues (fat tissues) are abundant in adult humans compared to other stem cell sources. In the limited clinical circumstances of high-risk patients such as elderly and chronic local infection, there is still opportunity of harvesting and processing the patient's own fat-derived stem cells successfully as seen in our case. Practically for emergency radiation injury cases, more abundant cell sources such as fat are the primary candidate for this purpose. The cell property and characterization of ADSCs are discussed and discussed either fresh or cultured [16]. The results from the clinical trial for acute myocardial infarction are expected and may be applicable for acute radiation injury treatment.

For treatment of systemic radiation injuries, stockpiled stem cells should be globally available through medical assistance network system under WHO-REMPAN, in which Nagasaki University is highly involved in its activity, or other international frameworks. Early resurfacing of the damaged skin and subcutaneous tissues is as important as hematological and intestinal system resuscitation [17].

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

Therapeutic regimens of radiation injuries used to be dependent on each subspecialty in the medical filed such as internal medicine, radiology, and surgery.

Recent establishment of wound care specialty was mostly led by plastic surgeons, but other supporting specialists such as nurses, dermatologists, and gastrointestinal physicians and surgeons may be practically handling these rare but of significant impact "radiation injuries" as a interdisciplinary approaches. Therefore, more specialization for "radiation injuries" may be required.

Acknowledgment

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References

[1] S. François, M. Mouiseddine, N. Mathieu et al., "Human mesenchymal stem cells favour healing of the cutaneous radiation syndrome in a xenogenic transplant model," *Annals of Hematology*, vol. 86, no. 1, pp. 1–8, 2007.

[2] S. Akita, K. Tanaka, and A. Hirano, "Lower extremity reconstruction after necrotising fasciitis and necrotic skin lesions usinga porcine-derived skin substitute," *Journal of Plastic, Reconstructive and Aesthetic Surgery*, vol. 59, no. 7, pp. 759–763, 2006.

[3] S. Akita, K. Akino, K. Tanaka, K. Anraku, and A. Hirano, "A basic fibroblast growth factor improves lower extremity wound healing with a porcine-derived skin substitute," *Journal of Trauma*, vol. 64, no. 3, pp. 809–815, 2008.

[4] D. Aquilina, F. X. Darmanin, J. Briffa, and D. Gatt, "Chest wall reconstruction using an omental flap and Integra," *Journal of Plastic, Reconstructive and Aesthetic Surgery*, vol. 62, no. 7, pp. e200–e202, 2009.

[5] L. Wong and J. Rehm, "Images in clinical medicine. Radiation injury from a fluoroscopic procedure," *The New England Journal of Medicine*, vol. 350, no. 25, p. e23, 2004.

[6] T. Thongpraparn, P. Chaudakshetrin, and P. Buranapong, "Lesson learned from Co-60 accident in Thailand," *Australasian Physical and Engineering Sciences in Medicine*, vol. 25, no. 4, pp. 172–174, 2002.

[7] C. E. Brandao-Mello, A. R. Oliveira, N. N. J. Valverde de, R. Farina, and J. M. Cordeiro, "Clinical and hematological aspects of 137Cs: the Goiania accident," *Health Physics*, vol. 60, no. 1, pp. 31–39, 1991.

[8] J. J. Lataillade, C. Doucet, E. Bey et al., "New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy," *Regenerative Medicine*, vol. 2, no. 5, pp. 785–794, 2007.

[9] H. Scherthan, M. Abend, K. Müller et al., "Radiation-induced late effects in two affected individuals of the Lilo radiation accident," *Radiation Research*, vol. 167, no. 5, pp. 615–623, 2007.

[10] G. Rigotti, A. Marchi, M. Galiè et al., "Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells," *Plastic and Reconstructive Surgery*, vol. 119, no. 5, pp. 1409–1422, 2007.

[11] P. A. Zuk, M. Zhu, P. Ashjian et al., "Human adipose tissue is a source of multipotent stem cells," *Molecular Biology of the Cell*, vol. 13, no. 12, pp. 4279–4295, 2002.

[12] D. Peroni, I. Scambi, A. Pasini et al., "Stem molecular signature of adipose-derived stromal cells," *Experimental Cell Research*, vol. 314, no. 3, pp. 603–615, 2007.

[13] M.-F. Chen, C.-T. Lin, W.-C. Chen et al., "The sensitivity of human mesenchymal stem cells to ionizing radiation," *International Journal of Radiation Oncology Biology Physics*, vol. 66, no. 1, pp. 244–253, 2006.

[14] E. Bey, M. Prat, P. Duhamel et al., "Emerging therapy for improving wound repair of severe radiation burns using local bone marrow-derived stem cell administrations," *Wound Repair and Regeneration*, vol. 18, no. 1, pp. 50–58, 2010.

[15] C. Valina, K. Pinkernell, Y.-H. Song et al., "Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction," *European Heart Journal*, vol. 28, no. 21, pp. 2667–2677, 2007.

[16] P. L. Sánchez, R. Sanz-Ruiz, M. E. Fernández-Santos, and F. Fernández-Avilés, "Cultured and freshly isolated adipose tissue-derived cells: fat years for cardiac stem cell therapy," *European Heart Journal*, vol. 31, no. 4, pp. 394–397, 2010.

[17] D. M. Weinstock, C. Case Jr., J. L. Bader et al., "Radiologic and nuclear events: contingency planning for hematologists/oncologists," *Blood*, vol. 111, no. 12, pp. 5440–5445, 2008.

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Efficacy of patients' own adipose-derived regenerative cells for chronic intractable radiation injuries

Abstract

Chronic radiation injury may lead to intractable wounds by demonstrating ulcer, prone to infection and stiffness. As the therapeutic radiation is often involve wider and deeper tissues and often require extensive debridement and reconstruction, which are not sometimes appropriate for elderly and compromised hosts. Recent advancement of understanding of efficacy of patient own (autologous) adipose-derived regenerative cells (ADRCs) is proved highly yielding and may indicate usefulness in clinical cell therapy. Using patients' own ADRC and artificial dermis with angiogenic growth factor resulted in successful wound healing. Due to high-yielding of cell number compared to the other mesenchymal stem cells from bone marrow origin, this methods does not require cell culture because of sufficient enough ADRCs are obtained through cell processing. Donor sites are minimally incised and the decrease of the subcutaneous tissue by liposuction was less harmful. Thus, the ADRC s treatment for decades-long radiation injuries is effective, safe and improves such otherwise difficult wounds.

Keywords: radiation injuries, patient-own, non-cell culture, artificial dermis, angiogenic growth factor

Introduction

Radiation injuries have increased fibrosis and decreased and insufficient vascularity, which can lead to tissue necrosis, infection, ulceration and fibrosis later in demonstrating chronic radiation injury syndrome.[1] Therefore radiation wounds tend to persist for a long time, be present impaired healing and prone to recurrence. Radiated wounds are treated by standardized, adequate debridement both in the depth and in the width and covered with well-vascularized tissues, however, the long-term outcome is not warranted and donor-site morbidity is sometimes concerned, especially for the aged patients or patients who somehow contain problems in harvesting the donors or limited due to the co-existing diseases.

Application of Stem cell therapies for repair and regeneration has recently been investigated at a clinical level in variously defected or injured tissues. Among which stem cells, adipose-derived regenerative cells (ADRCs) can be harvested with a minimally invasive procedure by liposuction through a small incision. The ADRCs contain several types of stem and regenerative cells, including Adipose-Derived Stem (or stromal) Cells (ADSCs), endothelial and smooth muscle cells and their progenitors and preadipocytes.[2] The ADSCs have the capacity to differentiate into multiple lineages and cell types including mesodermal tissues such as fat, bone, cartilage, endothelial cells of endodermal origin and neurons and epidermis of ectodermal origin as seen in the mesenchymal stem cells.[3]

We succeeded to treat 3 patients of chronically persisted radiation injuries with autologous ADRCs therapy.

Methods

This study was approved by the Ethics Committee of the Nagasaki University Hospital and written informed consent was obtained from all patients (approved number 08070296) and partly supported by the Global COE (Center of Excellence) program E08, Global Strategic Center for Radiation Health Risk Control, funded by the Japan Society for the Promotion of Science.

Patients

We have treated 3 patients presenting with chronic radiation injures with ulcers and severe fibrosis. The patients ranged in age from 52 years to 87 years. The mean age of patients was 68.6 years. The patients were all female. The average follow up was 8.3 months. Radiation injuries to sacral, neck and anterior chest after 50 Gy of adjuvant linac, 50 Gy-cobalt and 60Gy-linac radiotherapy, respectively were enrolled in this clinical treatment.

Harvesting of adipose tissue by liposuction and Isolation of ADRCs

A few 3-5 mm incisions were made on the abdominal region, the thigh, and the gluteal region. The subcutaneous layer was infiltrated with a lactated Ringer's solution with addition of 0.5 ml of epinephrine and 25 ml of 1% lidocaine per 500 ml. Adipose tissue was suctioned using an 18-G Becker cannula with a 50ml syringe. ADRCs were isolated from the suctioned adipose tissue by using the Celution system. (Cytori Thera-

putics, Inc., USA). Briefly, the suctioned adipose tissue was introduced into the Celution cell-processing device, which automatically and aseptically extracts and concentrates the mononuclear fraction of adipose tissue and removes unwanted or deleterious cells, cell and matrix fragments such lipids. By using the Celution system, a 5 ml solution containing concentrated ADRCs in about one and a half hour *(Figure 1, 2)*. The whole procedure is in a closed circuit and this reduces the chance of the contamination.

The small portion of processed ADRCs were used for the *ex vivo* cell culture and confirmed the proliferation and differentiation potential in all cases.

Adipose-Derived Regenerative Cell Grafting and Postoperative management

For the scaffold purpose, we used the artificial dermis (Terudermis®, Olympus-Terumo Biomaterials Co., Ltd., Japan) *(Figure 3)*. The Terudermis® is composed of two layers: a lower layer of bovine atelo-collagen, and an upper layer comprising a silicone sheet which protects against infection and dryness from the outside. After minimum debridement, the Terdermis® was multi-layered stacked over freshly debrided wounds. The silicone sheets were removed except top Terudermis®. The two-third of an isolated ADRCs alone was injected; around the debrided wounds, at the base of the wounds and into Terudermis®. Another one-third of ADRCs were mixed with the autologous adipose which was rinsed with a lactated Ringer's solution. After mixed, it was injected to a zone of hard fibrotic tissue around the debrided wounds.

One week after removing the silicone layer, human recombinant fibroblast growth factor (bFGF: Fiblast®, Kaken Co., Ltd., Japan) *(Figure 4)* was sprayed. The wound was covered with non-adherent occlusive foam dressing.

Results

CASE 1

An 89-year-old female underwent radiotherapy 40 years ago due to uterine carcinoma. She had suffered from chronic radiation enteritis. Recently ulcer occurred in the sacral region. On first examination the sacral bone was exposed, bad smell and plenty of exudates were also noted. 210 ml of adipose tissue was harvested by liposuction. The harvested adipose tissue contained 3.7x107 ADRCs. After debridement, ADRCS were injected around the debrided wound and at the wound base. Furthermore, Terudermis® and continuous negative pressure device were applied. The negative pressure (16 Kda) started 8 hours after completion of the surgery and continued for one week. At day 14, the wound base elevated and spotty granulation tissue was observed. In 4 weeks, the wound margins demonstrated new epithelialization. Finally in 82 days post-operation, complete wound healing with regeneration was obtained. At 27 months after surgery, there is no recurrence or worsening of the regenerated tissue *(Figure 5)*.

CASE 2

A 52-year-old female was suffering from intractable chronic radiation wounds, which limited her neck movement forward. 335ml of adipose tissue was harvested by liposuction. The harvested adipose tissue contained 4.1x107 ADRCs. After debridement, ADRCS were injected to the debrided wound margin and in the wound base. The adipose tissue which mixed

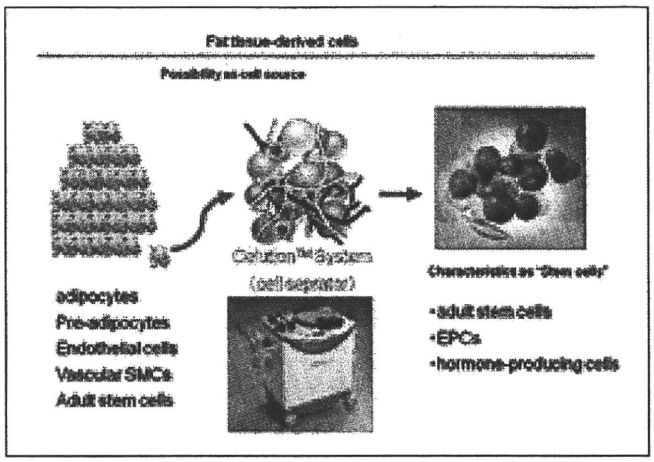

Figure 1. For the successful cell harvesting for effective cell application, our solution, through the use of a novel source of autologous tissue- fat tissue, we can obtain therapeutic quantities of regenerative cells all within one and half hour.

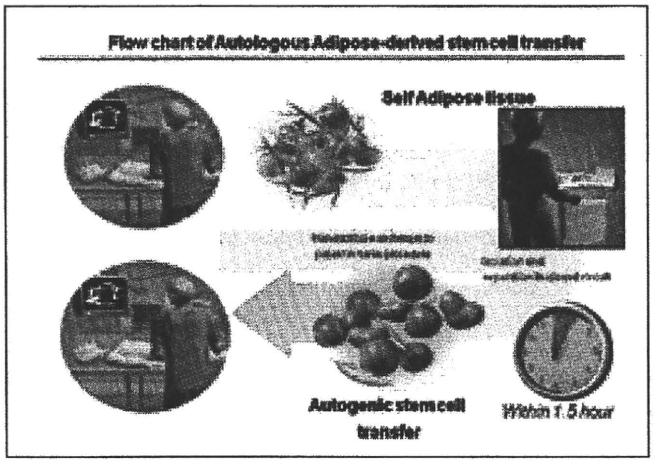

**Figure 2. The benefit of this procedure is that 1. the cell source is derived from the patient-own fat, which is less invasive and abundant in cell amount compared to other procedures such as bone-marrow aspiration, 2. the all procedure is in a closed circuit and this reduces the chance of the contamination,
3. and it is simultaneous operation in both harvesting and transferring the cell within 1 and half hour.**

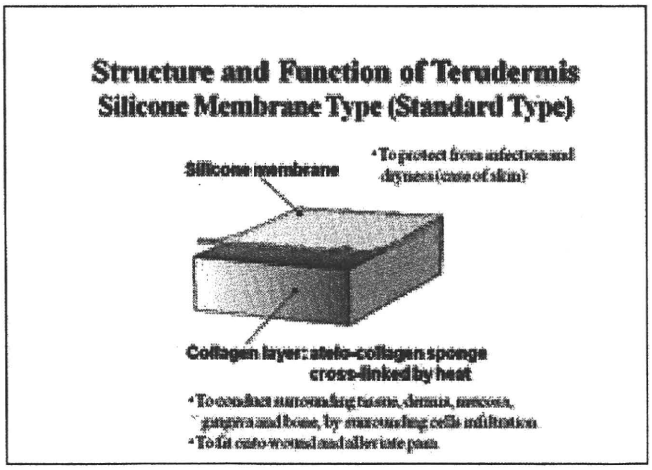

Figure 3. For the scaffold purpose, silicone Membrane Type Terudermis®. Collagen layer is made in bovine atelo-collagen sponge cross-linked by heat. It is conducted surrounding tissue, dermis, mucosa, gingiva etc, by surrounding cells infiltration. It has silicone membrane to protect against infection from the outside and dryness. This product is highly efficient in containing the exogenously applied cells as well as local neighboring cells.

→

with ADRCs was also injected to the subcutaneous tissue around the wound. In 75 days, the wound was healed and the neck forward movement was improved. At 5 months after surgery, there is small erosion and the injected subcutaneous lesion has still kept its soft texture *(Figure 6)*.

CASE 3

A 67-year-old female was suffering from irritable and burning pain in the anterior chest with sternal bone exposure after radiotherapy due to breast cancer. 173 ml of adipose tissue was harvested by liposuction. The harvested adipose tissue contained 1.7x107 ADRCs. After debridement, ADRCS were injected to the debrided wound margin and in the wound base. The adipose tissue which mixed with ADRCs was also injected to the subcutaneous tissue around the wound. In 75 days, the wound was completely healed and pain was dramatically decreased. At 5 months after surgery, there is no recurrence or worsening of the regenerated tissue. The injected subcutaneous lesion has still kept its soft texture *(Figure 7)*.

Discussion

Chronic radiation ulcer often demonstrates poor response to the conventional therapy. The standardized treatment of radiation ulcer is a wide-enough and deep-enough excision of potentially involved as well as grossly apparently affected tissues, followed by coverage with well-vascularised tissue such as musculocutaneous free or local flaps. However lack and limitation of tissue transplantation and inability of selecting such donor-site and possible donor-site morbidity lead to incomplete treatment in the long-term.

The adipose tissue can be easily harvested from patients by a minimally invasive method through the small port incisions. Freshly isolated adipose-derived stem cells in our method demonstrated significant subjective improvement such as pain, discomfort and irritability as well as remarkably improved wound healing.

The human subcutaneous adipose tissue is abundant and contains greater number of various stem cells than bone marrow [2,3]. As freshly isolated ADSC or ADRCs are greater in yielding, application of freshly isolated ADSCs are comparable to the cultured ADSCs, which demonstrates phenotypic and functional similarity in mice model [4]. ADRCs are the cellular fraction derived from the enzymatic digestion of the adipose tissue. ADRCs contain several types of stem and regenerative cells, including ADSCs, endothelial and smooth muscle cells, and preadipocytes. ADSCs delivered into an injured or diseased tissue may secrete cytokines and growth factors that stimulate recovery possibly in a paracrine manner. Indeed, human adipose stromal cells play a role in producing angiogenic and antiapoptotic growth factors such as vascular endothelial growth factor, hepatocyte growth factor and transforming growth factor-ß [5]. Also, fibroblast growth factor-2-induced hepatocye growth factor secretion form adipose-derived stromal cells are able to inhibit post-injury fibrogenesis through a c-Jun N-terminal kinase-dependent mechanism [6]. In animal models, although it is cultured, topical administration of adipose tissue-derived stromal cells (ATSCs) is effective for rat cutaneous wounds induced by mitomycin C-treatment [7] and in diabetic mice wounds [8]. Clinically purified autologous lipoaspirates were used as treatment for radiotherapy tissue damage of consecutive 20 patients. Indirectly, induced ADRCs have potential in cell therapy for radiation injury due to increasing neovascular-

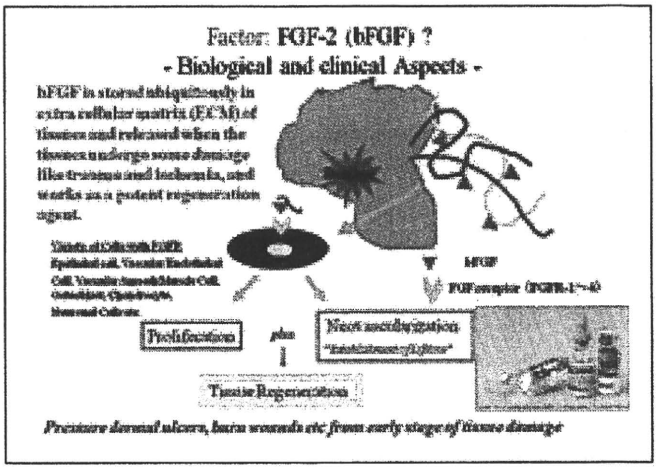

Figure 4. For factor with spray-form application, human recombinant basic fibroblast growth factor (bFGF) is used to promote local cell and tissue proliferation and neovascularization. This is commercially available growth factor is the single most-sold wound product in Japan since 2003.

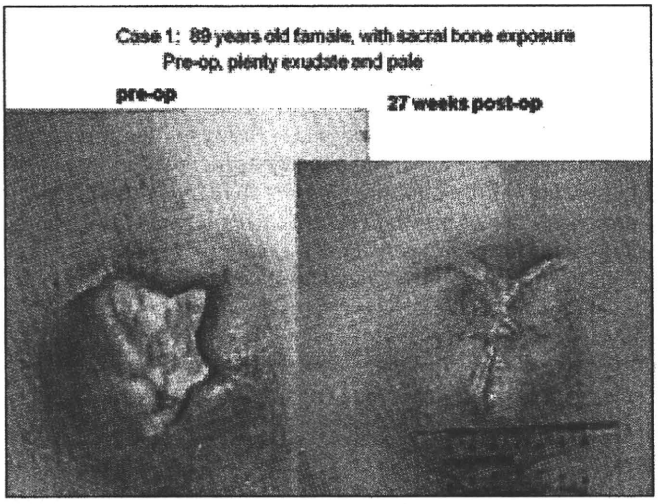

Figure 5. Case 1 of this method was applied to the 89-year-old female patient with over 40-year radiation injury and recently the sacral bone was exposed and bad smell and plenty of exudates were also noted (right). 27 weeks post-operatively, wound is closed with regeneration uneventfully and demonstrated no recurrence.

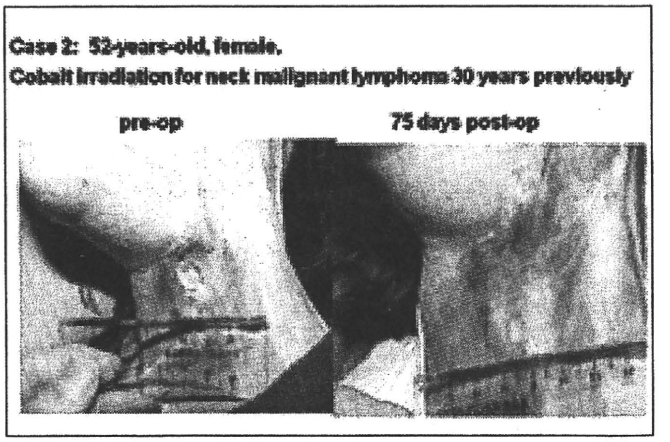

Figure 6. This case 2 patient was suffering from the intractable chronic radiation wounds, which limit her neck movement forward. After 335 grams of the patient-own lipo-suction, the processed Adipose-Derived Stem cells were injected post-debrided wound margin and wound base. The fat cells mixed with ADSCs were also injected in a circumferential manner. In 75 days, the wound was completely healed and the neck forward movement was improved as depicted in the right panel.

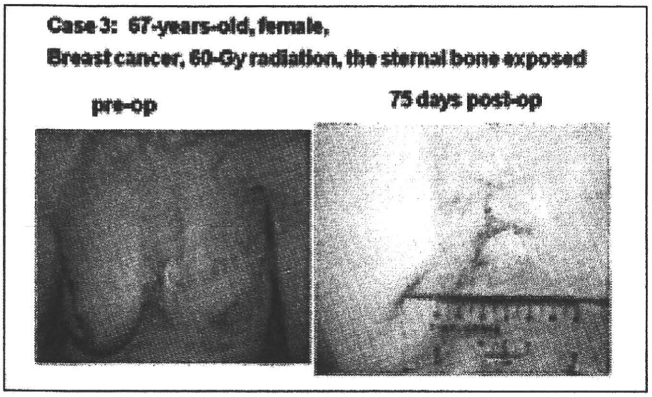

Figure 7. This case demonstrated long-term sequela of the post-breast cancer radiation. The patient was suffering from the irritable and burning pain in the anterior chest with sternal bone exposure. 173 grams of patient-own lipo-suction, the ADSCs are injected in the debrided wound margin and base. For the pain relief, wider subcutaneous fat cells mixed ADSCs were applied. In 75 days, the wound is completely healed and the pains were dramatically decreased (right).

ization and retention of the fat property [9].

We have also shown that administration of ADRCs or the fat with ADRCs is a highly effective method for treatment of chronic radiation injury after several decades. The transplanted subcutaneous lesion keeps its soft texture and subjectively more comfortable and activity of daily living markedly improved. Using ultrasonography, we detected an increase on local blood circulation at the site in which the ADRCs were injected or alternatively, where the fat with ADRCs was transplanted. (data not shown). Thus, ADRCs are highly accessible as a therapeutic option for intractable chronic radiation injury. ■

References

1. **Scherthan H, Abend M, Muller K, Braselmann H, Zitzelsberger H, Kohn FM et al.** Radiation-induced late effects in two affected individuals of the Lilo radiation accident. *Radiat Res,* 2007; 167: 615-623.

2. **Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang, JI, Mizuno H et al.** Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell,* 2002; 13: 4279-4295.

3. **Peroni D, Scambi I, Pasini A, Lisi V, Bifari F, Krampera M et al.** Stem molecular signature of adipose-derived stromal cells. *Exp Cell Res,* 2007; 314: 603-615.

4. **Bai X, Yan Y, Song Y-H, Seidensticker M, Rabinovich B, Metzele R et al.** Both cultured and freshly isolated adipose-derived stem cells enhance cardiac function after acute myocardial infarction. *Eur Heart J,* 2010; 31: 489-501.

5. **Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE et al.** Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation,* 2004; 109: 1292-1298.

6. **Suga H, Eto H, Shigeura T, Inoue K, Aoi N, Kato H et al.** IFATS collection: Fibroblast growth factor-2-induced hepatocyte growth factor secretion by adipose-derived stromal cells inhibits postinjury fibrogenesis through a c-jun N-terminal kinase-dependent mechanism. *Stem Cells,* 2009; 27: 238-249.

7. **Nambu M, Ishihara M, Nakamura S, Mizuno H, Yanagibayashi S, Kanatani Y et al.** Enhanced healing of mitomycin C-treated wounds in rats using inbred adipose tissue-derived stromal cells within an atelocollagen matrix. *Wound Repair Regen,* 2007; 15: 505-510.

8. **Nambu M, Kishimoto S, Nakamura S, Mizuno H, Yanagibayashi S, Yamamoto N et al.** Accelerated wound healing in healing-impaired db/db mice by autologous adipose-derived tissue derived stromal cells. *Ann Plast Surg,* 2009; 62: 317-321.

9. **Rigotti G, Marchi A, Galie M, Baroni G, Benati D, Krampera M et al.** Clinical treatment of radiotherapy tissue damaged by lipoaspirate transplant: a healing process mediated by adipose-derived adult cells. *Plast Reconstr Surg,* 2007; 119: 1409-1422.

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The efficiency and benefits of combined use of artificial dermis with growth factor in clinical cases

Abstract

Recent advancement of recombinant human product and wide application of "artificial dermis" made possible for effective skin defect coverage. Extensive burns, congenital pigmented nevi and congenital anomalies are otherwise very limited for successful reconstruction. The human gene-encoding growth factor such as human recombinant basic fibroblast growth factor (hr-bFGF) is useful in such clinical cases as well as single application. Terudermis® is one of most promising "artificial dermis" with very unique two distinctive types of bovine collagens, 90% is consist of fibrillar atelo-collagen and 10% of heat-denatured atelo-collage. When used together with hr-bFGF, since this product composes of heat-denatured atelo-collagen, which easily binds to small glycopeptides like hr-bFGF, enhanced synergism is observed clinically. Further basic mechanisms should be clarified in detail; however, here are some successful clinical application of hr-bFGF and artificial dermis.

Keywords: hr-bFGF, artificial dermis, atelo-colagen, heat-denatured

Introduction

The use of artificial dermis (or skin substitute) over tendon-exposed, bone-exposed wound surfaces or for temporary coverage before subsequent skin grafting for flap surgery in burns and other skin loss or damage is widely accepted.[1-3] Artificial dermis is easily used for temporary coverage for staged reconstruction in the case of emergency trauma as well as in elective cases. Since there is no cell involvement in the artificial dermis itself, it would be very beneficial with little ethical concerns related to cell therapy, given that ethics involve a very strict process in countries such as Japan where it is very difficult to pass the Internal Review Board (IRB) and national regulations.[4] For artificial dermis, there is evolutional improvement in such products with a composition of insoluble collagen fibers using telopeptide towards atelocollagens excluding telopeptides or a combination of fibrillar atelocollagens and heat-denatured atelocollagens.[5]

Basically, there are bi-layer compositions with an outer layer of silicone membranes and inner collagen sponges. Currently, there are two commercially available bi-layered collagen sponge-type skin substitutes in Japan. Both consist of atelocollagens, which are soluble in water and provide strength through the dermal structure with suitable polarity for adjacent host cell invasion. One is porcine-derived and the other is made from bovine material. Bovine atelocollagen artificial dermis is composed of both fibrillar atelocollagens and heat-denatured atelocollagens. Recently, numerous variant uses of artificial dermis have been implemented. One form is to use only the inner layer of the sponge with primary skin grafting or flap coverage over the collagen, and other uses include that with topical negative pressure devices.[6] Another application is to use the bi-layered artificial dermis as "mesh" grafting with drainage slit types for external administration of soluble factors such as growth factors.

Bovine-derived artificial dermis (Terudermis®)

Terudermis® is made of atelocollagen to minimize antigens and its atelocollagen has been cross-linked by heat-denaturing to obtain maximal biocompatibility. It consists of scaffolding for surrounding cells and heat-denatured atelocollagens that stimulate infiltration of the cells in the products. In Japan, this is clinically applicable for deep burns (third degree burns) and deep dermal burns, for release of contracted wound coverage, degloving injuries, temporal coverage for open fractures, fingertip amputees, for post-tumor resections, oral defects due to palatoplasty and tympanic membrane *(Figure 1)*.

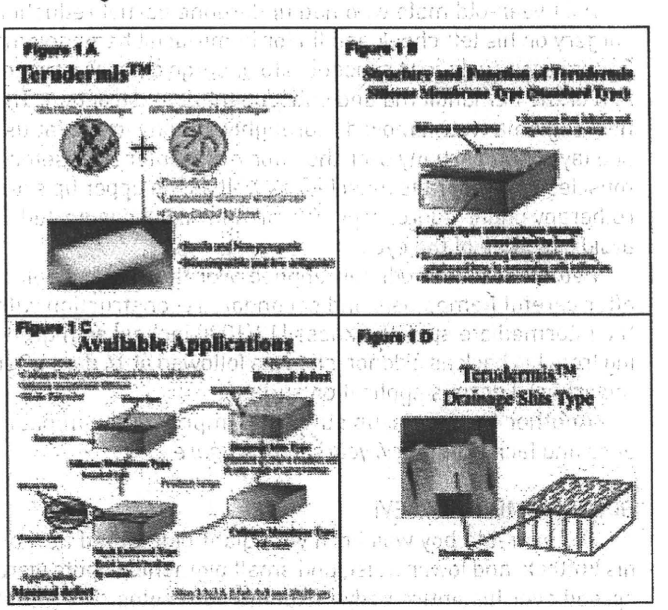

Figure 1. Basic structure and applications of Terudermis®. A: Composition of Terudermis®. B: Standard type structure and function. C: Various applications are indicated. D: Drainage slits type for bFGF application.

Porcine-derived artificial dermis (Pelnac®)

Indications for the use of artificial dermis were previously limited to deep tissue exposure such as tendons, bone or cartilage, in which wound closure was more difficult to achieve by skin grafting alone. All surgical debridement was confirmed as sufficiently deep and wide for clinically contaminated or infected lesions. An artificial dermis, a bi-layer with an outer silicone membrane and inner porcine tendon-derived collagen sponge (Pelnac®, Gunze Co. Ltd, Kyoto, Japan), was immediately applied to all wounds. Follow-up was ensured after the last procedure, which was either application of the artificial dermis or secondary split skin grafting with the exception of small areas of fingertips or toe tips.

Basic fibroblast growth factor (Trafermin, Fiblast Spray®)

Genetically recombinant human basic fibroblast growth factor (bFGF) was used right after completion of wound bed preparation in the operative ward by spraying. This product is the first approved human recombinant basic fibroblast growth factor in the world. bFGF induces wound bed angiogenesis and also stimulates fibroblast accumulation and organization, by constituting "good" wound bed preparation and before secondary split thickness skin grafting.

The concentration of bFGF was 30 µg of bFGF per 30 cm^2 area as 100 µg of freeze-dried bFGF dissolved in 1 ml of solution of minimal concentration of benzalkonium chloride, with 300 µl sprayed over a 30 cm^2 area at a distance of 5 cm, and 0.3 ml of such concentration solution was given by this method. Using bFGF with the artificial dermis has to be delivered as a solution with the same medium of spray use at concentration of 1 µg/µl. For use with the bi-layer artificial dermis, the solution is applied from the edge, also at a final concentration of 1 µg/ cm^2.

Clinical cases and benefits of using artificial dermis and bFGF

A CASE OF THE STURGE-WEBER SYNDROME

A 41 year-old male who had undergone partial reduction surgery on his left cheek and lip for hemi-facial hemangioma 2 years previously, was scheduled to undergo extensive resection of the hemangioma and subsequent reconstruction. The hemangioma was removed thoroughly, mostly in the fat tissue layer and partially over the supra-muscular (masseteric muscles or temporalis muscles) as well as the upper lip sclerotherapy under fluoroscopy. Tarsorraphy is also indicated to avoid exposure of the eyes.

Pelnac® was immediately applied over the surgical defect after careful hemostasis and secondary reconstruction with the intermediate split-thickness (12/1000 inches) skin grafting from his back as a donor-site was followed at 14 days after the artificial dermis application surgery.

Another two touch-up surgeries improved the appearance and facial contour 4 years later *(Figure 2)*.

GIANT PIGMENTED NEVI

A 2 year-old boy was born with giant pigmented nevi on his buttock, and lower waist, and small pigmented spots were spread over his entire body including both soles. The initial surgery was performed on his lower waist with an intermediate split-thickness (11/1000 inches) from his upper back. The

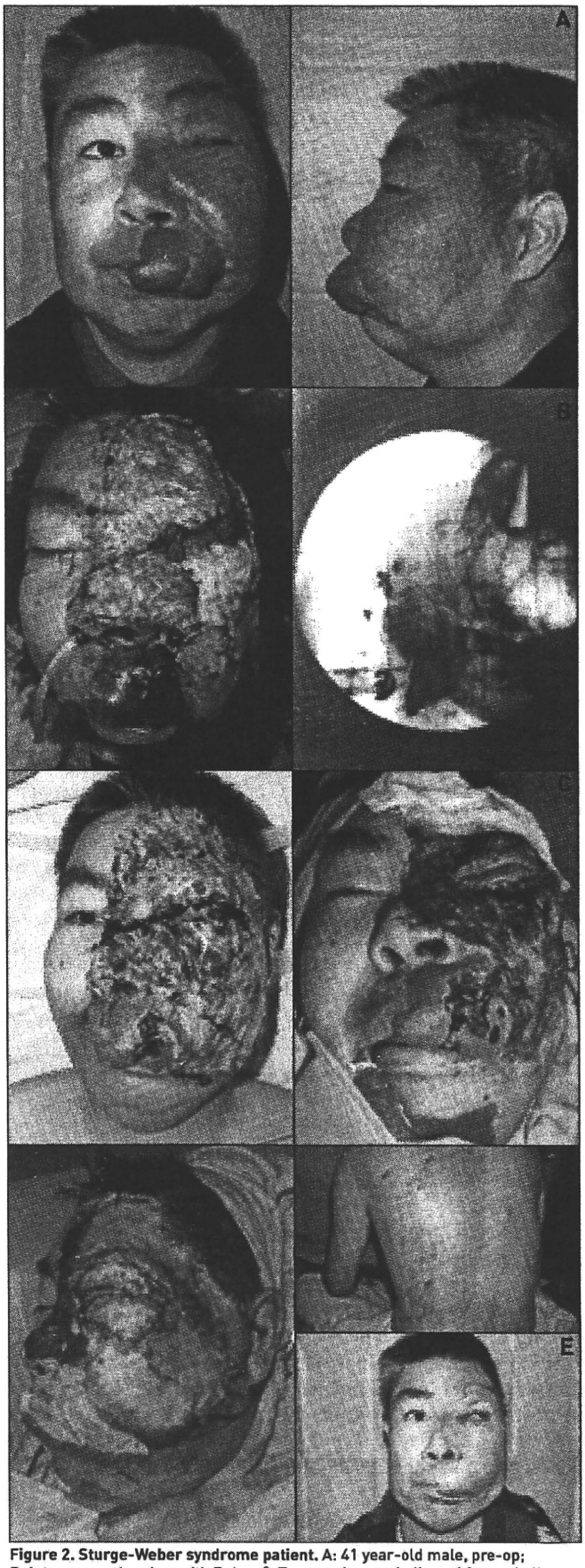

Figure 2. Sturge-Weber syndrome patient. A: 41 year-old male, pre-op; B: Intra-operative view with Pelnac®. Tarsoraphy was indicated for eyeball protection. Sclerotherapy under fluoroscopy in the upper lip was performed. C: 14 days after initial surgery. With the outer membrane on the left and after peel-off of the membrane (on the right). D: Immediately after split-thickness skin grafting on the left and the donor site is shown on the right.

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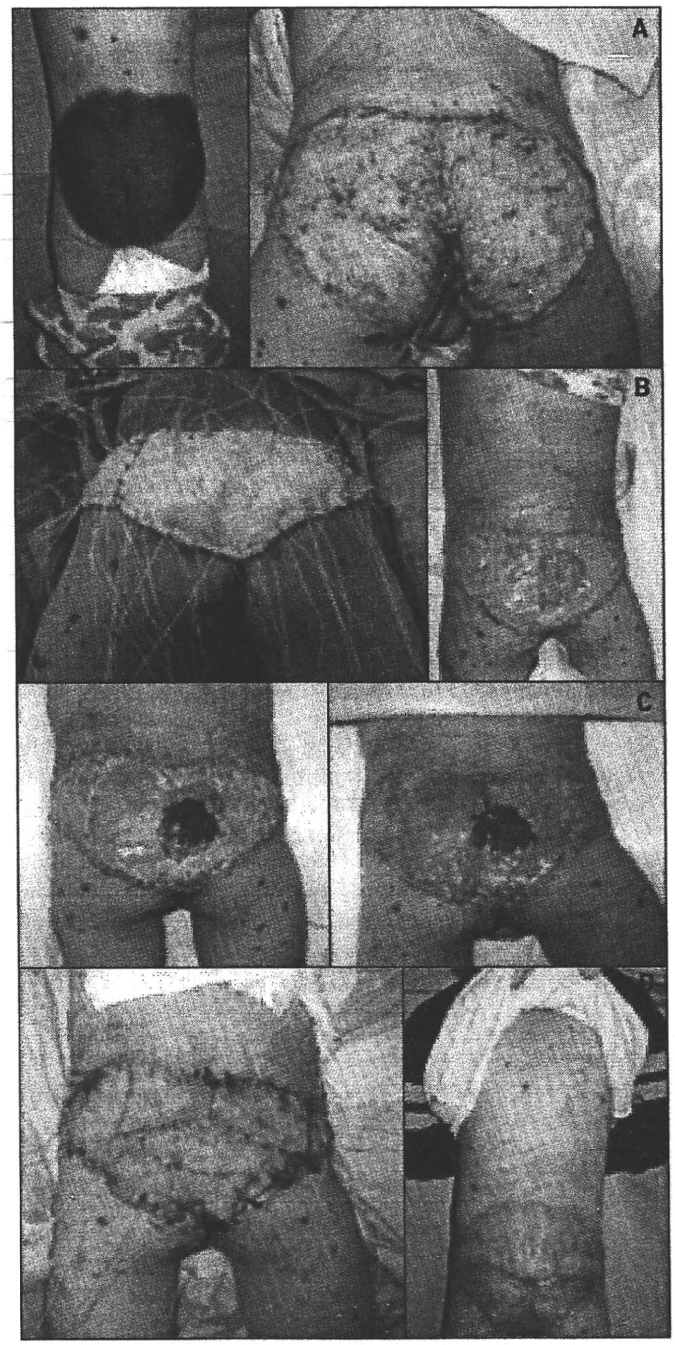

Figure 3. Giant pigmented nevi. A: 2-year-old boy, pre-op on the left and post-complete debridement on the right. B: Intra-operative view with Terudermis® and "tie-over" threads in the left and 11 days after Terudermis® and removal "tie-over" and the outer membranes of the central portion due to tear-off. bFGF was sprayed. C: 14 days after Terudermis™ application and 3 consecutive-day bFGF spraying on the left and peeled off the outer membranes on the right. D: Immediately after split-thickness skin grafting on the left and a 2 months-post op view on the right.

second surgical procedure was planned on the buttocks and peri-anal areas. Complete debridement was undertaken deep enough in the bottom half of the subcutaneous tissues followed immediately by Terudermis® application with "tie-over" dressings. Eleven days later, the "tie-over" was removed and a small portion of the outer membrane was torn. Thus, bFGF for accelerating the wound bed angiogenesis and optimization was used for 3 days right before the secondary intermediate split-thickness skin grafting (12/1000 inches) from his back. The post-operative clinical course was uneventful at 2 months *(Figure 3)*.

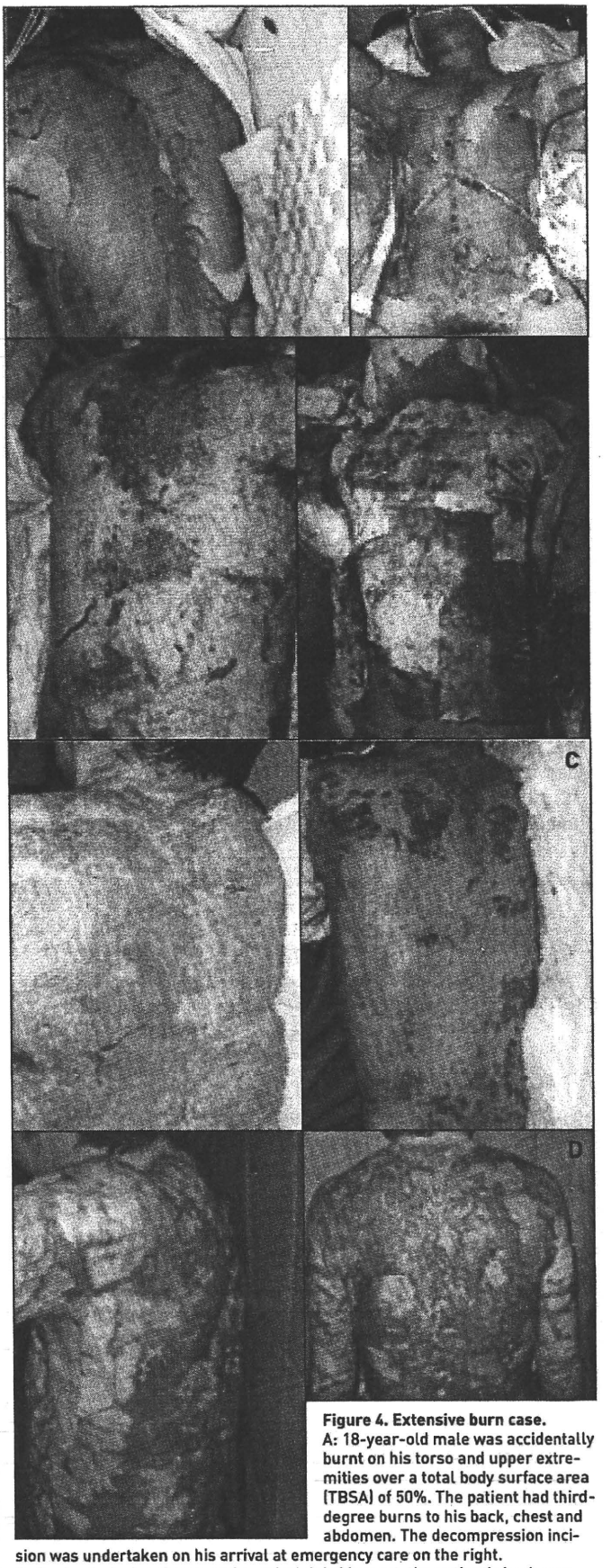

Figure 4. Extensive burn case. A: 18-year-old male was accidentally burnt on his torso and upper extremities over a total body surface area (TBSA) of 50%. The patient had third-degree burns to his back, chest and abdomen. The decompression incision was undertaken on his arrival at emergency care on the right. B: On day 6, during the second surgical debridement, the patient's back was completely debrided and bFGF was sprayed over the wound, then drainage-slit type Terudermis® applied over with daily continual application for 7 days. C: Post-operative view at 4 weeks (on the left) and 10 weeks (on the right). Re-epithelialization was uneventful. D: 8 months post-operative view. The mild scar is formed; however, the skin resurfacing involved no problems.

AN EXTENSIVE BURN

An 18 year-old male was accidentally burnt when his clothes caught fire during handling of machinery. The total body surface area of mainly deep burns was 50% on his chest, abdomen, back and bilateral upper extremities. The initial surgery was undertaken on Day 3 for the chest, abdomen and bilateral upper extremities with split-thickness skin grafting from his thighs, buttocks and leg. On Day 6, extensive debridement of his entire back and remaining upper arm tissue was performed, followed by covering drainage-slit Terudermis® with bFGF spraying over the slit on the surgical ward and daily dressing changes for 7 days and then good re-epithelialization was observed. At 10 weeks, the majority of the back was healed with spotted well-vascularized raw surfaces *(Figure 4)*.

Discussion

Artificial dermis is designed and planned for use as temporary wound coverage. In particular, healing of deeper less-vascularized tissues such as bones, cartilage and tendons is hard to achieve spontaneously. The bi-layer artificial dermis covered the defect by maintaining a moist wound environment and provide the optimal wound bed by inducing inflammatory cells, fibroblasts, endothelial cells and capillaries in the inner collagen sponges. The efficacy and benefits of the artificial dermis are demonstrated among patients suffering from severe affections such as necrotizing fasciitis and necrotic lesions immediately after extensive debridement[7] The artificial dermis also provides external stem cell differentiation of bone-derived epithelial regeneration in experimental models[8] Basic fibroblast growth factor is a clinically well-accepted potent angiogenic factor. This relatively inexpensive product for human recombinant growth factor, which costs 100 US dollars for 500 µg for 2 weeks use (7 US dollars daily), and is available for reimbursement in Japan; thus, each patient pays only 30 US dollars for a 2-week therapy. When bFGF is used for second-degree burns in adults, pediatrics and with split-thickness skin grafting in the early phase, wound healing is significantly accelerated compared with conventional treatment and the quality of the wound healing depicted by multiple clinical assessments and objective indicators such as scar hardness, stratum corneum function and mechanical properties such as elasticity and extensibility[9-11] In combined use of bFGF and artificial dermis provides esthetically better outcomes and better stratum corneum function.[12]

As demonstrated in the casesdescribed, the post-nevus removal use of bFGF in the artificial dermis, excluding the outer membrane, enhances vascularity and provides a secondary skin grafting wound bed. In extensive burns, with early administration after debridement, satisfactory healing is obtained.

As previously observed in the case of medical comorbidity in vascular and diabetic patients, surgically sufficient debridement with artificial dermis coverage would be a powerful wound healing procedure when bFGF is applied immediately after surgical debridement over the wound bed then daily at a concentration of 1 µg/ cm^2.[12]

The artificial dermis functions as a barrier against external microorganisms, maintains fluid levels and also provides the best available wound environment. Several modifications have been attempted in order to determine the most clinically appropriate use and this product is highly promising in surgical regenerative therapy when combined with topical negative pressure device[13] or purely synthetic materials avoiding use of animal-derived materials in complete clearance of contamination of animal origin.[14] ■

References

1. **Yurugi S, Hatoko M, Kuwahara M, Tanaka A, Iioka H, Niitsuma K.** Usefulness and limitations of artificial dremis implantation for posttraumatic deformity. *Aesthetic Plast Surg,* 2002; 26: 360-364.

2. **Suzuki S, Kawai K, Ashoori F, Morimoto N, Nishimura Y, Ikada Y.** Long-term follow-up study of artificial dermis composed of outer silicone layer and inner collagen sponge. *Br J Plast Surg,* 2000; 53: 659-666.

3. **Lee LF, Porch JV, Spenler W, Garner WL.** Integra in lower extremity reconstruction after burn injury. *Plast Reconstr Surg,* 2008; 121: 1256-1262.

4. **Yoshikawa T, Mitsuno H, Nonaka I, Sen Y, Kawanishi K, Inada Y, Takakkura Y, Okuchi K, Nonomura A.** Wound therapy by marrow mesenchymal cell transplantation. *Plast Reconstr Surg,* 2008; 121 : 860-877.

5. **Lee JW, Jang YC, Oh SJ.** Use of the artificial dermis for free radial forearm flap donor site. *Ann Plast Surg,* 2005; 55: 500-502.

6. **Pollard RL, Kennedy PJ, Maitz PK.** The use of artificial dermis (Integra) and topical negative pressure to achieve limb salvage following soft-tissue loss caused by meningococcus septicemia. *J Plast Reconstr Aesthet Surg,* 2008; 61: 319-322.

7. **Akita S, Tanaka K, Hirano A.** Lower extremity reconstruction after necrotizing fasciitis and necrotic skin lesions using a porcine-derived skin substitute. *J Plast Reconostr Aesthet Surg,* 2006; 59: 759-763.

8. **Nakagawa H, Akita S, Fukui M, Fujii T, Akino K.** Human mesenchymal stem cells successfully improve skin-substitute wound healing. *Br J Dermatol,* 2005; 153: 29-36.

9. **Akita S, Akino K, Imaizumi T, Hirano A.** A basic fibroblast growth factor improved the quality of skin grafting in burn patients. *Burns,* 2005; 31: 855-858.

10. **Akita S, Akino K, Imaizumi T, Tanaka K, Anraku K, Yano H, Hirano A.** The quality of pediatric burn scars is improved by early administration of basic fibroblast growth factor. *J Burn Care Res,* 2006; 27: 333-338.

11. **Akita S, Akino K, Imaizumi T, Hirano A.** Basic fibroblast growth factor accelerates and improves second-degree burn wound healing. *Wound Repair Regen,* 2008; 16: 635-641.

12. **Akita S, Akino K, Tanaka K, Anraku K, Hirano A.** A basic fibroblast growth factor improves lower extremity wound healing with a porcine-derived skin substitute. *J Trauma,* 2008; 64: 809-815.

13. **Helgeson MD, Potter BK, Evans KN, Shawen SB.** Bioartificial dermal substitute: a preliminary report on its use for the management of complex combat-related soft tissue wounds. *J Orthop Trauma,* 2007; 21: 394-399.

14. **Tanihara M, Kajiwara K, Ida K, Kamitakahara M, Ogata S.** The biodegradability of poly(Pro-Hyp-Gly) synthetic polypeptide and the promotion of a dermal wound epithelialization using a poly(Pro-Hyp-Gly) sponge. *J Biomed Mater Res,* 2008; 85: 133-139.

STEM CELLS AND GROWTH FACTORS

In this issue emerging "Stem cells" and "Growth factors" topics are highlighted.

Growth Factors or cytokines are extensively studied and now clinically widely practiced following technological innovation of protein or peptide gene recombination. Growth factors and cytokines in wound healing are ideal for beneficiaries of progress, thanks to their relatively small in size (dozens to hundreds of kilo-Daltons) with a simple glycoprotein structure. Recombinant human epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) have proven to be safe and effective in wounds in which it is otherwise difficult to obtain full healing In another approach, a "cocktail" application of the patients' own endogenous platelet-rich plasma (PRP), is used and comprises lots of good mediators that accelerate wound healing. The triune structure of cells, factors and scaffold are discussed together with factors (growth factors) and cells. Optimal and ideal combinations are is still being investigated but there are some great benefits associated with the selection of one type of "factor" with a specific "scaffold" and "cell" environment.

As you will readily recall, several experimental and theoretical "stem cells" are currently spot-lighted in medicine as well as by the mass media. However, "stem cells" such as Embryonic Stem (ES) cells involve ethical issues, there are fears of progressing into malignancy, or immune rejection may occur, even though these cells may have truly differentiated into multiple lineages. More recently, induced-Pluripotent Stem (iPS) cells are proving to be great candidates for the future with a variety of tissue and organ regeneration, but there is a risk of leading to tumor formation, because during the re-programming process, it is still necessary to use at least one oncogene.

Therefore, in current clinical settings, somatic stem cells from bone marrow, fat, or endothelial progenitor cells are used in direct application or after cell culture expansion. Finally, more intensively studied and clinically applied methods are used, and include expansion of autologous cultured epidermis, which is broadly accepted in many nations as one means of achieving powerful tissue regeneration, especially when the wound bed is sufficiently prepared using "scaffold" such as artificial dermis or traditionally with allo-grafting.

Readers of this issue, "Stem Cells and Growth Factors" will find out how new technology and innovation are being adopted in this field and how difficult it is to obtain such an optimal goal. The section editor will be very happy if this issue will be a milestone publication paving the way for future wound healing technology and practice.

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局所療法　外用剤と外用療法◎トピック

bFGF製剤を用いた局所療法

Topical treatment using bFGF

秋田　定伯*
Sadanori Akita

わが国において，2001年より世界で初めてヒト遺伝子組換え塩基性線維芽細胞増殖因子（basic fibroblast growth factor；bFGF，FGF-2）が発売され，外傷などにより傷ついた組織の再生，血管新生を目標として臨床使用されている。bFGFは，真皮線維芽細胞・炎症細胞を遊走化・活性化させ，局所に集中させるのみならず，表皮中のケラチノサイトを増殖・遊走させる。創傷治癒過程におけるサイトカインの役割は重要であり，受傷直後からの炎症細胞・線維芽細胞，血管内皮細胞などへの直接的，間接的作用により，創傷治癒を促進する[1]。皮膚創傷治癒過程において，局所のマクロファージを炎症・凝固期からリモデリング期まで数週間持続活性化させる。この際のFGF発現は時間・空間的にも制御されており[2]，Ⅱ度熱傷創および植皮創由来の浸出液中のbFGF濃度は，切開創と比較して低く，そのため血管内皮細胞の増殖性と走化性も低下している[3]。*in vitro*培養の成人および胎児線維芽細胞にbFGFをシリコンゲル中に含有させて投与すると，bFGF分泌は増加し，肥厚性瘢痕の予防になる。このことからbFGFは瘢痕形成に重要な因子と考えられている[4]。

臨床使用において，bFGF製剤は，難治性皮膚潰瘍，熱傷創，褥瘡に対する創傷治癒促進の作用に加え，瘢痕期における，創傷治癒の質（quality of wound healing）改善にも有用であることがわかってきた。このため，われわれは，臨床的に手術を含めた治療方針として流動的なⅡ度熱傷へのbFGF製剤の初期から治癒までの使用に対する効果を検討するために，臨床研究を行った。

bFGFの局所投与により，急性・慢性創傷治癒促進効果のみならず，瘢痕の質的改善がみられる。熱傷潰瘍治療におけるデブリードマン直後から創閉鎖までの植皮術後にbFGF製剤スプレーを用いると，創閉鎖後1年以上の経過観察で，臨床スコア評価およびデュロメータ（durometer）測定による瘢痕硬度の改善を認めている[5]。また小児Ⅱ度熱傷創における前向きランダム化臨床試験においても同様に，受傷後早期（受傷直後～96時間以内より開始）からのbFGF製剤スプレー使用により，創閉鎖後1年以上の経過で肥厚性瘢痕の軽減とバンクーバー瘢痕スケール（Vancouver Scar Scale）による臨床的評価改善，さらに経皮的水分損失量（trans-epidermal water loss；TEWL）など角質バリア機能の改善が得られている[6]。

さらに，153例中51例をbFGF治療，51例を対照とした成人Ⅱ度熱傷患者での前向きランダム化臨床検討でも，主に深達性Ⅱ度熱傷（deep dermal burn；DDB）創治療において，創閉鎖後1年でのバンクーバー瘢痕スケール評価，瘢痕範囲などの臨床評価，キュートメータ（cutometer）を用いた受動瘢痕硬度，瘢痕粘弾性，デュロメータによる直接瘢痕評価，角質水分測定によると，bFGF製剤スプレーの早期からの使用（受傷後～96時間以内で開始，平均48時間）では，平均2.2日早く創閉鎖し，瘢痕臨床評価でも有意にバンクーバー瘢痕スケールを改善し，TEWLの有意な改善とキュートメータ測定による最大伸展係数，最大収縮比率（粘弾性）はともに有意に正常化した[7]（図1）。

また，熱傷潰瘍などでしばしばみられる深部組織の腱・骨の露出した下肢創の再建例においても，bFGF製剤のデブリードマン直後からのスプレー使用と，二次植皮までの人工真皮周囲からのbFGF溶液注入により，bFGF製剤を用いなかった人工真皮単独の再建例と“歴史的対照”によりデュロメータ測定での硬度の改善と角質機能改善が得られた[8]。

bFGF製剤は皮膚軟部組織における創閉鎖促進のみならず，創・瘢痕再構築を含めた瘢痕軽減効

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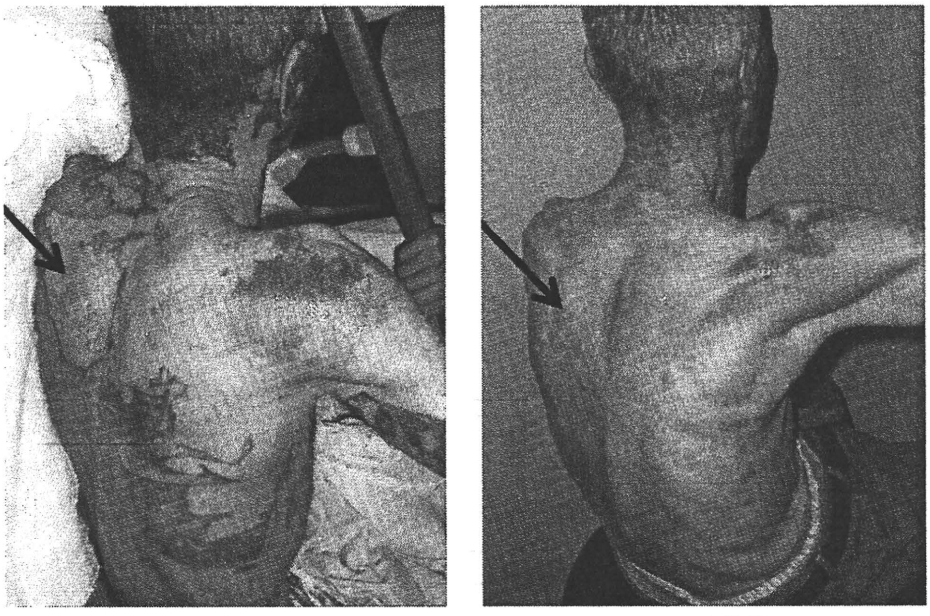

a　　b：6カ月後

図1　82歳，男性，背部・上肢に12%のⅡ度熱傷

果を認めており，手術直後のデブリードマン直後，保存的治療例における局所創管理で有効であり，長期結果（瘢痕程度）も臨床的に優れている。瘢痕硬度，角質機能の改善とともに手軽で安全かつ有効な創傷外用剤（スプレー）として外傷・熱傷創への使用が初期から可能であり，今後，ますます重要な治療薬剤の位置を占めると思われる。

【文　献】

1) Singer AJ, Clark RA：Cutaneous wound healing. N Engl J Med 341：738-746, 1999.
2) Kibe Y, Takenaka H, Kishimoto S：Spatial and temporal expression of basic fibroblast growth factor protein during wound healing of rat skin. Br J Dermatol 143：720-727, 2000.
3) Nissen NN, Gamelli RL, Polverini PJ, et al：Differential angiogenic and proliferative activity of surgical and burn wound fluids. J Trauma 54：1205-1210, 2003.
4) Hanasono MM, Lum J, Carroll LA, et al：The effect of silicone gel on basic fibroblast growth factor levels in fibroblast cell culture. Arch Facial Plast Surg 6：88-93, 2004.
5) Akita S, Akino K, Imaizumi T, et al：A basic fibroblast growth factor improved the quality of skin grafting in burn patients. Burns 31：855-858, 2005.
6) Akita S, Akino K, Imaizumi T, et al：The quality of pediatric burn scars is improved by early administration of basic fibroblast growth factor. J Burn Care Res 27：333-338, 2006.
7) Akita S, Akino K, Imaizumi T, et al：Basic fibroblast growth factor accelerates and improves second-degree burn wound healing. Wound Repair Regen 16：635-641, 2008.
8) Akita S, Akino K, Tanaka K, et al：A basic fibroblast growth factor improves lower extremity wound healing with a porcine-derived skin substitute. J Trauma 64：809-815, 2008.

＜総　説＞

創傷治癒・創傷治療における“幹細胞”の意義と役割

秋田定伯*

要旨

創傷治癒を促進し，理想的な瘢痕をもたらすために，さまざまな方法がとられるが，“幹細胞”生物学の発達とその臨床応用の拡大により，創傷への再生方法として注目をあびつつある。幹細胞のなかにはES細胞，iPS細胞など将来非常に有望な細胞源もあるが，現状では倫理的問題を含めた実務上の問題があるため，いわゆる“体性幹細胞”を応用する方法が開発されている。創傷への臨床応用は基礎的研究に基づき，臨床へ展開されている。創傷治癒・創傷治療における幹細胞の動態，発現状態，分化様式の基盤的知見が，理想的創傷治癒へどのように展開されているか，付属器を含めた皮膚再生に向けての研究の現状と問題点について述べる。自験例では幹細胞を用いた創傷治療のなかで自家脂肪組織由来幹細胞を用いての難治性創傷治癒への有効例も経験しており，ますますの発展が期待される。

Key Words：体性細胞，人工真皮，皮膚付属器

はじめに

骨髄由来幹細胞は組織修復，心筋[1]，血管[2]，受傷後の骨，腱，軟骨[3]，半月板，皮膚再生[4,5]に関与する。最近の研究では，骨髄由来細胞の多くは皮膚の細胞になると報告されている[6,7]。正常皮膚には骨髄由来細胞があり，宿主免疫と創傷治癒を含めた炎症課程にすると理解されている。一方では，骨髄由来細胞は皮膚を構成するケラチノサイトや線維芽細胞の生成に貢献するとの報告もある[6～8]。白血球の凝集と同様に，骨髄由来幹細胞は，骨髄前駆細胞を含めて皮膚などの障害を受けた部位に集合可能である。

また，脂肪由来幹細胞は骨髄由来幹細胞と非常に近い細胞特性を有し，多分化能をもつことと，優れた細胞増殖性のみならず，自家脂肪は培養過程を経ずに幹細胞分離直後に移植可能であり，臨床的にも効果的であると認められている[9,10]。本稿で体性幹細胞のなかでも骨髄由来幹細胞，脂肪由来幹細胞を用いた創傷治癒・創傷治療への意義を再生医療的視点から検討し，現状と将来への展望について解説する。

1．骨髄由来幹細胞による組織修復／再生

骨髄幹細胞には2系統あって，造血幹細胞（HSC）と間葉系幹細胞（MSC）がある。これまで成人骨髄由来HSCは造血系の全部に関与し，赤血球，血小板，白血球系列の幹細胞と考えられてきた。しかし，HSCが肝細胞[11]，血管内皮細胞，平滑筋細胞，心筋細胞[1]などの非造血系の細胞産生に関与するとの報告もある。骨髄由来MSCは自己再生し，非造血系組織の前駆細胞である。骨髄内の細胞比率においては，MSCは有核細胞の中に0.001～0.01％にすぎず，HSCと比較しても10％未満である。

2．骨髄由来幹細胞の損傷部位への定着

骨髄由来幹細胞が骨髄から出て行き，血流内を通って損傷部位に定着可能であることが示唆されている[2]。

骨髄の幹細胞の範疇と考えられている骨髄由来血管内皮前駆細胞（EPC）はHSC系列に加えられているものの，骨髄から末梢血に移動可能であり[12]，G-CSF（顆粒球コロニー刺激因子）などのサイトカインで動員可能である。循環血中のEPCは成人末梢血からCD34+細胞として分離，培養・増幅され，体外で血管内皮細胞に転換される。循環血中のEPCは正常成人血管内皮細胞を一定の割合で構成している[2]。

さらにEPCは，創傷治癒に関与すると考えられている。

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