Review Article

Novel Therapeutic Approach for Difficult Wound Healing

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Wound healing process is highly organized, regulated and orchestrated by cellular and molecular basis. As for biology, wound healing is very much attractive to multi-subspecialty fields of both basic and clinical medicine. Clinical problems of wound healing also extend a single subspecialty of medicine, not only surgeries but internal-, social-, psychological- medicine. Thorough comprehension of clinical and futuristic approaches for this complex entity is recommended. Based upon a number of clinical experiences of wound-related problems, starting with burns, trauma to chronic intractable wounds and subsequent unfavorable scar formation, the clinically relevant evaluations for wound healing is presented including life-saving modalities, minimizing the scar formation or scarless wound healing, prognosis predictors. Also, clinical related-basic research brought about regenerative medical solutions using artificial dermis, cytokine and adult stem cells. Acute and chronic difficult wound healing should be holistically approached and "emergency primary wound healing center" will be most appreciated for any form of biological, chemical, nuclear and radiation-related issues. In this context, Nagasaki University may be able to play a pivotal role in global view point.

Keywords: Wound; Scar; Clinical; Stem cell; Regenerative medicine; Emergency primary wound healing center

Introduction

No single person avoids a wound in his or her entire life. Usually wounds heal with no special attention on it, because it is a biologically highly organized and structured process. However, once devastating wounds which may be brought about to the body by significant impacts happened, it will result in lethal and severe consequences. As clinically often observed, extensive burns require intensive cares under stringent medical circumstances for life-saving, while minor wounds necessitate decent and refined medical and surgical management. In this context, wound healing deals with wide range of medical issues from emergency to aesthetical considerations. Also, there are increasing evidences of the difficult wound healing due to aging, change of life style and complicated medical conditions.

Surgery is one of medical category initially dealing with various kinds of trauma in many years in the history and developed in subspecialty. Plastic surgery is one of sub-specialties of surgeries and covers its clinical fields with wound healing-related problems. In plastic surgery, it is very important how to or where to incise the skin of the body to minimize the scar tissue as a result. Wound healing is in fact a transverse category extending solely from surgery to other medical sub-specialty with increasing phenomenal facts of chronic intractable wounds caused by pressure, diabetic, arterial ischemic and venous ulcers, affect one's quality of life as well as burden of medico-social issues. Therefore, understanding and interdisciplinary approaches from many sub-specialty of medicine is necessary for better treatment for wounds. When the quality of wound healing is concerned, there should be paid attention on scar formation as a consequence, which also interferes with one's quality of life.

In this review, the biological mechanisms of wound healing are presented and discussed, clinical approaches for better quality of wound healing are analyzed, future applications of regenerative modalities are reviewed and finally the concept of wound healing center is proposed.

Wound healing as biology

Wound healing process composes of an integral part of various molecules which are interacted and regulated both temporally and spatially. The soluble mediators, blood cells, extracellular matrices and parenchymal or mesenchymal cells are involved and there are

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three phases in normal wound healing process: inflammation and coagulation phase, tissue proliferation phase and tissue remodeling phase. The first phase of inflammation and coagulation starts within seconds and continue approximately weeks after initial injury. The reaction of the vessels begins within seconds, peaks at 3 to 7 days and returns to previous level within weeks. The blood coagulation and fibrin network formation are also initiated within seconds and complete in 24 hours. The inflammatory responses usually reach at peak within 3 to 5 days after injury and return to previous level by weeks. In this phase, most dynamic involvement of cytokine and growth factor interaction from local and systemic cell sources are presented.

Next, there are stromal and interstitial formations starting from approximately 10 to 12 hours after injury and continue up to 2 or 3 weeks. Scar formation is started in this phase and dramatic increase in tensile strength is observed mainly by collagen deposition and production, which reaches at peak around 2 weeks. This tissue proliferation phase continues to several months. Finally, with tissue remodeling phase, wound healing is complete. Most of the shallow and minor wounds will be re-epithelialized from 12 hours to 2 weeks. However, deeper and majority of the wounds will be resurfacing or re-epithelializing after completion of stromal (mesenchymal) tissue formation, which often diverses from wound to wound, because the provision of the stromal (mesenchymal) is a determinant factor. Tissue remodeling phase continues days to 2 years. More abnormal wound takes more time to finalize this phase. In other words, the accelerated wound healing may lead to more normal and conspicuous wound. Therefore, for holistic approach for wound management, it is stressed that primary medical intervention is most important as well as apprehensive overview of entire wound healing process.

Additionally, each phase is overlapped partly or sometimes intermingled; therefore, careful clinical relevance should be taken account in case of medical practice (Figure 1).

**Wound healing as a clinical fact**

Primarily the skin plays a major role in barrier against external environment. Loss of integrity of the skin may lead to severe topical tissue and systemic damage of the body. As seen in burn patients, there are profound and extensive burns which may lead to severe outcomes such as death by shock, multiple organ failures, or sepsis, even to lesser extent, may be prolonged wounds, namely burn ulcers. The long-term sequelae of the burn ulcer wounds tends to progress to the clinical fibroproliferative disorders (FPD) such as hypertrophic scars and keloids, which cause both esthetic problems as well as functional limitations of the articular range of motions (ROM). In acute wounds aside from the burns, there are several clinically concerned wounds such as friction injuries, biological, chemical, nuclear and radiation-induced (BCNR) injuries by traumatic or industrial accidents and even by fear from terrorist attacks.

On the other hands, there is a clinical entity, namely chronic wounds or chronic ulcers which increase by time correlated increase of life-style related diseases such as metabolisms disorders like diabetics, hyperlipidemia, atherosclerosis, obesity, blood vessel disorders and pressure ulcers. The vigorous and intensive wound cares such as normalizing the basic underlying diseases and relief of the interfering factors involved in each category may explain partly the revolving mechanisms of such disease but further integrated and beyond-the-boundary concept and clinical setting may be most useful for best wound healing care and management.

**Pediatric burns in Nagasaki University**

Pediatric burns are frequently observed and bring about significant consequences, because relative skin surface compared to adult counterpart is greater thus the results may be worsened. Also, pediatric burns associated with more long-term follow-ups are concerned.

Twenty-year burn analysis was performed in a single department, and 354 cases, aged 0 to 6 years old, were analyzed. The major cause of pediatric burns was scalding (68%) and hot water comprised over half of the scald burns. At 1 year old and younger, the total burn surface area (TBSA) was significantly smaller than from 1 to 6 years old (4.8±9.56% vs. 10.5±18.86%, respectively, \( p < 0.001 \)). TBSA of scald burns was significantly greater than contact burns (8.9±15.76% vs. 0.9±2.0%, respectively, \( p < 0.05 \)). Surgery was performed for 65 patients (18%) and 126 patients were hospitalized (34%). Compared to the first decade of analysis, the second decade had fewer patients (222 vs. 142 cases) and lower severity (7.4±14.6% vs. 6.6±13.26%, TBSA). In this way, pediatric burns in younger children should be studied closely as to their causes and with further follow-up.

**Pediatric burn wound management**

Pediatric burn wounds can be problematic since accurate evaluation is difficult due to anatomically immature vasculature or immobilization failure, especially in second-degree burns, and the burn
surface areas and the burn depth tend to worsen over time. Delayed wound healing results in unsightly scarring, such as hypertrophic scars, which are problematic both esthetically and functionally. Among cytokines and growth factors, basic fibroblast growth factor (bFGF) is clinically proven, having demonstrated accelerated acute and chronic wound healing.

Accelerated wound healing may lead to improved scarring. To elucidate the effects of bFGF on second-degree pediatric burn wounds, a comparative study was performed. A total of 20 pediatric patients, aged from 8 months to 3 years with the mean (=standard deviation) of 1 year and 3 months (=6 months) suffering from the burns by various causes, were divided into two groups of conventional (n=10) and bFGF treatment (n=10). A moisture meter for objective measurement of the stratum corneum and epithelial-mesenchymal functions was used to assess scars at least one year after wound healing. Conventional and bFGF-treated scars showed a significant difference (p<0.01) in the clinical evaluation of pigmentation (1.7±0.55 vs. 0.7±0.58, conventional vs. bFGF-treated), pliability (2.4±0.82 vs. 1.1±0.69), height (1.8±0.66 vs. 0.5±0.57) and vascularity (1.9±0.63 vs. 0.8±0.68), respectively. The effective contact coefficient was significantly greater (p<0.01) in conventional wounds (14.6±1.68%) than in bFGF-treated wounds (8.7±2.82%) and bFGF-treated wounds demonstrated significantly less (p<0.01) transdermal water loss (TEWL) values (5.7±1.85 g/m²/h) than conventional treatment (8.3±1.90 g/m²/h). BFGF-treated pediatric burns showed less damaging function of the stratum corneum after healing both in clinical assessment and moisture meter analysis.1

Effective wound healing in split-thickness skin grafting donor

Few comparative studies have been performed on the various wound-dressing materials or methods proposed for use. To clarify the efficacy of wound dressing, 35 patients (17 females aged 44.8±26.86 years and 18 males aged 35.4±29.70 years) were subjected to a prospective study comparing a polyurethane dressing and a hydrogel dressing for split-thickness skin donors from the lateral thighs. We examined their clinical usefulness such as accelerated healing time, frequency of changing the dressing, degree of pain, or amount of exudates, and performed moisture meter analysis at 1 month and 1 year after re-epithelialization, which reflects the quality of the stratum corneum and subsequent scarring. The polyurethane dressing was superior to hydrogel in the wound healing time, amount of exudates, and frequency of dressing changes; the hydrogel was better for regulating the degree of pain. There was a significantly positive correlation between transdermal water loss and the effective contact coefficient, which indicates skin barrier function and is affected by skin surface electrolytes and reflects water content, in moisture meter analysis (r=0.57, p<0.01). Transdermal water loss returned to the control level at 1 year after healing with both dressings. The effective contact coefficient of the polyurethane wound was significantly lower than that of hydrogel at 1 month (p<0.01), while both dressing wounds demonstrated significantly higher values at both 1 month and 1 year compared to the control (p<0.01).

The polyurethane dressing is therefore superior both clinically and in moisture meter analysis.1

Cytokine involvement and predictor for fatality of extensive burns

In order to investigate circulating cytokine responsiveness in major burns in association with the systemic stress response system, hypothalamic-pituitary-adrenal (HPA) axis markers were tested in extensive burn cases treated in the department of Plastic and Reconstructive Surgery, Nagasaki University.

The HPA axis is a major stress response system and leukemia inhibitory factor (LIF) may be a potent mediator of the HPA axis; circulating LIF levels in burn patients were therefore studied. Twenty patients (10 females and 10 males) with extensive burn (Burn Surface Area > 20%) aged 37 to 77 years with the mean (=standard deviation) of 59.1±12.10 years were assessed. Circulating LIF, adrenocorticotropic hormone (ACTH), other inflammatory markers and 24-hour urinary free-cortisol excretion levels were investigated. LIF levels were significantly higher (p<0.001) in patients who died (186.1±80.41 pg/mL) than in those who survived (83.5±64.49 pg/mL) at 36-hour after injury. ACTH levels were more significantly elevated (p<0.0001) in fatal cases (41.3±8.28 pg/mL) than in those who survived (25.2±7.84 pg/mL). Twenty-four-hour (24 to 48 hours after injury) pooled urinary free-cortisol excretion levels were also significantly higher (p<0.0001) in fatal cases (235.0±36.94 µg/day) than in the surviving patient group (69.0±18.04 µg/day). The correlation between serum LIF and urine free cortisol was significant (r=0.55, p<0.01) as was the correlation of serum LIF with plasma ACTH (r=0.49, p<0.01).

Serum LIF as well as HPA axis activity markers is a good marker of disease severity and prognosis in patients with extensive burns.1

Skin grafting with bFGF application

In accelerated and efficient treatment of burn wounds, skin grafting is often used and in order to avoid hypertrophic scars in burn wounds, the simultaneous application of basic fibroblast growth factor (bFGF) with regular surgical debriement and skin grafting was investigated for skin hardness by clinical examination and instrumental measurement. As little is known about the role of bFGF in wounds, burn wound scars were tested for hardness. Burn scars in various anatomical locations at least one year after final wound healing clinically demonstrated a significantly lower (p<0.01) hard score in bFGF-treated wounds (0.95±0.51) than in non-bFGF wounds (2.3±0.66). In addition, a durometer, which is widely used in industry to measure materials similar to skin such as rubber and thread-balls, demonstrated a significantly lower reading (p<0.01) in bFGF-treated wounds (7.9±3.64) than in non-bFGF wounds (15.5±4.39). The results demonstrated that burn wounds treated with clinically approved bFGF may contribute to a better cutaneous wound quality, at least in terms of hardness.1
Lower extremity reconstruction

There are significantly important surgical and medical management especially for lower limb wounds. Seven consecutive clinical cases of necrotizing fasciitis or necrotic skin lesions (42-78 years old; average 65.0 years of age, three females and four males) due to group A Streptococcus, group B Streptococcus, methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa in the lower extremities, which demonstrated rapid systemic symptoms, were investigated for the usefulness of a bilayer artificial dermis, derived from porcine tendons, over 4±2.4 years (1-7 years).

All surgical debridement was confirmed as sufficiently deep and wide for clinically infected lesions and artificial dermis was applied to all wounds. The average interval to secondary split-thickness skin grafting was about 17.5 days (17.5±2.44 days, 21 days max., 14 days min.) and the average thickness was 0.009±0.0022 in. (range: 0.006-0.012 in.). No clinical problems were noted during the application of the artificial dermis and the subsequent skin grafting. Since the donated skin graft was thin, the morbidity was minimized.

The use of artificial dermis after extensive surgical debridement in necrotizing fasciitis was easy and effective. Local or systemic infection was negligible.1

Sclerotherapy for vascular malformation

The venous malformation is the therapeutic target, even though it often demonstrates the difficult wound healing and required multiple surgical interventions, algorithmic approaches would turn out the better quality of life for the patients. Even though the precise mechanisms related to venous malformation are still unclear, the clinical manifestations sometimes threaten vital signs such as mastication, airway and phonics. Our therapeutic modalities were reviewed, and their effectiveness and related complications were analyzed.

Between March 1998 and February 2006, 11 patients (15 years to 59 years old; average 32.4±13.60 years, 4 females and 7 males) with craniofacial venous malformation were included in this investigation. All cases experienced some kind of surgery at least once during clinical follow-up. Direct puncture scintigraphy with technetium-99m Sn colloid-labeled demonstrated low-flow malformations in all cases. Two cases underwent bone surgery and another two cases had static suspensions for facial nerve paralysis. Blood loss was 1352±1115.0 mL from surgery alone, 400±244.9 mL from simultaneous procedures and 187±284.8 mL from sclerotherapy alone; blood loss was significantly larger from surgery alone than from sclerotherapy alone (p<0.01). Excellent sclerotherapy cases were when the malformation was localized, and the number of sclerotherapies in them (1.3±0.58) was significantly fewer (p<0.05) than in good cases (3.6±1.15).

Although there are difficulties in understanding the mechanisms and multiple therapeutic interventions are required, there have been satisfactory outcomes so far and the development of better sclerosants or a real-time navigation system may benefit more precise therapeutic effects and lower morbidity.2

Wound healing as an implication of regenerative medicine

Embryonic fibroblast with artificial dermal grafting

Embryonic wound healing demonstrates the "scarless" wound healing. Current clinical usage of an artificial dermis in less vascularized tissues on tendon, bare bone, ligament and articular joint would be a candidate for a wound healing promoter with assistance of embryonic fibroblast population. The combined application of cytokines on embryonic fibroblasts and dermal substitute were studied for optimal skin defect coverage. The mechanism of combined treatment of leukemia inhibitory factor (LIF)-transfected embryonic fibroblasts and vascular endothelial factor (VEGF) was elucidated and subsequently the in vivo applications of both were tested in an artificial dermal substitute.

Mouse embryonic fibroblast cells, BALB-3T3, were stably transfected with mouse full length LIF cDNA and added to various doses of VEGF for detection of signaling interaction. LIF-transfected cells and VEGF treatment were tested with pig-tendon derived collagen dermal substitute in the backs of BALB/c male mice up to for 14 days.

As a result, LIF-transfected cells as well as vector-transfected fibroblasts significantly proliferated by 1, 10, or 100 ng VEGF on days 3 and 5. Erk mitogen-activated protein (MAP) kinase phosphorylation was observed from 1 to 30 minutes in LIF-transfected and 10 ng of VEGF and 1 to 60 minutes in LIF-transfected and 100 ng VEGF treatments. The cellular fibronectin levels also increased in LIF-transfected cells with 10 and 100 ng VEGF additions. In vivo analyses, LIF-transfected embryonic fibroblasts with 50 μg of VEGF markedly enhanced collagen I expression and CD 34 angiogenic marker on days 7 and 14.

LIF transfection and VEGF treatment enhanced phosphorylated-Erk MAP kinase in vitro. In vivo study revealed that the combined application of LIF-transfection of embryonic fibroblasts with an angiogenic factor such as VEGF in the template of a dermal substitute induced greater skin collagen production and angiogenesis in the dermal substitute.20

Mesenchymal stem cell proliferation and differentiation by cytokine in vitro

After experimental facts that combination of cytokine, artificial dermis and cells are capable of inducing better skin defect healing were observed, further efficient cell type such as mesenchymal stem cells are tested for cell property and induction by cytokine in vitro. Human mesenchymal stem cells (hMSCs), obtained from a single donor from the iliac crest, were further investigated for cell proliferation, cell cycle profiles, gene expressions and ultrastructure using electron microscopy. The hMSCs significantly increased their cell number by day 2 after treatment with bone morphogenetic protein
(BMP)-2 alone, or basic fibroblast growth factor (bFGF) alone or combinations of both in the serum-free condition \( (p<0.01) \). The hMSCs demonstrated marked proliferation of cell nuclear antigen notably at day 1 and pituitary tumor transforming gene throughout the experiment, suggesting cell cycle progression by BMP-2 treatment in addition to the strong cellular nuclear BrdU expression by immunocytochemistry. The fluorescence-activated cell sorter also demonstrated a similar pattern of cell cycle progression between BMP-2 treatment in the serum-free and 10% fetal bovine serum treatment. The BMP-2 treated hMSCs demonstrated heterochromatin in the nucleus, suggesting cell differentiation and well-developed granular endoplasmic reticulum, indicative of protein production. The hMSCs successfully proliferated, the cell cycle progressed, and the cell ultrastructure morphology suggested marked nuclear and granular endoplasmic reticulum induction by BMP-2 treatment in the serum-free condition.\(^{11}\)

*Early cellular changes of mesenchymal stem cell and interaction with other cells*

As demonstrated, human mesenchymal stem cells characterization of proliferation, differentiation and electron microstructures in vitro, cellular interaction is investigated with other cells. To further analyze human mesenchymal stem cells, their cell-to-cell interaction with possible adjacent cells such as endothelial cells, dermal fibroblasts and epidermal keratinocytes was investigated. The modified Boyden dual chamber assay revealed significantly \( (p<0.01) \) more powerful cell migration of the human mesenchymal stem cells by human epidermal keratinocytes \( (345.0\pm61.60 \text{ cells/field}) \) than other cells, such as aortic endothelial cells \( (36.2\pm14.45 \text{ cells/field}) \) and dermal fibroblasts \( (63.8\pm24.81 \text{ cells/field}) \), in 8mm pores during 16-hour incubation, respectively. The ultrastructures under transmission electron microscopy demonstrated larger euchromatin nuclei in 10% fetal bovine serum, while basic fibroblast growth factor maintained immature cell morphology for 4 days. Scanning electron microscopy demonstrated hMSC migration through the pores with endothelial cells, fibroblasts or keratinocytes in the lower chambers. Monolayer co-culture demonstrated human mesenchymal cell changes in ultrastructural morphology in the vicinity of the epidermal keratinocytes. Human mesenchymal stem cells possibly interact with human epidermal keratinocytes to accelerate wound healing and coverage.\(^{13}\)

*Mesenchymal stem cell and artificial dermis improves wound healing*

In order to resolve clinical problems, human mesenchymal stem cells, cytokine and artificial dermis were attempted to test the possibility of skin wound healing. Large or deteriorated skin and cutaneous defects are sometimes life threatening. There is increasing evidence that adult stem cells are useful for tissue regeneration. Human mesenchymal stem cells (hMSCs) are self-renewing and potent in differentiating into multiple cells and tissues.

The effects of the hMSCs for cutaneous wound healing are unknown. The hMSC-populated porcine skin substitute has been investigated for wound healing, using a nude rat model to minimize immune reactions. 1.5 \( \pm \) 1.5 cm\(^2\) full-thickness skin and soft tissue defects, including the panniculus carnosus, were excised and covered with hMSCs and bFGF-soaked skin substitutes and the wound sizes, histology and protein expressions were evaluated 3, 7 and 42 days after injury. The wound size was significantly smaller in the hMSC-treated groups \( (p<0.01) \) and any dose of bFGF \((1, 10, 100 \mu g)\) significantly enhanced the healing \( (p<0.01) \). The re-epithelialization markers such as integrin a3 and skin-derived anti-leukoprotease (SKALP) were remarkably increased with the presence of bFGF in a dose-dependent manner, while mesenchymal cell surface markers such as CD29 and CD44 were reversely regulated in a time-dependent manner. The human pan-cytokeratin, which does not cross-react with the rat antigen, was observed by Western blotting at 38 KDa and 42 KDa from the hMSC-treated tissues on day 7. The expression levels were significantly elevated by 10 \( \mu g \) bFGF \( (p<0.01) \). The immunohistochemical expression of human pan-cytokeratin was only observed in the hMSC-treated groups. These data suggest that hMSC together with bFGF in a skin defect model accelerates cutaneous wound healing as the hMSC trans-differentiated into the epithelium.\(^{13}\)

**Wound healing center**

Although there is a wound healing center concept proposed, the main target is toward chronic wounds.\(^{13}\) In this chronic wound healing center concept, multidisciplinary approaches from various departments of sub-specialties are proposed. In Nagasaki, which holds a sad history of the unique atomic-bombed medical school, a concept of an emergency primary wound healing center is proposed. Rapid growing fears of unseen objects such as nuclear radiation, which is also an entire medical research concerns for Nagasaki University Medical School, as well as other factors such as biological, chemical and other wound causatives should be handled in this center. The content of this center is mainly focused on as follows;

3. Promotion of reconstruction and regeneration to biological and chemical injuries.
4. Assessment and regeneration of quality of wound healing with skin adnexa.

Energetically engaged new-comers involving in this field are expected to join for a holistic management and research for wound healing (Figure 2).
Conclusion

Wound healing is comprehensively reviewed in the context of biological, clinical cases and future regenerative medicine. As wound healing extends each sub-specialty boundary, finesse approaches which are supported by practical as well as basic knowledge-based expertise should be necessary. Also, a concept of an emergency primary wound healing center in Nagasaki University Hospital is proposed.

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Figure 2. The concept of Emergency Primary Wound Healing Center in Nagasaki University Hospital. The center should be available for any kind of wound healing problems from acute to scar problems.

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