The Burn Wound Microenvironment

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Significance: While the survival rate of the severely burned patient has improved significantly, relatively little progress has been made in treatment or prevention of burn-induced long-term sequelae, such as contraction and fibrosis.

Recent Advances: Our knowledge of the molecular pathways involved in burn wounds has increased dramatically, and technological advances now allow large-scale genomic studies, providing a global view of wound healing processes.

Critical Issues: Translating findings from a large number of *in vitro* and pre-clinical animal studies into clinical practice represents a gap in our understanding, and the failures of a number of clinical trials suggest that targeting single pathways or cytokines may not be the best approach. Significant opportunities for improvement exist.

Future Directions: Study of the underlying molecular influences of burn wound healing progression will undoubtedly continue as an active research focus. Increasing our knowledge of these processes will identify additional therapeutic targets, supporting informed clinical studies that translate into clinical relevance and practice.

INTRODUCTION

BURN INJURIES are a major public health problem in the United States.¹ Despite significant improvements in critical care and surgical management, there is little consensus regarding the underlying molecular basis for the temporally progressive late sequelae of burn wound healing, such as contraction and scarring. New and improved methods for assessing molecular responses to wound healing offer hope for genome-wide studies^{2,3}; however, the goal of finding straightforward molecular mechanisms that can be targeted in clinical practice has been elusive. The shift to study human burn patients reflects a growing understanding that the molecular mechanisms of healing in humans are not necessarily mirrored in animal and

in vitro models. Recent meta-analyses suggest that even when similarities exist at the macroscopic level, the underlying genomic responses are divergent.⁴ An overview of the mechanisms of burn wound healing and scarring, and the current state of clinical research highlighting the difficulties in translating basic research to clinical care of burn wounds, is herein provided.

TRANSLATIONAL RELEVANCE

A number of studies have looked at the link between inflammation and fibrosis; nevertheless, the often conflicting results pose a challenge for researchers attempting to show direct cause-and-effect relationships between specific molecular pathways

and clinical outcome. Numerous studies suggest that the expression of cytokines, chemokines, and growth factors in the wound environment determines the eventual outcome of the wound healing process. Translating this knowledge into effective treatments remains a challenge.

CLINICAL RELEVANCE

Although mortality rates from burns have improved, injuries often result in long-term consequences related to poor healing that affect appearance and function of the injured area.⁵ While we have greatly increased our knowledge of the processes and underlying pathways involved in wound healing and scar formation, (Fig. 1) potential advances observed in preclinical and *in vitro* studies have yielded very few effective therapeutic approaches.^{6–8}

Burn wound healing

Compared to nonburn trauma wound healing, burns are characterized by a fundamental damage to tissues that complicates the normal wound healing response. While the tissues damaged from

nonburn trauma are largely vital and fed by underlying blood supplies, in severely burned tissues, the cells and vasculature are often destroyed. Accordingly, burn wounds are characterized by a region of coagulative necrosis, referred to as the zone of coagulation, in which tissues are not sufficiently oxygenated to support survival or quick healing responses.⁹ This deficiency underlies many of the observed differences between healing of burn wounds and nonburn trauma.

Phases of wound healing

Surrounding the zone of coagulation, characterized by dead tissue incapable of restoration, is a region of less severely burned tissue referred to as the zone of stasis, characterized by decreased tissue perfusion (Fig. 2). The ultimate fate of tissues within the stasis zone is dependent upon the wound environment, leading to either survival or necrosis. Because the tissue is damaged but still perfused, the zone of stasis is associated with vessel leakage and vascular damage.¹⁰ This increased and generalized capillary permeability serves to distinguish burn wounds from other traumatic injuries, as the local

Figure 1. Multiple factors can impair wound healing, some of which are well-documented while others have only recently come to light. Inadequately debrided tissue can act as a nidus of inflammation as well as prevent proper vascularization of skin grafts placed over the necrotic tissue. Poor-quality skin replacement resulting from a less-than-ideal donor site or from repeated harvests is a well-characterized negative factor. The presence of burn eschar or infection can enhance local or systemic inflammation, leading to poor incorporation of skin grafts or enhanced contraction and fibrosis. Other factors may still be waiting discovery. The relative contribution to wound healing of any given factor can be difficult to ascertain, and the final outcome likely involves complex interactions among these factors.

Figure 2. The stages of wound healing coincide with the presence of specific cohorts of immune cells and stromal cells, each contributing an array of growth factors and cytokines to the wound microenvironment. In nonburn wounds, the cell populations and cytokine/growth factor milieu are fairly well-characterized. While it is generally accepted that burn wounds induce more inflammation and have a more complex microenvironment, the relative proportions of cells and cytokines throughout the healing process have not been well-studied. Although the numbers of specific cells may change over time, the growth factor environment may not directly coincide with the presence of any given cell. For example, platelets arrive on the scene first and secrete vasoactive substances as well as cytokines like transforming growth factor (TGF)- β 1. However, hemostasis and dissolution of the clot and loss of platelets does not coincide with a reduction of TGF- β 1 because subsequent cellular cohorts, such as macrophages, fibroblasts, and keratinocytes, all secrete TGF- β 1. Most inflammatory cytokines peak during the first few days following injury and are secreted by neutrophils and inflammatory macrophages. The resolution of inflammation coincides with apoptosis of neutrophils and transdifferentiation of inflammatory macrophages into tissue remodeling macrophages expressing TGF- β 1, fibroblast growth factor (FGF), and epidermal growth factor (EGF).

Figure 3. Jackson's burn wound model. The different zones of damage from a partial-thickness burn wound are shown in profile through skin layers. The determination of healing is primarily determined by outcomes in the zone of stasis. Insufficient perfusion can result in extension of necrosis within this zone.

inflammatory reaction can lead to persistent, progressive vasodilation and edema.¹¹

The outermost zone of burn injuries is the zone of hyperemia, characterized by vasodilation and inflammation in otherwise uninjured tissues. This tissue, clearly viable and at low risk of necrosis, forms the nucleus from which wound healing begins.

These zones of injury serve to highlight two major difficulties with healing burn wounds. First, in the center of the burn, the tissue is necrotic and must be sloughed or excised. Second, the initial injury may continue to progress if the zone of stasis trends toward necrosis. Successful wound healing proceeds through an orderly progression of phases typically classified as hemostasis, inflammation, proliferation, and maturation/remodeling. The end result of this process ideally leads to an imperceptible scar with little fibrosis, minimal contraction, and preservation of tissue architecture and function. However, this orderly process relies upon a vasculature by which cells and soluble factors can reach the wounded tissues.

In a traumatic, nonburn wound, healing begins with the arrival of platelets, which mediate hemostasis as well as releasing numerous vasoactive substances and mediators that stimulate the healing process, such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF)- β , fibroblast growth factor (FGF), epidermal growth factor (EGF), β -thromboglobulin, serotonin, bradykinin, prostaglandins, thromboxane, and histamine. Cytokines and growth factors follow typical patterns of expression throughout the time course of healing and remodeling (Fig. 3); however, these patterns may vary substantially, both temporally and in amplitude, for fetal healing or burns in ways that have not been well-characterized.

Having achieved hemostasis, vascular permeability increases, allowing an influx of leukocytes responding to various chemotactic signals. These early arrivals consist primarily of neutrophils that phagocytose any bacteria and secrete degradative enzymes to break down necrotic tissue.¹² Tissue oxygenation is important for neutrophil function in wounds because neutrophils generate antimicrobial oxygen radicals. The early activity of neutrophils may thus be critical for inhibiting bacterial colonization and wound infection.¹³ Burn wounds pose a distinct problem because much of the damaged tissue is nonviable and lacking significant blood flow. Not only does this inhibit neutrophil access, which requires blood vessels to reach proximal tissues, but it also results in low oxygen

Figure 4. Paradigm of underlying responses to different insults. The Venn diagram indicates that multiple causes can lead to systemic inflammatory response syndrome (SIRS). Burns over 30% of total body surface area are three times more likely to result in SIRS, which may in turn lead to multi-organ failure. Similar patterns of gene expression are observed from sepsis-induced SIRS, suggesting that underlying molecular pathways respond similarly to quite different stimuli.

tension in necrotic tissues should neutrophils successfully navigate to sites of bacterial wound contamination.

Typically, the neutrophil population is replaced within a few days by macrophages, which are key facilitators of normal wound healing, regulating fibroblast activity and deposition and remodeling of extracellular matrix (ECM) and granulation tissue.¹⁴ This activity involves secretion of numerous cytokines and growth factors, including PDGF, TGF- β , TNF- α , and various interleukins. As with neutrophils, the inability of monocytes to get to necrotic tissues may negatively impact the healing process.

The proliferative phase follows the inflammatory phase, with fibroblasts migrating into the wound and laying down additional ECM. Collagen levels rise steadily for several weeks before slowing to reach homeostasis. Initially type I collagen predominates but it is in due course replaced with type III collagen. The eventual tensile strength of the wound correlates with the amount of collagen deposition during this period. Angiogenesis also occurs at this point and is essential to wound closure, an area of particular importance during healing of burns. In most nonburn open wounds, the remaining tissue is itself vital and can supply nutrients to incoming fibroblasts and epithelial cells. Burned tissue is necrotic and surrounded by damaged tissue that may not be capable or efficient at responding to cues for angiogenesis, thus delaying normal wound healing processes. The cues for angiogenesis appear to originate from platelets and macrophages early during wound healing.

The maturation phase of wound healing takes place over months or years as collagen is reorganized into lattice structures that are determined by the molecular characteristics and mechanical properties of the particular wound. In addition to alterations of the ratio of type I-to-type III collagen, the new collagen forms additional cross-links, increasing the tensile strength of the healing wound. The maximum strength of the new tissue plateaus at about 70%–80% of undamaged tissue.¹⁵

Systemic response

A localized inflammatory response is critical for both healing and prevention of infection. However, if the trauma is sufficiently severe, then a systemic inflammatory response may ensue. Systemic inflammatory response syndrome (SIRS) describes an inflammatory response that shares common features with various types of injuries, including infection, trauma, burns, and pancreatitis (Fig. 4).

The causes of SIRS are not necessarily overlapping. For example, not all infections cause sepsis, and even if an infection does lead to sepsis, it does not follow that SIRS will result. For patients with burns covering more than 30% of the total body surface area (TBSA), even in the absence of infection, the risk of SIRS increases threefold and is characterized by elevated levels of IL-6, IL-2, and IL-8 and reduced levels of IL-10.¹⁶ The recognition that diverse causes underlie a single syndrome suggests that similar treatments may provide relief for such diverse injuries and also provides targets for anti-inflammatory molecules.

During a systemic response to burns, proinflammatory cytokines, such as IL-1 β and TNF- α , are produced by numerous cells in the wound vicinity. In addition to generalized induction of fever and acute-phase proteins, these cytokines also induce production of prostaglandin E₂ (PGE₂), IL-6, and platelet-activating factor by endothelial cells and macrophages.¹⁷ IL-6 contributes to activation of T cells,^{18,19} although it is not clear whether high levels of IL-6 drive a systemic response or are simply a reflection of burn severity. Activated T cells polarized toward a T_H1 response also produce the proinflammatory cytokine interferon gamma (IFN- γ), which is important for activation of macrophages.²⁰

In opposition to the inflammatory response in burn injuries is an anti-inflammatory and subsequent immunosuppressive response. Whereas the inflammatory response in a nonburn traumatic wound gradually diminishes to baseline levels, following the initial inflammatory response, immunosuppression is a characteristic feature of major burn injuries, predisposing burn patients to infections. This immunosuppression is evidenced by anergy of the lymphocyte population and consequent prolonged survival of allografts.^{21–23} Neutrophil dysfunction has also been reported during major burns,^{24,25} including reduction of both chemotaxis and degradation of phagocytosed pathogens.²⁶ Following burn injury, macrophages upregulate expression of PGE₂ and downregulate expression of the proinflammatory cytokine IL-12.²⁷ This shift away from a proinflammatory response results in reduced functionality and decreased MHC class II expression on antigen-presenting cells with consequent disruption of coordination of the adaptive immune response.²⁸ Suppression of lymphocyte reactivity resulting from increased expression of PGE₂ by macrophages²⁹ leads to altered numbers of CD4⁺ T helper cells relative to CD8⁺ suppressor T cells,^{30,31} and, after the initial proinflammatory phase, the T-cell response becomes polarized to an

anti-inflammatory T_H2 phenotype²⁰ with consequent production of anti-inflammatory cytokines, such as IL-4 and IL-10, and decreased production of IL-2 and IL-1 β . This in turn leads to decreased lymphocyte proliferation^{32,33} with decrease in T-cell-dependent immune functions.^{21–23}

The tilt toward an immunosuppressive response may involve a host of systemic hormone responses from the endocrine system as well as alterations of various signaling cascades, including increases in growth hormone, catecholamines, and cortisol.¹⁷ Increases in glucocorticoids also inhibit proinflammatory cytokine production but not anti-inflammatory cytokines.³⁴

CLINICAL PROBLEMS

According to the National Center for Injury Prevention and Control, ~1.2 million people in the United States report burn injuries.^{1,35–37} Of the 40,000 who require hospitalization, about 5,000 die each year from complications.^{35–39} In patients with more than 40% TBSA burns, most deaths are the result of sepsis from infection of the burn wound, other infection complications, or inhalation injury.^{40–44} Multiple-organ dysfunction syndrome (MODS) may also develop as a result of severe trauma and may be the major cause of death in cases of severe burns.⁴⁵ In one study, over 28% of severe-burn patients suffered from MODS with a mortality range up to 98%.⁴⁶

By comparison with other forms of open wounds, thermal injuries present particular challenges to the burn care specialist. Most nonburn open wounds present as trauma to the skin and associated proximal tissues. However, while most burn wounds typically involve only a single organ (skin), the systemic response engendered by the burn injury can result in a generalized disorder affecting multiple organs distant from the burned area. As a result, burn wounds are typically managed by a team consisting of multiple medical specialties. Although mortality rates from burns have improved, injuries often result in long-term consequences related to poor healing that can affect appearance and function.⁵ In the days and weeks following lifesaving medical procedures, the primary modality for treatment involves split-thickness skin grafting. Because of primary burn wound contraction, secondary graft contraction, or hypertrophic scarring, late deformities are common, with negative functional and aesthetic consequences delaying and/or preventing the survivor's return to normal function.⁴⁷ Late correction of these deformities can be difficult and

time intensive and even in best cases require repeated releases, grafts, and tissue transfers.

The standard of care for burn wounds begins with excision of necrotic tissue as early as possible following hemodynamic stability.^{48–50} Early debridement is primarily aimed at removing stimulators of the inflammatory response, which is correlated with increased hypertrophic scarring and reduced function and appearance of the final scar.⁵¹ Early wound closure by autograft, allograft, or skin substitute at the earliest opportunity also improves final outcome.⁵² Initially, grafts survive by diffusion of plasma fluids via imbibition. In addition to the nutrients provided to the graft tissue, growth factors to induce revascularization are also present. Vascular inosculation may begin within 24 h and a new blood supply is usually established within 3 to 5 days.⁵³ While our tools for administering these surgical procedures have increased in efficacy, the basic paradigm of wound treatment has remained relatively unchanged for decades.

Because humans lack a panniculus carnosus, the skin is unable to move freely over underlying tissues,⁵⁴ leading to distortion of surrounding tissues when the wound edges contract. The absence of the panniculus carnosus also represents a critical divergence between humans and other animals when establishing animals as wound healing models. Contraction of the skin graft is more significant in split-thickness grafts than in full-thickness⁵⁵ and the amount of contraction is correlated with the thickness of the graft. The condition of the wound bed also plays a role in the amount of contraction. Grafting onto more mobile tissues, such as hypodermis, results in reduced contraction compared with grafts placed directly onto fascia.⁵⁵ The extent of granulation tissue also seems to relate to graft contraction.⁵⁶

THERAPEUTIC APPROACHES

While numerous studies have greatly increased our knowledge of the processes and underlying pathways involved in wound healing and scar formation, very few have yielded effective therapeutic approaches.^{6–8} An overview of the status of current clinical and preclinical trials is shown in Table 1. The ability of fetuses to heal without scarring has been noted for decades, suggesting that modification of the wound environment in adults could recapitulate the phenotype. In fact, the types of genes expressed and the distribution of growth factors in scar-free healing compared with scar-forming healing are quite different.^{57–60}

Table 1. Overview of the status of preclinical and clinical trials

Class	Trial Type	Author	Year	Remarks
Cytokines/growth factors				
TGF- β 3	Phase I-III	Renovo ⁹⁰	2011	Phase III trial stopped
	Phase I-III	Occleston <i>et al.</i> ¹¹¹	2008	Phase III trial stopped
IL-10	Pilot study	Renovo ⁹²	2012	Clinical trials stopped
PDGF	Phase II	Mansour ¹¹²	2008	Results unpublished
EGF	Phase II-III	Aramwit <i>et al.</i> ¹¹³	2013	
Interferon alpha 2b	Phase III	Wang <i>et al.</i> ¹¹⁴	2008	
Platelet rich plasma	Animal	Henderson <i>et al.</i> ¹¹⁵	2003	
	Phase I	Patel ¹¹⁶	2013	Recruitment phase
	Phase III	Guerid <i>et al.</i> ¹¹⁷	2013	
	Phase II-III	Prochazka <i>et al.</i> ¹¹⁸	2013	
EPO	Phase IIa	Gunter <i>et al.</i> ¹¹⁹	2013	
Cytokine neutralization				
TGF- β	Animal	Huang <i>et al.</i> ⁸⁵	2006	
	Phase I-II	Mead <i>et al.</i> ⁸⁷	2003	Ineffective
	Phase III	Denton <i>et al.</i> ⁸⁶	2007	Ineffective
TNF- α /IL-6	Animal	Sun <i>et al.</i> ⁶⁸		
Anti-inflammatory				
NSAIDs	Animal	Wilgus <i>et al.</i> ¹²⁰	2003	
	Animal	Chong <i>et al.</i> ¹²¹	2014	
	Animal	Sio <i>et al.</i> ⁷¹	2010	
	Phase I-III	Pavliv <i>et al.</i> ⁷²	2011	Phase III results unpublished
Corticosteroids	Phase II-III	Porro <i>et al.</i> ¹²²	2012	
Cell-based				
hUCMSCs	Phase I-II	Shenzhen Beike Biotechnology ¹⁰²	2011	Recruitment phase
hMSCs	Phase I	Schulman	2014	Prerecruitment
Fibroblasts	Phase II	Smith <i>et al.</i> ⁹⁶	2013	Recruitment phase
	Phase I-II	Rubin ⁹⁷	2011	Recruitment phase
ADSCs	Animal	Altman <i>et al.</i> ¹⁰⁴	2009	
	Animal	Nambu <i>et al.</i> ¹⁰⁵	2009	
Tissue engineering/scaffolds				
		Scuderi <i>et al.</i> ¹⁰⁹	2009	
	Animal	Gholipour-Kanani <i>et al.</i> ¹⁰⁸	2012	
	Phase II-III	Herndon ¹²³	2007	Trials stopped
	Phase I	Stratatech ¹²⁴	2011	Ongoing
	Phase I-II	Centanni <i>et al.</i> ¹²⁵	2011	
	Case study	Mitchell <i>et al.</i> ¹²⁶	2012	

ADSCs, adipose-derived stem cells; EGF, epidermal growth factor; hUCMSCs, human umbilical cord mesenchymal stem cells; NSAID, nonsteroidal anti-inflammatory drugs; TGF, transforming growth factor; PDGF, platelet-derived growth factor.

However, relatively few of these factors are potential therapeutic targets as many of the differences turn out to be phenomena that are associated with the fact that embryos are still expressing genes required for development and which are not causative of the scar-free-healing phenotype. On the other hand, some gene products have been clearly shown to play a role in scarless healing and suggest themselves as therapeutic targets. While PDGF levels and localization are similar in both fetus and adult following wounding, in fetal wounds PDGF levels attenuate rapidly and are gone by 48 h. PDGF levels in adult wounds remain for up to 4 days.⁵⁷ TGF- β 1 is detected within the wound bed in both neonatal and adult tissues but it is absent in fetal wound tissues, and a similar pattern is observed for FGF.⁵⁷

Large-scale genetic studies also demonstrate differences in gene expression. Numerous genes are located in functional pathways associated with superoxide radical degradation, melanocyte development and pigment signaling, and nitric oxide synthase signaling.⁶¹ Further, the Wnt signaling pathway, known to positively regulate TGF- β 1, is upregulated in scar tissue and may contribute to loss of regenerative ability.

Burn progression

One of the more difficult issues to resolve in burn wound healing involves the progressive tissue damage associated with unexcised partial-thickness burns and the zone of stasis in excised full-thickness burns. Clinical practice usually adopts a wait-and-see approach to partial-thickness burns to

avoid unnecessary removal of tissue. However, the inflammatory response within the injured tissue and within the initially non-necrotic zone of stasis can lead to additional tissue death or fibrosis. Potent vasoconstrictors, such as thromboxane A₂, are typically present in high concentrations in burn wounds.^{62,63} This tends to reduce perfusion of the tissue in the zone of stasis, which may lead to progressive necrosis. Inhibitors of thromboxane A₂-induced vasoconstriction can improve blood flow and increase survival of the tissue.⁶⁴ Application of antioxidants, bradykinin antagonists, and negative pressure therapies can also improve blood flow and improve outcome for this tissue.^{65,66} Inflammation in the zone of stasis also plays a role in determining survival of the tissue by virtue of the effect of these mediators on vasoconstriction. Anti-inflammatory treatment of the zone of stasis immediately after injury improves tissue perfusion and tissue survival in animal models. This may be tied to inhibition of activities mediated by neutrophils and macrophages, as animals treated with a fibronectin peptide that interferes with attachment of infiltrating leukocytes show enhanced blood flow and reduced necrotic progression in the zone of stasis.⁶⁷

Inhibition of specific proinflammatory cytokines also has potential to reduce burn progression. In a rat model, application to a partial-thickness burn of anti-TNF- α or anti-IL-6 antibodies conjugated to hyaluronic acid resulted in significant reduction of burn progression.⁶⁸ Both reduction in macrophage infiltration and reduced levels of IL-1 β were observed, suggesting inhibition of inflammatory inducers. However, clinical studies are lacking and must address the increased risk of infection that may result from inhibition of the inflammatory response.

Anti-inflammatories

When considering the role of inflammation in wound healing, it is important to recognize that it is not always clear whether the inflammatory response initiates and perpetuates pathology or whether it is a reaction to pathogenic stimuli. Certainly it is commonly acknowledged that prolonged inflammation is correlated with poor wound healing, but it is worth considering whether reduction of inflammation results in improved wound healing or whether treatments alter underlying processes that subsequently result in reduction in inflammation.

Because of the obvious correlation, in addition to use of anti-inflammatories to reduce burn progression, inflammatory factors have long been a target for therapeutic approaches to wound healing and scar-

ring in general. While the role of TGF- β in promoting collagen synthesis and deposition leading to scar formation is well-documented,⁶⁹ TGF- β likewise potentially stimulates chemotaxis of lymphocytes, fibroblasts, monocytes, and neutrophils.⁷⁰

The COX-2 pathway also appears to play a role in wound healing and scarring in response to burns. Indeed, COX-2 is significantly upregulated in response to acute burn injuries leading to production of PGE₂ and subsequent expression of proinflammatory cytokines. Application of the COX-2 inhibitor parecoxib in mice attenuates the proinflammatory cytokine response and reduces remote acute lung injury.⁷¹ However, studies of nonsteroidal anti-inflammatory drugs for treatment of burns and similar trauma in humans have typically not focused on the wound healing process itself but on alleviation of pain and fever.⁷²

The use of corticosteroids has likewise been limited beyond preclinical studies. While glucocorticoids are potent anti-inflammatory agents, they also inhibit epidermal proliferation. This suggests that use of such agents for wound healing may be contraindicated, though their use in reduction of hypertrophic scar subsequent to wound closure and for treatment of keloids is not without potential.⁷³

Numerous *in vitro* studies have looked at the use of opioids to modulate tissue inflammation. However it is unclear to what extent these studies inform potential *in vivo* studies. Much of the literature on opioids and inflammation is contradictory and mechanisms are lacking. Some researchers have suggested that leukocytes express opioid receptors and may respond directly to the drug. Others have been unable to detect the receptors or receptor transcripts in peripheral blood mononuclear cells. Other studies indicate that opioids may indirectly suppress inflammation by modulating the hypothalamic-pituitary-adrenal axis, leading to upregulation of glucocorticoids. Further complicating the analysis, opioids have highly species-specific and duration-dependent (acute vs. chronic administration) effects. Thus, although opioids have been studied intensively, definitive evidence tying their involvement with specific immune pathways or molecules to specific *in vivo* anti-inflammatory effects is lacking. For a comprehensive review of current research on opioids and immune modulation, see Al-Hashimi *et al.*⁷⁴

Beyond their role in modulating inflammation, there is some evidence that opioids may affect wound healing processes more directly. Several studies that look at effects on acute wound healing have described increased granulation tissue formation and re-epithelialization following

application of opioid receptor agonists. These are linked to enhancement of keratinocyte migration; upregulation of FGF-2, -7, and -10; upregulation of matrix metalloproteinase-1; and increased collagen synthesis and angiogenesis.⁷⁵⁻⁷⁸ There are also indications that opioids can affect the maturation and remodeling phase of wound healing via inhibition of myofibroblasts and wound contraction.⁷⁹ This may be the result of inhibition of PDGF and TGF- β 1. While extensive preclinical studies have been published on the use of opioids, clinical studies that investigate the role opioids may play in directly influencing wound healing are rare. The primary concern for use of opioids is their immunosuppressive effects. Chronic use of opioids is associated with delayed healing and infection-related complications, and infectious complications are often cited as evidence of opioid-mediated immune suppression. However most of these reports are observational or epidemiological and controlled trials are lacking. For a comprehensive review of opioids in wound healing, see Stein and Kuchler.⁸⁰

Scarring

While the primary focus of research efforts to date has been on life-saving strategies and wound closure, reduction of scarring has begun to receive more attention. Fetal scarless-healing models have pointed to TGF- β isoforms as a potential point of intervention. Numerous animal models have demonstrated that TGF- β 1 and -2 upregulation can lead to excessive scarring, including models of human fetal skin in nude mice and rats.^{81,82} Exogenous application of TGF- β 1 to rabbits induced scarring even in fetal tissues.⁸³ *Ex vivo* studies of human scar tissue also demonstrate enhanced expression of this cytokine.⁸⁴ These studies indicate that TGF- β 1 by itself can induce scarring in tissues that are otherwise resistant to scarring, pointing to TGF- β 1 as a potential target for modulating wound healing. While one study in a murine burn wound model has shown that neutralization of TGF- β 1 improves the ability to clear infections,⁸⁵ the utility of such treatments for improvement of scarring and wound healing in humans has proven ineffective.^{86,87} It is unclear whether this failure is the result of redundant profibrotic factors, or whether the TGF- β pathway in humans operates differently than in rodents. The differences in genomic responses of mice and humans to injury suggest that studies of therapeutics in mice may not be predictive of efficacy in humans.⁴

In contrast to low or absent expression of TGF- β 1 and -2, fetal wounds express high levels of TGF- β 3. This pattern is the reverse observed in adult wound

healing,^{57,58} and has led to testing of recombinant TGF- β 3, trademarked as Avotermin by the biopharmaceutical company Renovo,⁸⁸ as a scar preventative. Avotermin progressed successfully through controlled phase I/II trials.⁸⁹ However, phase III trials showed that Avotermin failed to meet its primary and secondary endpoints of scar reduction and studies were terminated.⁹⁰

Anti-inflammatory cytokines have also been examined for their antiscarring effects. IL-10 has been demonstrated to provide protection against TGF- β 1-induced fibrosis in mice.⁹¹ Application of recombinant IL-10 resulted in reduced levels of α -smooth muscle actin-positive myofibroblasts, reduced collagen deposition, and significantly inhibited wound contracture. However exploratory clinical trials in humans were less successful. Though human recombinant IL-10, trademarked as Pervascar by Renovo, showed improved scar reduction over placebo at 1 month, by 13 months the trend had reversed and placebo scars showed reduced scar over drug treatment, and consequently, further work was halted.⁹²

The use of retinoids has demonstrated some efficacy in treatment of various skin problems associated with photoaging, acne scarring, keratin disturbances, and hyperpigmentation, the latter commonly associated with split-thickness skin grafting following burn. The combination treatment of tretinoin and glycolic acid is thought to increase the dermal elastin content of skin.⁹³ In patients with perioral burn sequelae resulting in restricted mouth opening, a similar treatment regimen led to increased elasticity of the perioral skin and ease of mouth opening.⁹⁴ In a separate study, patients with whole-face scarring were treated with tretinoin alone, and measures of skin quality showed decreased resistance and increased elastance.⁹⁵ Interestingly, there were no obvious differences in total protein content or histology noted between treated and nontreated individuals, making it difficult to assess how the treatment altered the wound healing process.

Beyond the application of drugs or recombinant cytokines, cell-based therapies have been explored. A recent entry to the burn treatment field is Azfcil-T, which consists of autologous dermal fibroblasts, marketed as LAVIVTM by Fibrocell Science, Inc. Azfcil-T has been approved by the Food and Drug Administration for use in cosmesis, namely, bilateral nasolabial folds, acne scarring, and general wrinkle amelioration. Fibrocell is currently recruiting for a Phase II clinical trial to determine efficacy in reducing restricted mobility caused by burn scars.⁹⁶ A similar clinical study

sponsored by the University of Pittsburgh Medical Center is currently recruiting for Phase I/II trials using allogeneic human dermal fibroblasts.⁹⁷ The use of cells as opposed to direct application of individual molecules may represent a more useful approach, since the complex networks of interacting factors known to influence wound healing have proven resistant to reductionist approaches to the development of therapeutics.

Stem cell therapies

Adult stem cells may be potent modulators of the wound healing process and much effort is being undertaken to test their utility in wound healing. Preclinical evidence is accumulating for their ability to attenuate systemic inflammation following severe burns, thereby enhancing survival, enhancing the rate of healing, and improving final skin quality.⁹⁸ The primary challenges for application of stem cells to wound healing lie in determining optimal sources, processing methods, and administration.⁹⁹ For a comprehensive review of the current status of stem cell research related to burns and wound care and how these challenges are being addressed, see Huang and Burd.¹⁰⁰

Human umbilical cord mesenchymal stem cells (hUCMSCs) are the most readily available and most abundant stem cells. They have increased expansion capacity compared with bone-marrow-derived stem cells (BM-DSCs) and retain multipotency and immune regulation functions.¹⁰¹ One clinical trial, currently recruiting for phase I/II trials, is focused on the uses of hUCMSCs in acute burns, measuring stem cell effects on healing time, contraction, skin quality, and graft take.¹⁰²

Adipose-derived stem cells (ADSCs) are also currently under study for skin-regenerative applications. ADSCs are readily isolated from lipoaspirates and are much more easily obtained than BM-DSCs. A number of experimental models have demonstrated their potential both *in vitro* and *in vivo*. Three-dimensional skin substitutes incorporating ADSCs along with fibroblasts and keratinocytes differentiate into well-defined dermal and epidermal layers compared with those lacking ADSCs.¹⁰³ The presence of ADSCs also results in more complexity of the ECM. Several animal models incorporate ADSCs. A murine full-thickness skin defect model demonstrated that seeding ADSCs within a silk fibroin-chitosan scaffold enhanced healing and demonstrated differentiation into vascular, endothelial, and epithelial components compared with scaffolds lacking ADSCs.¹⁰⁴ Another model utilized healing-deficient diabetic mice, demonstrating that ADSCs incorporated into an atelocollagen matrix enhance

granulation tissue formation, capillary formation, and reepithelialization.¹⁰⁵

Despite the promise of stem cells in these and other translational studies, the use of allogeneic stem cell therapies in clinical wound healing modalities still faces a number of regulatory hurdles.

Skin substitutes

One of the major problems in large TBSA burns is the limited amount of donor sites capable of providing skin for coverage of the wound. The use of tissue-engineered scaffolds in place of, or in conjunction with, skin grafts has been a clinical tool since the early 1990s. Skin substitutes can be broadly subdivided into decellularized allogeneic skin substitutes, such as Alloderm®, or tissue-engineered skin substitutes, such as Integra®. The various products available on the market differ based on their composition and method of preparation.

Integra® is made from cross-linked bovine tendon collagen chondroitin-6-sulfate. The standardization associated with engineered substitutes includes large controlled pore size, conducive to cellular infiltration, and the presence of a silicone layer to help prevent fluid loss. Integra® is noncytotoxic and nonpyrogenic. On the other hand, revascularization of the matrix is unpredictable. Alloderm®, by contrast, preserves the natural ECM, including collagen, glycosaminoglycans, and elastin, and it has been shown to support vascularity and epithelialization without inflammation.¹⁰⁶ However, the process of cellular ingrowth may be slower than Integra® due to the density of the ECM.¹⁰⁷

Many of the clinical and preclinical studies associated with the various skin substitutes involve preparation of the matrix with various cell types, including fibroblasts, keratinocytes, or stem cells. Several of these studies are described in more detail in the stem cell therapies section. There is also a continuing effort to explore different scaffold compositions, such as chitosan¹⁰⁸ and esterified hyaluronic acid¹⁰⁹ among others. However, the basic modality of treatment utilizing skin substitutes remains relatively unchanged.

SUMMARY

Our understanding of the molecular mechanisms that influence wound healing has expanded greatly over the last 50 years. However, this increased understanding has primarily demonstrated how complex the underlying processes are, often raising more questions than providing answers. Attempts to take a reductionist approach and apply our knowledge of individual molecular

pathways or their products have yielded limited success. And despite potential advances observed in preclinical and *in vitro* studies, the standard of care for burn wounds has remained relatively unchanged over the last 30 years. The causes of this failure (technical, business, and philosophical) are beyond the scope of this review, but for a thorough analysis of these barriers, see Hollister and Murphy.¹¹⁰

To what extent does a given molecular pathway contribute to the final outcome of wound healing? Are various molecular pathways that appear to influence similar aspects of wound healing truly redundant or are they mediating many separate effects? To what extent do animal models accurately inform clinical studies? These questions remain to be answered; however, they also allude to problems for translation into clinical application. Thus we are left with a number of critical points that highlight both the pitfalls and the opportunities of translational science.

- Correlation of macroscopic observations of wound healing with molecular observations has not established causation.
- Even in cases where strong correlations suggest causation, as with the role of TGF- β 1, care must be used when translating results of animal studies to humans. Though animals may demonstrate clinical similarities to human healing, their genomic and molecular responses may not mirror the human response.
- Numerous molecular pathways underlie wound healing processes, and the degree to which they are redundant and interconnected may explain the lack of efficacy in clinical trials focused on modulating the activity of any particular cytokine or growth factor.
- It may be more effective to focus on ways to manipulate signaling networks by employing cells that are already capable of holistically altering molecular networks.

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Abbreviations and Acronyms

ADSCs = adipose-derived stem cells
 BM-DSCs = bone-marrow-derived stem cells
 ECM = extracellular matrix
 EGF = epidermal growth factor
 FGF = fibroblast growth factor
 hUCMSCs = human umbilical cord mesenchymal stem cells
 IFN- γ = interferon-gamma
 MODS = multiorgan dysfunction syndrome
 PDGF = platelet-derived growth factor
 PGE₂ = prostaglandin E₂
 SIRS = systemic inflammatory response syndrome
 TBSA = total body surface area
 TGF- β = transforming growth factor beta
 TNF- α = tumor necrosis factor alpha