Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics

Part I. The molecular basis of scar formation

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Cutaneous scarring is often the epicenter of patient-related concerns, and the question “Will there be a scar?” is one that is all too familiar to the everyday clinician. In approaching this topic, we have reviewed the pathology, the embryology, and the molecular biology of cutaneous scarring. (J Am Acad Dermatol 2012;66:1-10.)

Key words: cutaneous scar; early growth response protein-1; homeobox13; interleukins; mechanisms of scarring; platelet-derived growth factor; transforming growth factor—beta; Wnt pathway.
People with exaggerated skin scarring may face substantial physical and psychosocial consequences. In this first installment of a two-part continuing medical education series, we aim to explore the pathophysiology underlying cutaneous scarring and the molecular mechanisms governing scar formation. Understanding the biology of scarring will allow for a better understanding of the scientific basis of scar-reduction strategies. The latter shall be discussed in Part II of this series.

METHODOLOGY

In preparing this work, we used PubMed to perform literature searches on scar-related research. Key terms used in the search were “scarring,” “wound healing,” “prevention,” and “treatment.” Review articles were used as an initial source of information and, where relevant, information from primary research papers was obtained.

PATHOPHYSIOLOGY OF THE CUTANEOUS SCAR

Key points

- The inflammatory phase of wound healing aims to contain the injury and prevent infection
- The proliferative phase is characterized by granulation tissue—composed of macrophages, fibroblasts, and epithelial tissue
- The remodeling phase is the lengthy process of extracellular matrix reorganization around the site of injury
- Embryonic cutaneous wounds in the first third of gestation heal without a scar

Disruption of cutaneous epithelial continuity results in a characteristic pathophysiologic response. This response has been traditionally subcategorized into the three phases of normal wound healing. These phases are the inflammatory, proliferative, and remodeling phases (Fig 1). Wound healing, however, is a dynamic process, and at any point in time, processes occurring in one phase overlap with those occurring in another.

Inflammatory phase (days 1-3)

After the disruption of epithelial integrity, the immediate priority is hemostasis. This is achieved by activation of the extrinsic clotting pathway. Ultimately, this results in formation of a fibrin hemostatic plug, which is further solidified by the arrival of platelets from the local microcirculation.

Once the danger of exsanguination subsides, the next priority is the removal of dead tissue and the prevention of infection. Inflammatory cells are crucial to this process. For the first 5 days, neutrophils enter the fibrin-rich zone of injury. Through their actions of phagocytosis and protease secretion, neutrophils kill local bacteria and help degrade dead tissue. On the third day after injury, macrophages also enter the injury zone. In addition to phagocytosing pathogens and tissue debris, these cells secrete a multitude of growth factors, chemokines, and cytokines. These
signaling molecules are vital for the coordination of downstream events occurring during the proliferative phase.\textsuperscript{2}

\textbf{Proliferative phase (days 4-21)}

Granulation tissue—composed of macrophages, fibroblasts, and endothelial cells—is the hallmark of the proliferative phase. This tissue replaces the fibrin hemostatic plug set up during the inflammatory phase. Macrophages, via the secretion of platelet-derived growth factor (PDGF) and transforming growth factor--beta 1 (TGF\textsubscript{B1}), induce fibroblasts to proliferate and lay down type III collagen. This provides a structural framework for endothelial cells to proliferate and lay down new vessels by the process of angiogenesis.\textsuperscript{2}

Reepithelialization is essential for the reestablishment of tissue integrity. Keratinocytes adjacent to the wound edge and in hair follicles undergo dedifferentiation and reorganize their adhesion molecules. This loosens their connections to both the basement membrane and to each other, and subsequently allows them to migrate across the wound surface and close the skin defect.\textsuperscript{2}

\textbf{Remodeling phase (day 21 to year 1)}

During the remodeling phase, formation of granulation tissue ceases through apoptosis of the responsible cells. This process is important, because its aberration leads to hypertrophic scarring and keloids.\textsuperscript{2}

With maturation of the wound, the composition of the extracellular matrix undergoes change. The type III collagen deposited during the proliferative phase is slowly degraded and replaced with stronger type I collagen. This type of collagen is oriented as small parallel bundles, which differs drastically from the basket-weave orientation of collagen present in normal dermis.\textsuperscript{2}

Towards the later stages of healing, the wound undergoes a contractile response through the action of myofibroblasts. By virtue of their multiple attachment points to collagen, these actin-rich cells contract and reduce the surface area of the scar.\textsuperscript{2}

\textbf{Macroscopic considerations}

The immediate macroscopic appearance of the wound after injury is that of a skin defect with a glistening surface attributed to the fibrin exudate. A few days later, eschar—a collection of dead tissue—replaces this defect. In the coming weeks, a red and indurated cutaneous scar forms. The scar remodels in the year that follows to become soft and slightly lighter in color than the surrounding skin.\textsuperscript{3,4}

The abnormal architecture of collagen that results following the remodeling phase is the cause of the visible cutaneous scar. Within the
abnormal collagenous network, there is a notable absence of hair follicles, sebaceous glands, and sweat glands. The extent to which this abnormal dermal phenotype arises depends on the depth of injury; deeper cutaneous injuries give rise to more scar tissue.5

Embryonic regenerative healing

Cutaneous wounds made during the first third of gestation in mammals heal via tissue regeneration. This type of healing uses reactivation of the developmental pathways that originally gave rise to the tissue. Ultimately, the original tissue constituents are replaced and healing occurs without scar formation.6 Consequently, this prompts the question, “Why should there be a scar-mediated healing response?”

In evolutionary terms, most wounds encountered by animals were the result of postcombat trauma or falls. Consequently, the common wounds faced by mammals were bites, blows, contusions, and degloving injuries. These injuries involved widespread areas of tissue and were frequently contaminated with bacteria and foreign bodies. In such a scenario, evolutionary pressure favored a healing response that would control infection, wall off foreign bodies, and seal off the injured area from the environment. The inflammatory and proliferative phases witnessed in normal adult wound healing are remnants of this evolutionary selection. The multitude of leukocytes in the inflammatory phase helps prevent systemic infection, and the fibroblast activity during the proliferative phase walls off foreign bodies and reestablishes tissue integrity. In stark contrast to the scar-forming healing response, regenerative healing—as witnessed in early embryos and in liver regeneration—has a notable absence of fibroblast or inflammatory cell activity. In an evolutionary context, this attribute of regenerative healing would have been negatively selected for, because animals would face a high risk of septicemia and death.7

Wound healing today is likely to take place at an injury site that has been debrided, sterilized, and approximated with the use of suture material. As a result, the coarse and dirty wounds encountered throughout evolution are less of a commonality and the abundance of inflammatory cells and fibroblasts seen in scar-mediated healing are less of a necessity. In fact, in such controlled conditions for wound healing, a regenerative response would provide the advantage of a superior cosmetic result and would avoid the potential for aberrant scarring as seen in hypertrophic scars and keloids.7

Molecular biology of wound healing

Key points

• TGFβ1 and TGFβ2 expression leads to increased scarring, whereas expression of TGFβ3 reduces scarring
• Proinflammatory cytokines interleukin-6 (IL-6) and IL-8 enhance scarring, whereas the anti-inflammatory cytokine IL-10 decreases the amount of scar tissue
• Homeobox b13, the Wnt signaling pathway, early growth response-1, and platelet-derived growth factor all favor fibroplasia

The mammalian cutaneous wound is enriched with a multitude of extracellular matrix proteins, growth factors, and cytokines not normally present in intact skin.8 Because the literature on molecules proposed to have a role in wound healing is vast, the following account will concentrate on molecules that are noted to have a significant influence on tissue scarring.9,10 Preference was given to molecules with robust research evidence. Key molecules in the wound healing machinery and their pathologic significance are summarized in Table I.

Transforming growth factor—beta

Signaling. TGFβ is crucial in the regulation of wound scarring. The three isoforms, TGFβ1–3, exert their effects by binding to dimeric TGFβ receptor complexes. Upon activation, this receptor complex phosphorylates SMAD2 and SMAD3 proteins, which subsequently form dimers with SMAD4. In its dimeric form, this SMAD aggregation is able to translocate into the nucleus and function as a transcription factor (Fig 2). TGFβs β1 and β2 activate the receptor complex and thereby downstream signaling, whereas TGFβ3 is a receptor antagonist and thereby blocks signal transduction.9,10

Expression. Expression studies have strongly implicated TGFβs in scarring, because TGFβs and their receptors are expressed prominently in adult scar forming wounds. Conversely, in nonscarring fetal wounds, their expression is only transient.11 In addition, fibroblasts from both hypertrophic scars and keloids consistently overexpress proteins involved in TGFβ signal transduction.12–16

Animal studies

Genetically engineered animal models have provided invaluable information about the involvement of TGFβ signaling in cutaneous wound healing. Knock-out mouse animal models for TGFβ1 have
revealed a characteristic defect in healing during the proliferative phase: at 10 days after incisional injury, histologic analysis of the wounds reveals reduced granulation tissue bulk and collagen deposition.\textsuperscript{17} Conversely, in a transgenic mouse model where TGFβ\textsubscript{1} was overexpressed in keratinocytes, type I collagen expression was significantly up-regulated and scar tissue bulk increased.\textsuperscript{18}

The downstream mediators of TGFβ signaling have also been the subject of intense investigation. Because SMAD\textsubscript{3} is an important molecule in TGFβ signaling, SMAD\textsubscript{3} knock-out mice have been created to decipher its role in wound healing. These studies show that animals deficient for SMAD\textsubscript{3} have enhanced cutaneous healing with reduced deposition of scar tissue.\textsuperscript{19}

Recent research indicates that the scar-inducing signal transduction mechanism of TGFβ\textsubscript{1} is mediated via the Wnt pathway (Fig 3). The application of exogenous TGFβ\textsubscript{1}—a scar inducer—on the wounds of β-catenin conditional knock-out mice results in minimal scarring.\textsuperscript{20} In addition, the constitutive expression of an active form of β-catenin in SMAD\textsubscript{3} knock-out mice results in scarring not normally seen in the animals with SMAD\textsubscript{3} deficiency alone.\textsuperscript{20} These data imply that TGFβ\textsubscript{1} induces SMAD\textsubscript{3} to transcribe proteins that activate the Wnt pathway to induce scarring.

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**Table I.** Key molecules in the wound healing machinery and their pathologic significance

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Function</th>
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<tbody>
<tr>
<td>TGFβ\textsubscript{1} and TGFβ\textsubscript{2}</td>
<td>Key in the proliferative phase of wound healing; promote signaling via SMAD and Wnt-dependent pathways to enhance scarring</td>
</tr>
<tr>
<td>TGFβ\textsubscript{3}</td>
<td>Receptor antagonist; reduces scarring</td>
</tr>
<tr>
<td>IL-6 and IL-8</td>
<td>Proinflammatory cytokines expressed immediately after cutaneous injury; recruit and activate inflammatory cells, thereby promoting scarring</td>
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<tr>
<td>IL-10</td>
<td>Antinflammatory cytokine that reduces scarring; it inhibits the infiltration of neutrophils and macrophages towards the wound site and dampens the expression of proinflammatory cytokines</td>
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<tr>
<td>Homeobox13</td>
<td>Transcription factor; absent from the scar-free healing wounds of foetuses; favors fibroplasia</td>
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<tr>
<td>Wnt signaling pathway</td>
<td>Aberrant transduction has been implicated as causative factor of aggressive fibromatosis; hypertrophic scars and keloids display excessive signaling via the Wnt pathway</td>
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<tr>
<td>PDGF</td>
<td>Secreted by macrophages during the proliferative phase of wound healing and induces fibroblasts to produce type III collagen and exocytose osteopontin; overexpressed in hypertrophic scars and keloids</td>
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<tr>
<td>Osteopontin</td>
<td>Extracellular glycoprotein that enhances fibroplasia; connects integrins on cell surfaces to collagen within the extracellular matrix and promotes cell adhesion and cellular migration; abolition of osteopontin reduces the trafficking of both inflammatory cells and fibroblasts and also leads to a larger number of these cells dying by apoptosis</td>
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<tr>
<td>EGR\textsubscript{1}</td>
<td>A zinc-finger transcription factor; upregulates the expression of TGFβ\textsubscript{1} and PDGF, mediators of enhanced fibroplasia</td>
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EGR-1, Early growth response protein-1; IL, Interleukin; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor—beta.
Transforming growth factor–beta modulation studies. Studies using strategies to alter the concentration of TGFβs during healing have revealed that the adult scar-forming mechanism can be altered. The application of neutralizing antibodies to TGFβ1 and TGFβ2 in incisional rat wounds results in a significant reduction of extracellular matrix deposition and, by extension, subsequent scarring. Conversely, TGFβ3 promotes signaling via SMAD and Wnt-dependent pathways to enhance scarring. Collectively, these expression studies suggest that scar tissue deposition may be reduced by dampening the proinflammatory cytokine profile of healing wounds. Importantly, the administration of IL-6 to fetal healing wounds results in scarring that would not normally occur.

Inflammatory response transcription factors. The modulation of the inflammatory response transcription factors in cutaneous injury models has also produced promising data. Knock-out mouse models for the PU.1 transcription factor lack macrophages and functional neutrophils. Incisional wounds in neonates with this genetic background revealed normal healing with absence of any obvious scarring. This adds weight to the hypothesis that the inflammatory response in wound healing is a remnant of evolution and in many instances counterproductive to the healing of incisional wounds.

Fibroplasia

The molecular biology behind fibroblast proliferation and collagen deposition is central to the study of wound healing. Consequently, several
transcription factors and growth factors have been implicated for the control of these two processes.

**Homeobox b13.** The homeobox (hox) b13 transcription factor is notably absent from the scar-free healing wounds of fetuses. Conversely, in adults, hoxb13 expression is consistently high. Consequently, in an attempt to recreate this fetal parameter in adult healing wounds, hoxb13 knockout animals were engineered. In these animals, healing is strongly enhanced with increased wound tensile strength and reduced scarring (Fig 5). Structural analysis of the healed wounds in these animals revealed collagen architecture that resembles that of uninjured dermis. This implies that switching off hoxb13 is an important trigger to activating regenerative healing.

**Wnt signaling pathway**

Aberrant transduction via the Wnt signaling pathway has been implicated as a causative factor of aggressive fibromatosis; a connective tissue disorder leading to multiple subcutaneous nodules—desmoid tumors—and an excessive scar-forming healing response. Hypertrophic scars and keloids, in individuals without this disorder, also display excessive signaling via the Wnt pathway (Fig 3). The incidence of hypertrophic scars and keloids is higher in certain body areas, and regional variation in the activation of this pathway would be a reasonable speculation—one that has not been addressed in the scientific literature.

In aggressive fibromatosis, there is a mutation of the β-catenin gene, which leads to stabilization of the catenin protein product and thereby continuous activation of the Wnt signaling pathway. Transgenic mice that constitutively express this mutant form of β-catenin develop a phenotype similar to aggressive fibromatosis. In addition, experimental cutaneous injury in these animals gives rise to excessive scarring reminiscent of keloids and hypertrophic scars.

In order to further assess the outcome of manipulating β-catenin levels during wound healing, a conditional knock-out mouse was engineered. In this animal model, the β-catenin gene could be specifically deleted at the site of experimental injury. In comparison, wild-type mice, animals with the conditional deletion of β-catenin, display much smaller wounds and have fewer fibroblasts in their granulation tissue.

**Early growth response protein-1.** Early growth response protein 1 (EGR-1) is a member of the zinc-finger transcription factor group. Upon cutaneous injury, its concentration within fibroblasts rises acutely. In mouse embryos that have
been modified to overexpress EGR-1, excisional wounds result in enhanced collagen deposition and wound contraction. Because EGR-1 up-regulates the expression of TGFβ and PDGF, these growth factors are the likely mediators of this response.33

**Platelet-derived growth factor.** PDGF exists in several isoforms, and signals via the activation of dimeric transmembrane tyrosine kinase receptors. Within cutaneous wounds, it is a strong inducer of fibroblast-mediated collagen deposition, and it has been observed that both PDGF and its receptor are markedly overexpressed in hypertrophic scars and keloids.34,35 Importantly, recent studies have shown that PDGF secreted by macrophages during the proliferative phase of wound healing induces fibroblasts to produce and exocytose osteopontin.36 Osteopontin is an extracellular glycoprotein that connects integrins on cell surfaces to collagen within the extracellular matrix. This acts to promote cell adhesion and enhance cellular migration. In addition, via integrin-mediated intracellular signaling, osteopontin provides an important antiapoptotic signal via the nuclear factor κB group of transcription factors.37 Importantly, the application of osteopontin antisense oligonucleotides in mouse incisional wounds results in accelerated healing with reduced granulation tissue and scarring.36 These results suggest that the abolition of osteopontin from the wound environment reduces the trafficking of both inflammatory cells and fibroblasts and also leads to a larger number of these cells dying by apoptosis.

**CONCLUSION**

An increased understanding of the molecular mechanisms governing regenerative cutaneous healing would be expected to dispel the age-old notion that scarring is an inevitable consequence of injury or
surgery. Numerous approaches have been taken to successfully manipulate the adult scar-forming wound environment in the context of dermatologic surgery with the aim to recreate a scar-free healing environment. So, “Will there be a scar?” Part II will discuss the evidence-base of the available scar reduction modalities and attempt to answer this question.

REFERENCES