Fibroblasts and wound healing: an update

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“Increased comprehension of dermal fibroblast heterogeneity may yield both mechanistic insights into existing therapies and inspiration for novel therapeutics targeting specific cell populations in wound healing and scarring”

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Wound healing and regenerative medicine are intimately linked. While any dermal wound in an adult human, even if treated, will result in scarring [1], the ‘holy grail’ of wound healing is ‘scarless wound healing’: wound repair via the regeneration of functional, native tissue. Scarring and pathological wound healing states, such as hypertrophic scarring and keloids, represent an enormous clinical and financial burden on our healthcare system. Unfortunately, there are few truly effective therapies that hasten healing while reducing scar burden.

In the setting of skin injury, wound healing following hemostasis occurs in three overlapping stages: inflammation, proliferation and remodeling [2]. Fibroblasts are critical in all three phases, playing a key role in the deposition of extracellular matrix (ECM) components, wound contraction and remodeling of new ECM. Since our previous review [3], recent work continues to show the striking heterogeneity of skin fibroblasts. The concept that dermal fibroblasts represent multiple distinct subpopulations is an important advancement in our understanding of skin pathophysiology and serves as a new perspective from which the innovation of novel wound therapies may be possible. Herein, we will discuss recent advancements in the understanding of fibroblast heterogeneity as it pertains to cutaneous wound healing and relevant developments in clinical wound therapies.

Defining fibroblast subpopulations

Recent basic science research in wound healing has increased its focus on understanding the lineages, identities and roles of fibroblasts in various tissues. Rigorous characterization of dermal fibroblast heterogeneity based on cell surface markers has proved challenging, as cell surface marker expression is highly variable and no single marker identifies this cell type [4]. However, in recent years, distinct populations of dermal fibroblasts have been elucidated.

In 2013, Driskell *et al.* showed that dermal fibroblasts arise from two different lineages. The upper dermal lineage is involved with hair follicles, while the lower synthesizes ECM and engages with adipocytes [5]. Notably, the lower lineage was found to be largely responsible for dermal repair following wounding, explaining why scar tissue in humans is particularly ECM-rich and lacks hair follicles [5].

In 2015, Rinkevich *et al.* described the discovery of a ‘scarring fibroblast’ lineage responsible for depositing the vast majority of dorsal scar tissue in mice [6]. These cells are defined by lineage positivity for the homeobox transcription factor *EN1*. The authors found that these same cells could be reliably identified via expression of the marker CD26 and that ablation of these cells reduced scarring, although this also delayed wound healing. More recently, Hu *et al.* found that *PRRX1* demarcates the ventral lineage of murine scar-producing fibroblasts [7].

Novel insights into stem cell contributions to wound healing

In order to fully understand fibroblast heterogeneity, these cells’ lineages must be characterized. Plikus *et al.* showed that during wound healing, adipocytes can be generated from myofibroblasts (activated fibroblasts involved in wound contraction), suggesting significant lineage plasticity in what were previously understood to be terminally

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Figure 1. Wound healing pathophysiology and novel therapeutics for wound healing. An overview of cutaneous wound healing pathophysiology with a summary of recent wound healing therapeutics of note (discussed in depth in the text). **(A–C)** A review of cutaneous wound healing pathophysiology. **(A)** During the first stages of wound healing, platelets are recruited to the open wound and deposit fibrin (which serves as a preliminary extracellular matrix) to arrest bleeding. **(B)** During the next stages of wound healing, immune cells including neutrophils followed by macrophages are recruited to the wound and clear dead tissue and debris in preparation for healing. New blood vessels sprout around the site. Fibroblasts are recruited to the site in anticipation of scar formation. Keratinocytes begin to migrate to cover the cutaneous wound surface. **(C)** Finally, during the remodeling phases of wound healing, the keratinocytes have covered the site. Below the fibroblasts deposit new extracellular matrix replacing the fibrin plug, which is then remodeled to form the final scar. New blood vessels are pruned and nerves begin to regenerate to the site. **(D–F)** Novel therapeutics for wound healing. **(D)** Growth factors such as PDGFs can be provided directly to the wound to stimulate fibroblast proliferation and accelerate wound closure. **(E)** Cell-based skin substitutes (e.g., Graftix®, Osiris Therapeutics, MD, USA) can be applied directly to the wound site to protect it and directly provide factors including cells involved in wound healing. **(F)** Fibroblast mechanotransduction plays a role in stimulating scar production. Treatments that target fibroblast mechanotransduction, such as modulation of the FAK pathway, are being explored in the wound setting to decrease scar fibrosis and potentially improve cosmesis. Key for cell types used in illustrations provided in box at bottom.

differentiated fibroblasts [8]. Ge *et al.* also demonstrated significant lineage infidelity among cells involved in wound healing (including epidermal and hair follicle cells). They showed that this lineage infidelity is induced by stress-response-related transcription factors and is transient in healing but persistent in the setting of cancer [9]. In this regard, cancer cells can co-opt regenerative mechanisms seen in healing.

Such examples of lineage plasticity are dismantling the concept of distinct populations of stem cells that supply each cell type in a wound. For example, it is possible that under conditions of homeostasis, epidermal and hair follicle fibroblast lineages are distinct but under stress conditions this distinction is blurred [9]. These findings might explain why response to tissue injury can be highly variable across different individuals and pathological states.

Fibroblast heterogeneity across pathological wound healing states

Human wound healing may be viewed as a spectrum, with typical scar formation representing the 'normal' phenotype; chronic wounds at one extreme and hyperproliferative scarring and even keloids at the other. Previous studies have begun to investigate mechanisms by which fibroblast dysfunction could contribute to pathological wound healing states.

Multiple studies have shown that fibroblasts from chronic nonhealing wounds display abnormal phenotypes, including decreased proliferation, early senescence and altered patterns of cytokine release [10], as well as abnormal MMP and TIMP activity [11]. Conversely, keloid fibroblasts are known to exhibit increased proliferation and decreased apoptosis [12], and it has been suggested that keloid fibroblasts induce an abnormal phenotype in surrounding quiescent fibroblasts via paracrine signaling [13], explaining the observation that keloids outgrow the initial wound boundaries. Early observations, such as that made by Wang *et al.* that hypertrophic scar fibroblasts most closely resemble fibroblasts from deeper dermal layers [14], have alluded to the potential significance of different fibroblast subgroups in pathological healing states. However, this complex biology and its clinical implications have yet to be fully elucidated. We hope that research efforts will continue to explore these pathologies through the lens of fibroblast heterogeneity in the skin, to shed new light on the mechanisms behind scarring disease.

Fibroblast-focused therapeutics

There have been many wound therapeutic innovations in scar modulation since our previous review. We will limit our discussion to those most relevant to fibroblasts, including therapies involving the delivery of viable fibroblasts to the wound site and manipulation of fibroblast behavior.

Growth factor therapies

Several growth factors, including PDGF, FGF and TGF- β , are known to stimulate fibroblast division, activity and/or differentiation. But to date, only one growth factor, a recombinant human PDGF formulation (REGRANEX Gel, Smith & Nephew, London, UK), has been approved for chronic wounds. Other growth factors including FGF and TGF- β have failed to robustly demonstrate improved wound healing in human patients [15,16]. The general failure of growth factor therapies is likely due to multiple limitations including short half-life following delivery. While attempts have been made to integrate biomaterials for controlled growth factor release [17], major breakthroughs have yet to make their way into clinical practice.

Cell-based therapies

Effective wound treatments demand an agent that reflects the complex *in vivo* milieu of cell types and growth factors. One approach has been to deliver viable allogeneic cells, including fibroblasts, to the wound site. These cells do not persist indefinitely [18], but instead serve as a source of growth factors and cytokines to support the function of the patient's own cells.

Cell-based therapies are most often used for chronic wounds, perhaps because as previously mentioned these patients' own cells may be incompetent for wound healing. Cell-based therapies approved for use in wounds incorporate a varying range of cells, from fibroblasts only to fibroblasts plus keratinocytes, or even fully cryopreserved skin [19,20,21]. A more recent development in cell-based wound therapies, Grafix (Osiris Therapeutics, MD, USA), consists of cryopreserved placental tissue including placental ECM and fibroblasts [22].

While all of these products are used clinically for a large variety of wound types, their full mechanism of action remains unknown and in many cases their efficacy has not been robustly established *in vivo* [23]. Increased characterization of the different skin cell populations may enable the development of increasingly effective cell-based treatments.

Novel directions: targeting regeneration & fibroblast mechanotransduction

In recent years, growing mechanistic understanding of fibroblast function has led to therapeutically promising discoveries. Wnt signaling is known to be critical for skin differentiation and Wnt-responsive dermal fibroblasts play a key role [24]. As such, Wnt-3a and FGF-9 (which triggers and amplifies Wnt expression and activation in dermal fibroblasts) are being developed as therapies with the potential to achieve scarless healing with features of regeneration [25,26,27].

It has also been established that mechanical forces play a role in the development of pathological scars [28]. Mechanotransduction in wound fibroblasts occurs via focal adhesion complexes, which link the ECM to the intracellular cytoskeleton [29]. In 2011, Wong *et al.* demonstrated that FAK activation occurs following cutaneous injury and that fibroblast-specific FAK inhibition decreases scarring in mice [30]. These results suggest that fibroblast mechanotransduction may be a rich target for novel antiscarring wound therapies, and elucidating the molecular pathways linking mechanotransduction and fibrosis is an active area of current research. Figure 1 illustrates the stages of wound healing and fibroblast-related wound therapies.

Conclusion & future perspective

Understanding dermal fibroblast heterogeneity is a lofty but important goal. Major advancements have been made in the last several years to expand our knowledge of the different populations, signaling pathways and cellular niches of the diverse fibroblasts in mouse and human skin. Further work is needed to fully elucidate the contributions of different dermal fibroblast lineages to wound healing, characterize the most specific markers for each cell population and translate these populations from mice to humans. Increased comprehension of dermal fibroblast heterogeneity may yield both mechanistic insights into existing therapies and inspiration for novel therapeutics targeting specific cell populations in wound healing and scarring. We anticipate that in the near future, the field of wound healing therapeutics will expand to mirror our growing understanding of the diversity of cells involved in this biology.

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MT Longaker is a co-founder of, has an equity position in, and serves on the board of Neodyne Biosciences, Inc., a startup company which developed a device to reduce mechanical tension on wounds to minimize post-operative scarring. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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