Published in final edited form as:

Curr Pathobiol Rep. 2017 December ; 5(4): 333–342. doi:10.1007/s40139-017-0152-8.

Black, White, and Gray: Macrophages in Skin Repair and Disease

Melanie Rodrigues, PhD and Geoffrey Gurtner, MD

Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford University, USA

Abstract

Purpose of Review—Macrophages alter their responses during the temporal stages of wound healing. During the inflammatory phase macrophages perform phagocytosis. During neovascularization macrophages activate angiogenesis. In the proliferation phase of wound healing, macrophages deposit extracellular matrix and during wound resolution macrophages phagocytize excessive cellular components. This review addresses how these changing phenotypes affect skin repair and disease.

Recent Findings—Macrophages can determine the outcome of repair and can shift the normal wound healing response into fibrosis or chronic wounds. Emerging single cell technologies for the first time provide us with tools to uncover macrophage origin, heterogeneity and function.

Summary—Macrophages may exist as one population where all cells alter their phenotype in response to signals from the microenvironment. Alternatively, macrophages may exist as distinct subsets that can control wound outcomes. A clarified understanding will strengthen our knowledge of skin biology and aid in the development of wound healing therapies.

Keywords

Macrophages; Heterogeneity; Wound Healing; Skin Fibrosis; Macrophage Subsets; Chronic Wounds

INTRODUCTION TO MACROPHAGES

Macrophages are highly plastic cells of myeloid lineage that are an integral part of every tissue. They have the unique ability to adapt to local signals, respond to mechanical cues in the microenvironment and tailor to functional demands¹. Ever since their discovery by Elie Metchnikoff in 1882, for which he jointly received the Nobel Prize in 1908, these cells have been a fascination to scientists². Until three decades ago, macrophages were studied as a generic population in various tissues for their phagocytic activity and role in the immune

Correspondence to: Melanie Rodrigues, PhD, 257 Campus Drive West, GK-210, Stanford University CA 94305, Phone: 412-897-3519, melanie7@stanford.edu.

Conflict of Interest

Melanie Rodrigues has no conflicts to disclose.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

response. However, this concept has undergone several changes and with the evolution of cellular heterogeneity and development of single cell technologies, there have been significant revelations in their biology and function^{3,4}.

The M1 and M2 dichotomous states derived in vitro have largely formed the basis of macrophage research over two decades and a wide range of surface markers have been garnered to define both subsets⁵. This dichotomous classification resulted from two major studies. First, in 1992, Siamon Gordon's group showed that Interleukin (IL)-4 can alternatively activate macrophages to bring about a phenotype distinct from the inflammatory macrophages generated by Interferon (INF)- γ activation⁶. Subsequently, in 2000, macrophages were grouped into M1 and M2 based on their divergent effects when stimulated in Th1 (C57/B1) or Th2 (BALBc) mice⁷. Over the years, these two findings were combined and macrophages came to be grouped into M1 or classically activated macrophages with a pro-inflammatory phenotype, and M2 or alternatively activated macrophages that attenuate inflammation and participate in angiogenesis and tissue repair^{5,8}.

The rigid M1 and M2 classification is based on in vitro studies and does not accurately represent macrophages in vivo, where the cells modify their role based on the need of the tissue⁵. In vitro, M1 macrophages are derived following LPS/INF- γ stimulation and STAT1 activation⁹. M2 macrophages are derived following IL-4 stimulation and STAT6 activation⁹. Whether macrophages exist in these extremes, or as a continuum of phenotypes in vivo is actively being studied⁵. Regardless, there is now an agreement that several macrophage subsets exist. Some of these subsets contribute to steady state homeostasis, some subsets facilitate tissue repair and others exacerbate disease.

The origin of macrophages has also received considerable attention. Recent advances have revealed that all macrophages are not necessarily derived from a constant supply of monocytes through bone marrow hematopoiesis^{10–12}. Instead, there is a dynamic balance of macrophages derived from two unique pools: those that develop in the yolk sac from erythro-myeloid progenitors (EMPs) and persist in adult tissue as 'resident macrophages, and those that form in adult tissue from bone marrow-derived monocytes^{10,12,13}. The differences in macrophage origin might have a correlation with differences in their function.

MACROPHAGES IN SKIN HOMEOSTASIS

In the skin, macrophage populations can be distinguished based on their location within the epidermis, the dermis or the hypodermis. The epidermis, which is the outermost water-impermeable layer, consists of stratified epithelium, hair follicles and appendages. The interfollicular region is where resident macrophages called Langerhans cells (LCs) reside¹⁴. LCs are antigen presenting cells involved in antimicrobial immunity, immune surveillance and contact hypersensitivity¹⁴. They originate from EMPs in the embryo and undergo extensive proliferation between postnatal days 2 and 7^{15–17}. This proliferative burst is sufficient to maintain their numbers throughout adulthood where under steady-state they proliferate locally in response to signals generated by keratinocytes^{14,16}. Upon acquiring antigens, LCs downregulate e-cadherin with which they are bound to keratinocytes¹⁸. This allows them to migrate into the dermis, where they enter lymphatic vessels and proceed to

the draining lymph nodes to initiate a T-cell mediated adaptive response^{17,19,20}. It is interesting to note that migratory LCs are indistinguishable from migratory dendritic cells, the antigen presenting cells of the dermis¹⁴.

The dermis is the region of skin below the epidermis that is rich in extracellular matrix deposited by fibroblasts. The upper region or the papillary dermis is separated from the epidermis by the basement membrane. The deeper layer called the reticular dermis is rich in vasculature and contains both macrophages and antigen-presenting dendritic cells that can be difficult to distinguish from macrophages². The primary role of dermal macrophages under homeostatic conditions is to scavenge cellular and macromolecular degradation intermediates and microorganisms in the dermis²¹. Unlike dermal dendritic cells, macrophages have a poor antigen presenting capacity, are unable to activate T-cells and are not found to migrate into the draining lymph nodes in mice²¹. Macrophages also have a longer half-life compared to dendritic cells, which are usually replaced by progenitors from the blood in seven days²². Dermal macrophages consist of two subsets: those that are present prenatally as resident macrophages and those that develop from incoming LY6C^{high} monocytes following repeated episodes of inflammation during adulthood¹⁷.

Below the reticular dermis, the hypodermis or the superficial fascia consists of adipocytes and stromal vasculature. Macrophages constitute 5% of all cells in the subcutaneous adipose tissue under lean conditions²³. However, under pathological conditions such as obesity and obesity-induced diabetes, macrophages are found to constitute approximately 50% of the adipose tissue and this increase is predominantly through infiltration of bone marrow-derived monocytes²⁴. How macrophages under steady state conditions in the skin undergo change and are activated to perform distinct functions following injury are fields that are actively being studied.

MACROPHAGES IN WOUND HEALING

Restoring tissue integrity following epidermal damage is relatively simple and is achieved by compensatory proliferation of epidermal cells and local progenitor cells. Any damage to the skin that breaks the basement membrane and involves injury to the dermis and below results in an orchestrated cellular response in four sequential and overlapping stages of inflammation, angiogenesis, proliferation and remodeling²⁵. In mammals, skin injury induces an accumulation of macrophages (CD11b+/F/480+) within the first 24–48 hours after injury²⁶. In mice where wounds are completely closed by day 14, the number of macrophages within the wound reach a maximum at day 3, begin to decrease at day 5 and reach near-baseline levels by day 10, being involved as the most abundant inflammatory cells in each stage of wound repair^{27,28}.

To understand the importance of macrophages in wound healing, murine skin from transgenic models have been depleted of macrophages and then wounded. Macrophage-depleted wounds show an influx of neutrophils as a compensatory immune response, and demonstrate delayed wound closure^{29,30}. The impairment in the healing response correlates with decreased angiogenesis, reduced granulation tissue formation, lesser collagen deposition and reduced growth factor release in wounds, corroborating the importance of

macrophages in every stage of the wound healing response²⁹. To more specifically characterize the role of macrophages in each stage of wound repair, macrophages have also been conditionally depleted in each sequential stage³¹. Depletion of macrophages during inflammation is found to impair the subsequent stages of re-epithelialization and granulation tissue formation but results in lesser scar tissue^{31,32}. During neovascularization and the growth stage, depletion of macrophages results in severe hemorrhage and non-healed wounds³¹. Depletion of macrophages has been suggested to not impact remodeling³¹, however wound remodeling remains relatively unstudied and the influences of macrophages in this stage are now emerging.

Interestingly, following limb amputation in salamander, systemic depletion of macrophages during the first 24 hours results in permanent failure to regenerate the limb³³. Fascinatingly, the regenerative capacity of this limb is restored following re-amputation and replenishment of endogenous macrophage populations³³. In this section, macrophage contribution to the various stages of wound healing will be reviewed (Figure 1).

Role of Macrophages in the Inflammatory State of Wound Healing

To prevent blood loss, any injury to the skin immediately induces the coagulation response with reinforcement of a platelet plug²⁵. This generates a hypoxic microenvironment^{34,35}. Degranulation of platelets and mast cells in the plug along with hypoxia-inducible factors release more than three hundred active substances including cytokines, chemokines, inflammatory mediators and bioactive molecules within minutes to 5 days after injury, drawing in neutrophils and monocytes, and activating macrophages within the wound^{36,37}. Macrophages also possess pattern recognition receptors that bind to damage- and/or pathogen-associated molecular patterns (DAMPs and PAMPs) in the wound activating classical inflammation pathways through toll-like receptor and inflammasome signaling^{38,39}. Macrophages can also produce MCP-1, a potent chemoattractant for monocytes. Thus, wound macrophages may themselves recruit additional monocytes and macrophages exacerbating the inflammatory response^{40,41}.

Macrophages in this early stage of wound healing are pro-inflammatory and microbicidal with the surface marker profile CCR2^{high}/Ly6C^{high} and expressing interleukin (IL)-6, IL-1b and TNF- α ⁴². The pro-inflammatory macrophages recognize and engulf bacteria into phagosomes that become a nutrient-limiting environment high in reactive oxygen species and reactive nitrogen species, rapidly killing most bacteria⁴³. These macrophages also release matrix metalloproteinases to aid their migration through the extracellular matrix (ECM)⁴⁴. Digested ECM acts as an immunostimulatory DAMP, exacerbating the pro-inflammatory environment⁴⁴. Apart from being bactericidal, macrophages also phagocytose other cells and matrix debris in a process called efferocytosis^{45,46}. In particular, neutrophil infiltration ceases within 3–4 days of wounding, and expended neutrophils are phagocytized by macrophages⁴⁵. Decreasing neutrophils and a reduction in DAMPs and PAMPs at the end of the inflammatory stage yield alterations in the tissue microenvironment. Consequently, the inflammatory macrophage phenotype changes into a predominantly anti-inflammatory cell type in anticipation of the next stage of healing⁴⁷. How this transition occurs is being

investigated. There is evidence that microRNAs can mediate suppression of one macrophage phenotype and activation of another⁴⁸.

Role of Macrophages during New Blood Vessel Formation

Macrophages, especially of the alternatively-activated type, have long been known to contribute to new vessel formation during tissue repair and tumor angiogenesis^{49,50}. Both in wound healing and tumors, increased macrophage numbers correlate with high microvessel density⁵¹. This is supported by experiments where inhibition of colony stimulating factor 1 (CSF-1), a major chemokine that regulates both homing of monocyte into wounds and proliferation of macrophages, leads to significantly reduced neovascularization in the wound⁵². Furthermore, the state of macrophage metabolism determines if the new blood vessels are smoothly aligned, pericyte covered and functional, or leaky and abnormal^{53,54}.

Angiogenic macrophages in the wound are Tie2+ and contribute to new vessel formation in two ways⁵⁵. First, they form vessel anastomosis, fusing the independently sprouting endothelial vessels during neovascularization and connecting them to the systemic vasculature^{55,56}. Macrophages can also form lymphatic vessels during this stage of healing⁵⁷. This process of forming structurally primitive non-endothelial vessels is called 'vascular mimicry'⁵⁸. Second, macrophages release VEGF important for blood vessel sprouting⁵⁹. VEGF releasing macrophages are mostly replenished by Gr1-/Ly6^{low} monocytes that exhibit long-range crawling on the luminal side of blood vessels, where they survey endothelial cells and surrounding tissue for damage⁶⁰. During the late stage of skin remodeling VEGF release is taken over by keratinocytes and this transition is important for the progression of healing⁵⁹.

Over the past two decades, there has been a lot of attention garnered by the putative endothelial progenitor cell to new blood vessel formation in wounds⁶¹. However, there is sufficient evidence to demonstrate that such cells are not present either in wound healing or in tumors^{52,62,63}. While there is contribution of circulating cells such as monocytes to neovascularization through release of angiogenic growth factors, the bone-marrow derived cells are not found to differentiate into endothelial cells in wounds^{52,64–66,62}. Monocytes that differentiate into proangiogenic macrophages most likely constitute most of the circulatory proangiogenic cells.

Role of Macrophages and Fibrocytes in the Proliferative Phase of Wound Healing

In addition to supporting neovascularization, macrophages also affect the phenotypic state of dermal fibroblasts⁶⁷. M2 macrophages and particularly a subset that is CD206+/CD301b+ increase fibroblast proliferation and collagen deposition through conversion of fibroblasts into a-smooth muscle actin depositing myofibroblasts^{68,69}. Conversely, pro-inflammatory macrophages increase the production of inflammatory cytokines in fibroblasts and promote extracellular matrix breakdown⁶⁷. Macrophages can also deposit extracellular matrix themselves, directly contributing to the fibrotic response during granulation tissue formation. Such macrophages are referred to as fibrocytes⁷⁰. Fibrocytes share similarities with fibroblasts and can alter the balance of healing to that of a scar^{71,72}.

Pathways such as CCR1, CX3CL1–CX3CR1 and FAK-ERK-MCP1 have been implicated in promoting a fibrotic response through macrophage activation^{73–75}. While it is evident that pro-fibrotic macrophages contribute to fibroblast activation and extracellular matrix deposition, it is unclear whether the proangiogenic macrophages and the profibrotic macrophages can be distinguished, especially since new vessel formation and proliferation in the wound are temporally congruent. It may well be that macrophage plasticity creates continuous variant shifts depending on cells and juxtacrine signals neighboring the macrophages, rather than creating definite and separate phenotypes.

Role of Macrophages during Wound Resolution and Remodeling

Skin remodeling is the final stage of skin repair where the wound resolves and ‘stops healing’. The dermis is cleared of most cells and pro-growth matrices such as collagen 3, fibronectin and tenascin-C, and is replaced by type 1 collagen^{25,76}. In humans, the final remodeling phase starts 2–3 weeks after injury and lasts for a year²⁵. Changes occur incrementally over this large timeframe. Although skin remodeling is a significant part of the healing response, it remains the least studied of all stages of wound healing.

It is suggested that macrophages take on a “fibrolytic” and phagocytic profile during the remodeling phase where they can: (i) breakdown excessive fibrotic tissue through the release of proteases and (ii) phagocytize excessive cells that are no longer required following wound closure⁷⁷. If there is no timely resolution of excessive cells and matrix, there is scar formation and fibrosis. Fibrolytic macrophages remain to be characterized, however, a recent study has shown that macrophages can be therapeutically activated to take up and degrade excessive cellular components present in the fibrotic microenvironment. This study shows that similar to cancer cells, fibrosis is marked by increased CD47 on fibroblasts or the “don’t eat me signal” through activation of the c-Jun pathway⁷⁸. Presence of this protective signal allows fibrotic cells to evade the immune response. Inhibition of CD47 can resolve fibrosis through blocking the “don’t eat me signal” and allowing phagocytosis by macrophages⁷⁸.

MACROPHAGES IN SKIN FIBROSIS

Aberrations in cutaneous wounding can lead to fibrosis and hypertrophic scarring (HTS). Scar tissue has a unique structural makeup with a constitutively active proliferative phase⁷⁹. It is highly vascular, concentrated with inflammatory cells and contains fibroblasts that deposit abundant and disorganized extracellular matrix⁸⁰. While there can be several factors that shift wound healing into scar formation, one of the primary reasons for scarring is alteration of mechanical forces in the wound bed⁷⁵. The mechanisms by which mechanical forces are converted into molecular and cellular responses are collectively termed mechanotransduction. Alterations in mechanotransduction such as changes in focal adhesion kinase (FAK) signaling can bring about abnormalities in immune responses causing excessive ECM deposition, decreased apoptosis of cells and HTS^{72,75,79,81–83}.

Macrophages are an integral part of the abnormal immune response during scar formation. Increased macrophage numbers are a hallmark of fibrotic diseases such as keloids and HTS^{72,84}. The increased macrophage numbers result from increased monocyte chemoattractant protein (MCP-1) and a heightened T-cell mediated response^{72,75}. Since

macrophages exhibit such complexity, it is important to uncover the macrophage subsets that undergo alterations and the stage of the wound healing response when this occurs. Most studies in mice have focused on the early inflammatory stage of wound repair to study macrophage contribution to fibrosis. These studies indicate that early pro-inflammatory macrophages are necessary for normal repair while shifts towards either excessive or reduced pro-inflammatory macrophages are associated with HTS^{85–87}. However, the key alterations in macrophages that contribute to scar formation seem to occur after day 13, during the proliferation and remodeling phase⁴. A recent study showed that atypical macrophages derived from circulating granulocyte/macrophage progenitors, but not from macrophage/dendritic cell progenitors, increase in numbers 13 days after injury and are found to be critical for fibrosis⁴. Since these cells are derived from the circulation, they can be used both as a diagnostic for fibrosis and a biomarker to determine health following treatment.

A recent study analyzing various tissues has demonstrated that fibrosis results from fibroblasts that overexpress the CD47 “don’t eat me” and c-Jun signals that prevent them from being phagocytized by macrophages⁷⁸. Inhibiting the “don’t eat me” signal is a promising strategy to bring about scarless healing from two perspectives: (i) patients with fibrosis are usually presented in the altered skin remodeling stage when the wound has already “overhealed” and (ii) in the earlier stages of wound healing, the transitions of macrophage function and surface markers make these cells a difficult target.

Scleroderma is an overarching term that includes systemic sclerosis and localized scleroderma. Systemic sclerosis is an autoimmune disease with high mortality. It is characterized by vascular injury and progressive fibrosis of the skin and other organs. Localized scleroderma consists of fibrotic lesions limited to the skin and subcutaneous tissue. While these two diseases occur through biologically distinct processes, there is evidence of activation of similar immune pathways dominated by macrophages and T-cells around perivascular regions⁸⁸. Most studies conclude that circulating monocytes infiltrate the skin and abnormally differentiate into pro-fibrotic macrophages⁸⁸. These macrophages produce fibrogenic cytokines such as TGF β and promote neovascularization^{89,90}. Many of these studies are outcomes of in vitro observations or analysis of a limited number of surface markers, although more recently they are supported by unbiased genome-wide analyses⁹¹. Despite the promising findings, the pathways that govern activation of the macrophages in scleroderma are still unknown and remain an active area of investigation.

MACROPHAGES IN CHRONIC WOUNDS

Chronic wounds fail to proceed through the normal stages of wound healing. These wounds commonly represent as: (i) venous leg ulcers, (ii) wounds in patients with hemoglobinopathies such as sickle cell disease and Thalassemia, (iii) wounds in patients with autoimmune diseases, (iv) diabetic foot ulcers or (v) pressure sores.

Venous leg ulcers and hemoglobinopathies are characterized by iron overload⁹². Excessive iron activates the pro-inflammatory M1 macrophage phenotype⁹². These macrophages release tumor necrosis factor (TNF- α) and hydroxyl radicals, bringing about senescence in

fibroblasts⁹². Iron chelators such as deferoxamine are FDA-approved for systemic use in patients with hemoglobinopathies³⁵. Their indication can be extended to chronic wounds with iron-overload to reduce local macrophage-induced inflammation and promote the healing response.

Patients with autoimmune diseases constitute roughly 20% of all cases of lower extremity ulcers and do not usually respond to standard wound care⁹³. Patients with diseases such as rheumatoid arthritis, systemic lupus erythematosus, mixed connective tissue disease and pyoderma gangrenosum are especially susceptible⁹³. In rheumatoid arthritis for example, macrophages that accumulate in the synovium play a central role in the pathogenesis of the disease while fibroblasts and other immune cells display secondary dysfunctions⁹⁴. The persistent pro-inflammatory macrophages in the synovium release cytokines that most likely are the cause of the impaired wound healing responses⁹⁴. In systemic lupus erythematosus, there is an intrinsic defect in macrophage efferocytosis. Macrophages from these patients have a reduced ability to clear dying cells, especially neutrophils compared to patients without the disease⁹⁵. Persistently circulating apoptotic waste may alter signaling pathways within these patients.

Diabetic patients exhibit impaired repair responses and chronic non-healing wounds whose etiology can be multifactorial. Hyperglycemic cellular alterations, high reactive oxygen species, impaired neovascularization and chronic inflammation in the diabetic wound contribute to the chronic wound state³⁴. Macrophage activation in diabetes is temporally altered. There is delayed expression of chemokines, such as MCP-1 and macrophage inflammatory protein-2 (MIP-2), responsible for early recruitment of monocytes and activation of macrophages⁹⁶. This temporal lag causes diabetic wounds to display impaired efferocytosis⁹⁷. Thus, when the inflammatory stage of healing needs to transition into the proliferation and growth stage, there is a higher burden of apoptotic cells in the diabetic wound tissue and higher expression of inflammatory cytokines⁹⁷. The cytokines persist until the late stage of wound repair resulting in elevated numbers of macrophages even in the remodeling wound⁹⁶. Additionally, dysfunctional macrophages in diabetes result in compromised macrophage-derived vascularization⁵⁷. Combined, these effects have a significant decelerating on the healing outcome.

Targeting macrophage dysfunction during diabetic wound healing could be an avenue to enhance healing outcomes but how this can be achieved remains to be demonstrated. Use of topical stem cell therapies that can release growth factors and modulate macrophage responses is one strategy⁹⁸. In addition to modulating the immune response, stem cell-based therapies are associated with a myriad of benefits including improvement of neovascularization by influencing endothelial cells, promoting ECM deposition by influencing fibroblasts and increasing recruitment of progenitor cells into wounds^{99,100}.

IDENTIFICATION OF MACROPHAGE PHENOTYPES IN WOUND HEALING

In terms of phenotypic transitions during wound healing, macrophages in the initial inflammatory state that are pro-inflammatory and perform phagocytosis have been termed M1. Macrophages that are activated subsequently and produce growth factors have been

termed M2. In addition to these two phenotypes, macrophages that deposit extracellular matrix important for wound healing are termed M2a and macrophages important for fibrolytic responses during matrix remodeling are referred to as M2c or M_{reg}-like¹⁰¹. Similar to the M1 and M2 grouping, the classification into M2a or M2c arises from in vitro studies where macrophages are exposed to cytokines that are predominant during a particular phase of healing¹⁰¹. These studies indicate that exposure to IL-4 and IL-13 generates M2a macrophages by activating the STAT-6 pathway, while M2c macrophages are activated by IL-10, TGF- β or glucocorticoids through the Jak1/STAT3 signaling pathways^{102,103}. As noted earlier, the strict classification of macrophages into one subtype or another is based predominantly on in vitro studies. In vivo, wound macrophages display “hybrid” phenotypes since the wound is never strictly in one phase of healing¹⁰¹.

A robust way of analyzing the various macrophage subsets during wound healing is by isolating the wound tissue at various temporal stages and collagenase digesting the tissue to obtain single cells⁷⁰. These cells can be subjected to fluorescence assisted cell sorting (FACS) to isolate all macrophages based on the surface marker profile. In mice, the surface marker combination CD45+/CD11b+/F4/80+ identifies all macrophages in the skin^{10,70}. The sorted macrophages can then be subjected to single cell analysis in the form of RNA sequencing or quantitative polymerase chain reaction (qPCR). The transcriptional data obtained from these tests can be subjected to hierarchical and k-means clustering to generate subsets of cells based on their intracellular transcriptional profile that can include genes for inflammation, growth factor release, extracellular matrix deposition or matrix lysis^{70,104,105}. Correlation analyses can be conducted to reveal the best surface marker or surface marker combination unique to each cell subset^{106,107}. Finally, the surface marker combinations can be used to isolate unique macrophage subsets by FACS.

While current single cell RNA sequencing methods generate a large pool of data with transcriptional information of approximately 5000 genes per single cell, these techniques suffer from technical and biological noise that is difficult to distinguish from phenotypic variations and heterogeneity¹⁰⁸. Single cell qPCR results in data that is both accurate and precise. However, it is much less comprehensive compared to RNA sequencing and the primers used to probe the single cells need to be carefully selected. Despite the disadvantage, the reliability of the technique makes it more suited for clinical diagnoses^{106,107}.

From a biological perspective, single cell technologies can answer several important questions about macrophage heterogeneity in wound healing that cannot be addressed by population-based analyses. While there is clear evidence that macrophages are heterogeneous, the heterogeneity is currently classified either based on macrophage origin or based on discrete functions that these cells undertake in response to signals from the wound microenvironment (Figure 2). However, several important questions remain to be addressed. It is unclear if the same macrophage transitions into the subsequent stages of wound healing and takes on a different function based on signals from the wound, or if various macrophage subsets exist in different proportions at any given time and only some are activated in response to the spatiotemporal wound signal. How macrophage origin affects these discrete functions also remains unclear, but is actively being investigated.

CONCLUSIONS

The healing wound is a highly orchestrated tissue with several cell types coming together to bring about healing at distinct spatiotemporal stages. Macrophages are the predominant inflammatory cells in the healing wound in each of these stages and exhibit specialized functions. In the early stages, they are inflammatory and perform efferocytosis, taking up dead cells and debris from the wound. As the wound progresses from an inflammatory to a growth state, macrophages begin to support new vessel formation and matrix deposition. When the wound progresses into the resolving state, macrophage numbers in the wound return to baseline and there is healing without excessive scar formation. Since the M1 and M2 macrophage classification, it has been considered that the macrophage phenotype at any given moment reflects the state of the wound. However, macrophage heterogeneity might also be able to determine healing outcomes.

Dermal macrophages have a long half-life and macrophages are found until the stage of wound resolution. However, it is still unknown if the same inflammatory macrophage that is activated at the start of the wound healing response can transition into the subsequent wound healing phases, taking up unique functions. Simplistically, in this model, each macrophage is intrinsically similar to the other at a temporal stage and a different macrophage phenotype is formed in response to changing signals from the microenvironment. Alternatively, there could be distinct macrophage subsets in the wound at any given moment that are geared to perform discrete functions. These subsets could arise based on differences in their origin. Some of the discrete subsets get activated, while others are silenced based on the need of the microenvironment. In this model, macrophages are still dependent on signals from the microenvironment but they can also control outcomes of the microenvironment.

If the first model holds true, the microenvironment largely controls disease outcomes such as fibrosis and non-healing wounds. If the second model holds true, macrophages have a role in determining whether the wound heals or shifts into a diseased state. This knowledge of macrophage function is critical for the development of wound healing therapies. Single cell technologies such as RNA sequencing and multiplexed qPCR for the first time provide us with the ability to unravel these questions and deepen our understanding of skin macrophages in wound healing.

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Figure 1. Macrophage Phenotypes During Wound Healing

Wound healing is a highly orchestrated event with various cell types coordinating temporally. Macrophages are among the most fascinating since they play a unique role in each stage of the healing response. During the inflammatory stage, macrophages perform phagocytosis and release proinflammatory cytokines. During neovascularization, they release growth factors and fuse sprouting endothelial cells. During the proliferative phase of healing, macrophage-fibroblast interactions are important for matrix deposition. Macrophages themselves can deposit extracellular matrix in this stage. Finally, during remodeling, macrophages perform phagocytosis to eliminate excessive cells and cellular components. Dysregulation of these unique temporal macrophage functions can lead to fibrosis or chronic wounds.

Figure 2. Macrophage Heterogeneity in the Skin

(A) Macrophages in the adult skin can be categorized as tissue resident or monocyte-derived based on their origin. Macrophages can also be subdivided based on their function. Tissue resident macrophages originate in the embryo and persist in the adult skin. Monocyte-derived macrophages originate in the adult bone marrow and are recruited into the skin through the circulation. There is evidence that following injury, monocyte-derived macrophages increase in number to facilitate wound healing. Whether tissue-resident macrophages proliferate and whether they take up distinct functions during wound healing remain to be demonstrated. (B) During each sequential stage of wound healing, the majority

of macrophages are performing a unique function. Whether all macrophages starts as a pro-inflammatory cells and adapt to distinct functions temporally or whether distinct subsets with discrete functions exist, remains to be demonstrated.

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