Molecular Therapy
Review

Macrophages as a therapeutic target to promote diabetic wound healing

Maryam Sharifiaghdam,[1,2] Elnaz Shaabani,[1,2] Reza Faridi-Majidi,[2] Stefaan C. De Smedt,[1] Kevin Braeckmans,[1,3] and Juan C. Fraire[1]

[1]Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmacy, Ghent University, 9000 Ghent, Belgium; [2]Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran; [3]Center for Advanced Light Microscopy, Ghent University, 9000 Ghent, Belgium

It is well established that macrophages are key regulators of wound healing, displaying impressive plasticity and an evolving phenotype, from an aggressive pro-inflammatory or "M1" phenotype to a pro-healing or "M2" phenotype, depending on the wound healing stage, to ensure proper healing. Because dysregulated macrophage responses have been linked to impaired healing of diabetic wounds, macrophages are being considered as a therapeutic target for improved wound healing. In this review, we first discuss the role of macrophages in a normal skin wound healing process and discuss the aberrations that occur in macrophages under diabetic conditions. Next we provide an overview of recent macrophage-based therapeutic approaches, including delivery of *ex-vivo*-activated macrophages and delivery of pharmacological strategies aimed at eliminating or re-educating local skin macrophages. In particular, we focus on strategies to silence key regulator genes to repolarize wound macrophages to the M2 phenotype, and we provide a discussion of their potential future clinical translation.

OVERVIEW OF WOUND REPAIR

The skin is the first line of protection against chemical and physical assault, pathogen invasion, and prevention of unregulated dehydration. At the same time, it is involved in the transmission of sensory information and has a significant role in maintaining homeostasis.[1] When the integrity of the skin is compromised, it can affect the normal function of many cells, including endothelial cells, fibroblasts, and keratinocytes in each layer of the skin.[2] Therefore, proper recovery of the skin by wound healing is one of the most complex and tightly regulated processes involving evolutionarily developed genetic, epigenetic, and molecular processes to accomplish the goal of reorganizing different cells in space and time.[3] This process occurs in four overlapping phases: hemostasis, inflammation, proliferation, and remodeling.[4,5] A considerable amount of evidence has positioned macrophages as key regulators of these steps, playing an essential role during the different healing phases by readily adapting their phenotype according to spatiotemporal cues they receive during each phase.[6] In general, these phenotypes have been differentiated into pro-inflammatory M1-like macrophages (M1) on the one hand and pro-healing/anti-inflammatory M2-like macrophages (M2) on the other hand.[7]

An overview of the healing process is presented in Figure 1. Upon injury, the accumulated blood plug at the site of skin injury promotes the initial invasion of immune cells, specifically polymorphonuclear leukocytes (PMNs).[3,8] The arrival of these cells characterizes the beginning of the inflammatory phase.[9] This rapid, initial influx of PMNs is followed by successive waves of infiltrating monocytes that, in response to wound microenvironmental cues (e.g., low oxygen levels, microbial products, damaged cells, and activated lymphocytes), differentiate into M1-type macrophages for scavenging, phagocytosis, and antigen presentation during the inflammatory phase of healing.[10] When the acute injury is under control, macrophage gene expression gradually shifts from M1 (pro-inflammatory) macrophages to an M2 (pro-healing) phenotype with the capability for recruitment of stem cells and secretion of cytokines and growth factors that stimulate keratinocytes, fibroblasts, and endothelial cells to proliferate, differentiate, and migrate.[11] Through this plasticity, a positive feedback loop is established in which increasing normalization of the tissue microenvironment drives a progressively more pro-healing role for macrophages, which, in turn, promotes tissue repair.[12–14] This process also involves massive angiogenesis, which is needed for the supply of oxygen and nutrients necessary for fibroblasts and other cells to reconstitute the tissue matrix in the remodeling phase.[15–19]

The importance of macrophages in coordination of the wound healing response becomes apparent when their activation is disturbed. A study using genetically modified *LysMCre/DTR* mice, which allow inducible depletion of macrophages by diphtheria toxin (DT) administration, provided strong evidence that these cells are required for normal wound healing, including promoting angiogenesis, collagen deposition, and wound closure.[20] Other studies have found that properly regulated numbers and phenotypes of macrophages are crucial for efficient wound repair and that dysregulation of either of these may lead to chronic skin inflammation and impaired wound

https://doi.org/10.1016/j.ymthe.2022.07.016.

Correspondence: Kevin Braeckmans, Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmacy, Ghent University, 9000 Ghent, Belgium.
E-mail: kevin.braeckmans@ugent.be
Correspondence: Juan C. Fraire, Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmacy, Ghent University, 9000 Ghent, Belgium.
E-mail: juan.fraire@ugent.be

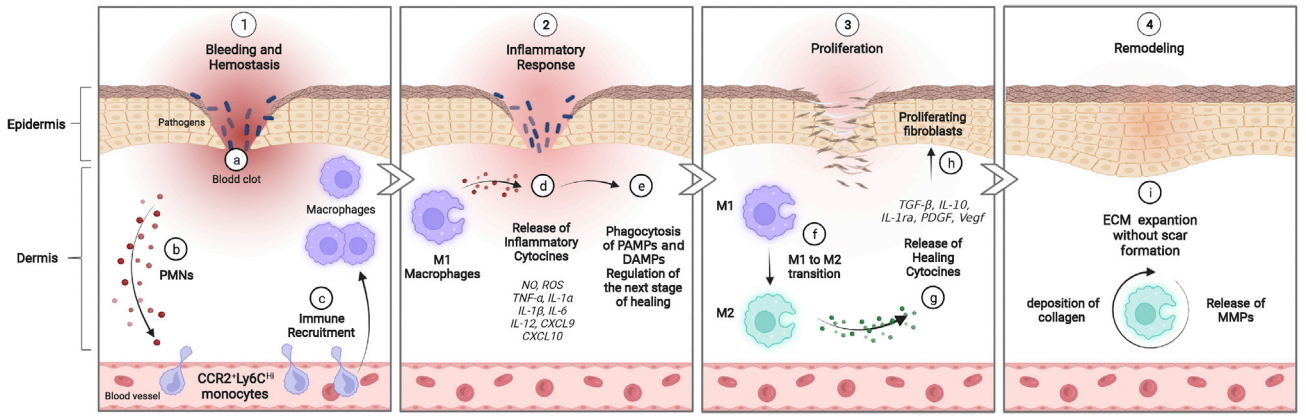


Figure 1. The role of macrophage cells in wound healing processes

(1) a–c: hemostasis phase. An initial influx of polymorphonuclear leukocytes (PMNs) in the blood clot is followed by successive waves of infiltrating monocytes that differentiate into macrophages in the wound. Infiltration of immune cells characterizes the beginning of the inflammatory phase. (2) d and e: during this phase, the differentiated M1 macrophages clear pathogens and cell debris, like pathogen-associated modifying proteins (PAMPs) and damage-associated modifying proteins (DAMPs), and release nitric oxide (NO), reactive oxygen species (ROS), and pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1α (IL-1α, IL-1β, IL-6, IL-12, CXCL9, and CXCL10, to prepare the wound bed for the next stage. (3) f–h: during the proliferation phase, the macrophage phenotype changes from an inflammatory to a healing state, releasing healing cytokines, such as transforming growth factor β (TGF-β), IL-10, IL-1 receptor antagonist (IL-1ra), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and vascular endothelial growth factor (Vegf). Proliferation, differentiation, and migration of keratinocytes, fibroblasts, and endothelial cells also take place. (4) i: the remodeling phase is the last stage of wound healing, in which macrophages still play a key role by releasing matrix metalloproteinases (MMPs) to the deposited ECM to restore tissue strength with minimal scar formation. Created with BioRender.

healing.[21,22] A particular case is chronic wounds, which are defined as wounds stalled in a constant and excessive inflammatory state with increased numbers of M1 macrophages, inactivated growth factors, increased oxidative damage, and deficient local angiogenesis, which prevents the normal tissue repair program to be executed.[23,24] For example, the hyperglycemic environment in diabetic wounds leads to high reactive oxygen species (ROS) levels and stimulation of the macrophages to secrete pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and IL-6, which encourages the vicious cycle of maintaining M1 macrophage polarization and chronic inflammation.

Because of a better understanding of the crucial role of macrophages in the different phases of the wound healing process, shifting the macrophage phenotype from the pro-inflammatory M1 to the pro-healing M2 phenotype has become an active area of research to promote wound healing[25] and diabetic wound healing in particular.[26,27] The objective of this review is to discuss the current macrophage-based therapeutic strategies in diabetic wound healing. We first go more deeply into the origin of skin macrophages and their plasticity, flexibility, and heterogeneity under physiological and diabetic wound conditions. Then we provide an overview of current developments of wound healing strategies controlling macrophage function or phenotype to accelerate wound healing.

ORIGIN AND ROLE OF MACROPHAGES IN SKIN WOUNDS

Macrophages in skin wounds originate from two primary sources: tissue-resident macrophages (Langerhans cells and dermal macrophages), stemming from yolk sac hematopoietic tissue in the uterus,[28] and macrophages that are generated from blood monocytes (recruited macrophages).[29] The major function of resident macrophages is maintenance of skin homeostasis and integrity.[30] The development of resident macrophages requires colony-stimulating factor 1 (CSF1; also known as macrophage colony-stimulating factor [M-CSF]).[31,32] After an injury, these cells are likely early responders, leading to subsequent recruitment of $CCR2^{+}Ly6C^{Hi}$ blood monocytes into the wound.[33–35] These recruited monocytes differentiate into $Ly6C^{Hi}F4/80^{+}$ macrophages in the inflammatory phase of wound healing and later switch to $Ly6C^{Lo}F4/80^{+}$ macrophages to accelerate wound healing.[33,36,37]

The first phase after injury is hemostasis, in which platelets accumulate at the site of tissue injury to form a blood plug.[8] The initial plug, which is stabilized by fibrin fibers, can act as a matrix for the various infiltrating cells.[3] The interactions of coagulant factors, along with activation of the endothelium, promote the initial invasion of immune cells, specifically PMNs. The arrival of these cells characterizes the beginning of the inflammatory phase (Figures 1A–1C).[9]

As mentioned previously, this rapid, initial influx of PMNs is followed by successive waves of infiltrating monocytes that differentiate within the wound into macrophages. During this step, to accommodate recruitment of a significant number of monocyte cells from the blood, there is an increase in myeloid-lineage-committed multipotent progenitors and monocytes in the bone marrow that results in a 70% increase in monocytes in the blood circulation on day 2.[38] After recruitment in the wound, these monocytes differentiate into activated M1

macrophages with an inflammatory phenotype.[39] These macrophages can recognize pathogen-associated modifying proteins (PAMPs) on the surfaces of bacteria or fungi and damage-associated modifying proteins (DAMPs), like extracellular DNA, RNA, and ATP, which are released because of cell death.[40,41] M1 macrophages produce nitric oxide (NO), ROS,[42] and pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-12 (Figures 1D and 1E), C-X-C motif chemokine ligand 9 (CXCL9), and CXCL10,[40] to clear pathogens and cell debris and stimulate proliferation of wound cells, such as fibroblasts and keratinocytes, to be able to regulate the next stage in the repair process.[43]

In vitro studies have shown that, after activation by lipopolysaccharide (LPS) and cytokines, like interferon gamma (IFN-γ) and TNF-α, macrophages become polarized into M1 macrophages, as characterized by Toll-like receptor (TLR)-2, TLR-4, CD80, CD86, inducible nitric oxide synthase (iNOS), and major histocompatibility complex (MHC) class II surface phenotypes. M1 macrophages release various cytokines and chemokines (such as TNF-α, IL-1α, IL-1β, IL-6, IL-12, CXCL9, and CXCL10) that exert positive feedback on unpolarized macrophages to change to the M1 state. Key transcription factors, such as nuclear factor κB (NF-κB), STAT1, STAT5, IRF3, and IRF5, have been shown to regulate expression of M1 genes.[42,44]

Transition from the inflammation phase to the proliferation phase is characterized by repolarization of M1 macrophages to the M2 phenotype.[3,45,46] The M2 phenotype is known to be a healing-associated macrophage with downregulated inflammatory factors and ROS levels and upregulated anti-inflammatory cytokines (e.g., TGF-β, IL-10, IL-1 receptor antagonist [IL-1ra], and the IL-1 type II decoy receptor), growth factors (e.g., platelet-derived growth factor [PDGF], epidermal growth factor, and vascular endothelial growth factor [Vegf]). The M2 phenotype promotes angiogenesis as well as proliferation, migration, and differentiation of fibroblasts, as required for tissue repair[47–49] (Figures 1F–1H). M2 macrophages are also involved in recruitment of stem cells to wounds, an important driving force in tissue repair.[50] M2 polarization occurs in response to downstream signals of cytokines, such as IL-4, IL-13, IL-10, IL-33, and TGF-β.[51] M2 macrophages can be identified by their expression of surface markers, such as the mannitol receptor, CD206, CD163, CD209, FIZZ1, and Ym1/2, and up-regulation of cytokines and chemokines, such as IL-10, TGF-β, CCL1, CCL17, CCL18, CCL22, and CCL24.[42,44,52]

During the final remodeling phase, fibroblasts and other cells reconstitute the tissue matrix via deposition of collagen type III and, later on, collagen type I,[16] and the number of macrophages starts to decline, but the remaining macrophages still play a role by releasing matrix metalloproteinases (MMPs) (Figure 1I).[53–55] MMPs are major proteolytic enzymes involved in turnover of the extracellular matrix (ECM) during cutaneous wound repair and restoration of skin functionality with minimal scar formation.[56] Impaired ECM turnover results in an abnormal remodeling phase that leads to unregulated cell proliferation as well as impaired apoptosis, cell differentiation, and physiological functions of the skin with excessive scar formation.[57–59]

MACROPHAGE DYSREGULATION IN DIABETIC WOUNDS

In the early stages of repair, around 85% of macrophages in the wound have the M1 pro-inflammatory phenotype, which switches to around 80%–85% anti-inflammatory M2 macrophages by days 5–7.[60] Studies have shown that pre-injury depletion of macrophages in mouse models causes defects in re-epithelization, formation of granulation tissue, angiogenesis, wound cytokine production, and myofibroblast-associated wound contraction.[20,61,62] Macrophage depletion during formation of granulation tissue, about 3 days after injury, is also associated with vascularization defects, delay in wound closure, and granulation tissue maturation.[62] Another study of isolated macrophages during the wound healing process on days 5, 10, and 20 after injury in wild-type mice indicated that pro-inflammatory macrophages were detectable until day 5. These macrophages expressed high levels of IL-1β, MMP-9, and NO synthase (NOS). By day 10, the expression of these pro-inflammatory factors decreased, concurrent with an increase in expression of the anti-inflammatory markers CD206 and CD36 and growth factors insulin growth factor (IGF)-1, TGF-β, and Vegf with efficient wound repair. Wound closure was completed after 20 days, when elevated expression for CD206, CD36, and TGF-β was still detectable, but not of IGF-1 or Vegf.[63]

More and more evidence is accumulating, and the pivotal role of macrophage phenotype switching is emerging as a key process to achieve successful wound healing. Any perturbation of this process could result in stalling of the repair process in the inflammatory phase, leading to a delay in wound closure and increased risk of infection.[23] Pathological conditions that can induce such perturbations include aging, obesity, infection, and diabetes.

Diabetes is a major and increasing health problem that leads to hyperglycemia as a consequence of cellular insulin resistance and insulin deficiency.[64] An elevated blood glucose level has a significant effect on the immune system and could lead to alterations in the number and phenotype of wound-associated macrophages. It has been reported that the diabetic microenvironment leads to increased myelopoiesis in hematopoietic stem cells, which results in elevated levels of circulating blood monocytes ($Ly6C^{Hi}$), which enter wounds and differentiate into macrophages with characteristic M1-like signatures (IL-1β, TNF-α).[65] This finding was supported by a recent study reporting that the number of infiltrating monocytes is higher in wounds of type 2 diabetic (T2D) mice compared with healthy mice[22] (Figure 2A). In addition, it was found that wound-derived macrophages in diabetic mice showed a lack of phenotypic switching to the M2 phenotype. The hyperglycemic condition in diabetic wounds decreases the inflammation-resolving functions of macrophages, such as their phagocytic activity (Figure 2B). Phagocytosis plays a role in the switch of the wound macrophage phenotype from M1 to pro-healing M2 (Figure 2C) and in release of IL-10, TGF-β, and all pro-healing factors.[60,63] Increased pro-inflammatory signals in diabetic wounds amplify the pro-inflammatory effect of M1 macrophages, which continue to release

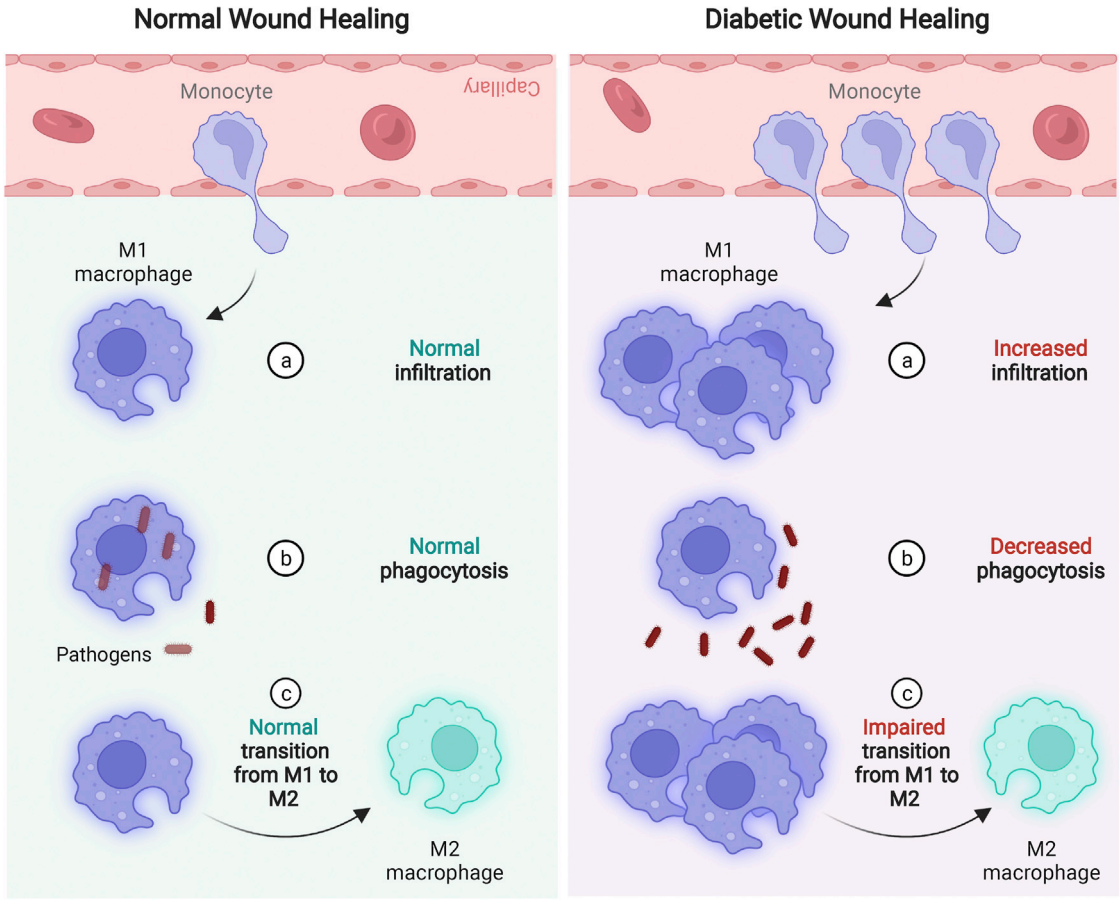


Figure 2. Macrophage dysregulation in diabetic wounds leads to delayed wound healing

Compared with normal wounds, increased numbers of infiltrated BM-produced monocytes are present in diabetic wounds, which, in turn, leads to a higher number of macrophages (a). The capability of M1 macrophages to phagocytose pathogens is decreased in diabetic wounds (b). Macrophage transition from M1- to M2-like phenotypes is impaired in diabetic wounds, leading to an accumulation of inflammatory M1 macrophages (c). M1, macrophage with a pro-inflammatory phenotype; M2, macrophage with a pro-healing phenotype. Created with BioRender.

inflammatory cytokines, proteases, and ROS, leading to degradation of the ECM. This phenotypic imbalance of macrophages causes a persistent state of inflammation in the diabetic wound that hinders the pro-healing activity of endothelial cells, keratinocytes, and fibroblasts.[60]

Diabetic foot ulcers (DFUs) are a very severe and common complication in diabetic patients and are the reason for approximately 80% of lower limb amputations.[66] In spite of many advances in wound healing, such as using growth factors in wound dressings, hyperbaric oxygen therapy, and negative pressure, complete healing of DFUs remains challenging. The growing number of amputations because of DFUs underscores the importance of continued investigation in this field.[67,68] Strategies that target macrophages as key regulators of wound inflammation and healing are considered promising new therapeutic approaches. In the next sections, we discuss these different approaches in the context of wounds, in particular diabetic wounds.

MACROPHAGE-BASED THERAPIES

Given the role of macrophages in the progression of wound repair, macrophage-based therapies aim to inhibit inflammation or enhance pro-healing anti-inflammatory activities. The therapeutic strategies can be divided into 2 main categories: pharmacological approaches to influence macrophage phenotype or products directly in the wound, and transplantation of *ex-vivo*-activated macrophages. Both therapeutic strategies are discussed below.

Pharmacological approaches to influence macrophage phenotype or products

Pharmacological strategies to modulate the macrophage phenotype or products in wounds can be divided into the following general categories: inhibition of the activity or effects of inflammatory M1-like macrophages, M2 phenotype promoting strategies, and manipulation of macrophage phenotype. They are discussed below (Figure 3).

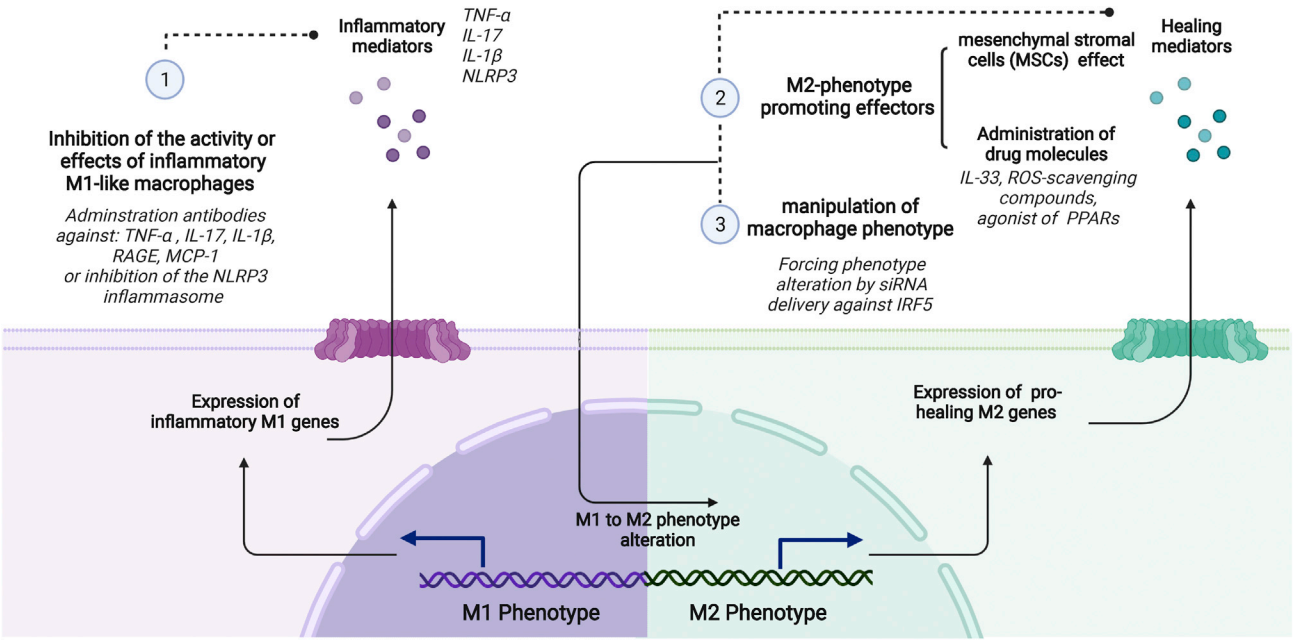


Figure 3. Pharmacological approaches to influence macrophage phenotype or activity

(1) Inhibition of the activity or effects of inflammatory M1-like macrophages can be achieved by administration of therapeutic antibodies that inhibit effects of inflammatory mediators like TNF-α, IL-1β, IL-17, advanced glycosylation end products (AGEs), and the monocyte chemoattractant protein 1 (MCP-1) or Nod-like receptor protein 3 (NLRP3) inflammasome. (2) M2 phenotype-promoting strategies include use of mesenchymal stromal cells (MSCs) to induce anti-inflammatory M2 like characteristics in macrophages or administration of drugs like IL-33, ROS-scavenging compounds, and agonist of peroxisome proliferator-activated receptors (PPARs) to promote the M2 phenotype. (3) Manipulation of macrophage phenotype can be achieved by delivery of siRNA against interferon regulatory factor 5 (IRF5), which enforces phenotype alteration. Created with BioRender.

Inhibition of the activity or effects of inflammatory M1-like macrophages

In all chronic wounds, despite their different etiologies, macrophages tend to get stuck in an M1-like phenotype, leading to increased levels of pro-inflammatory mediators like TNF-α.[69,70] This, in turn, leads to recruitment of more immune cells and also affects MMPs, leading to destruction of the ECM scaffold cells require to repopulate and close the wound.[71] It has been demonstrated that administration of therapeutic antibodies that inhibit TNF-α (e.g., infliximab topically or adalimumab subcutaneously) accelerates wound closure in a diabetic mouse model[64] and considerably improved wound repair in chronic venous ulcers (CVUs) of individuals whose wounds failed to respond to any previous conventional treatment.[69,72] In addition, using a secretory leukocyte protease inhibitor (SLPI)-deficient null mouse model with an imbalanced M1/M2 macrophage phenotype, it was found that neutralizing TNF-α at wound sites could accelerate the rate of healing macroscopically (Figure S1A, i and ii) and microscopically (Figure S1A, iii) by altering the balance between M1 and M2 macrophages on day 3 in a dose-dependent manner.[69]

There is compelling evidence that accumulation of advanced glycosylation end products (AGEs) in diabetic wounds, a common consequence of persistently elevated blood glucose, contribute significantly to the pathology associated with impaired wound healing.[73] It has been shown that M1 macrophages express high numbers of the receptor for AGEs (RAGE) under the diabetic condition and that AGE-RAGE signaling underlies the impaired phagocytosis of macrophages. As mentioned before, there is evidence to suggest that the switch from M1 to M2 phenotype may in part be regulated by phagocytosis of apoptotic cells.[25] *In vitro* work has shown that dysregulated phagocytosis by macrophages is followed by a decrease in expression of pro-healing factors, such as TGF-β1, which is believed to contribute to the observed subsequent disruption of macrophage phenotype switching.[74] The disrupted phenotype transition negatively affects wound repair through development of chronic inflammation.[75] Topical administration of anti-RAGE antibodies to full-thickness excisional wounds in diabetic mice results in an increase in phagocytosis of M1 macrophages, which has been associated with a gradual transition to the M2 phenotype and improved wound healing.[76]

In diabetic wounds, in which IL-1β, an inflammatory cytokine, is upregulated, it was found that inhibiting the IL-1β pathway with a neutralizing antibody induced the switch from pro-inflammatory to healing-associated macrophages and improved healing of these wounds.[77] Figure S1B, i, shows hematoxylin-and-eosin-stained cryosections of skin tissue on day 10 after injury. The wounds treated with antibodies against IL-1β significantly increased re-epithelialization and granulation tissue (gt) in IL-1β antibody (IL1ab)-treated samples compared with control immunoglobulin G (IgG)-treated samples

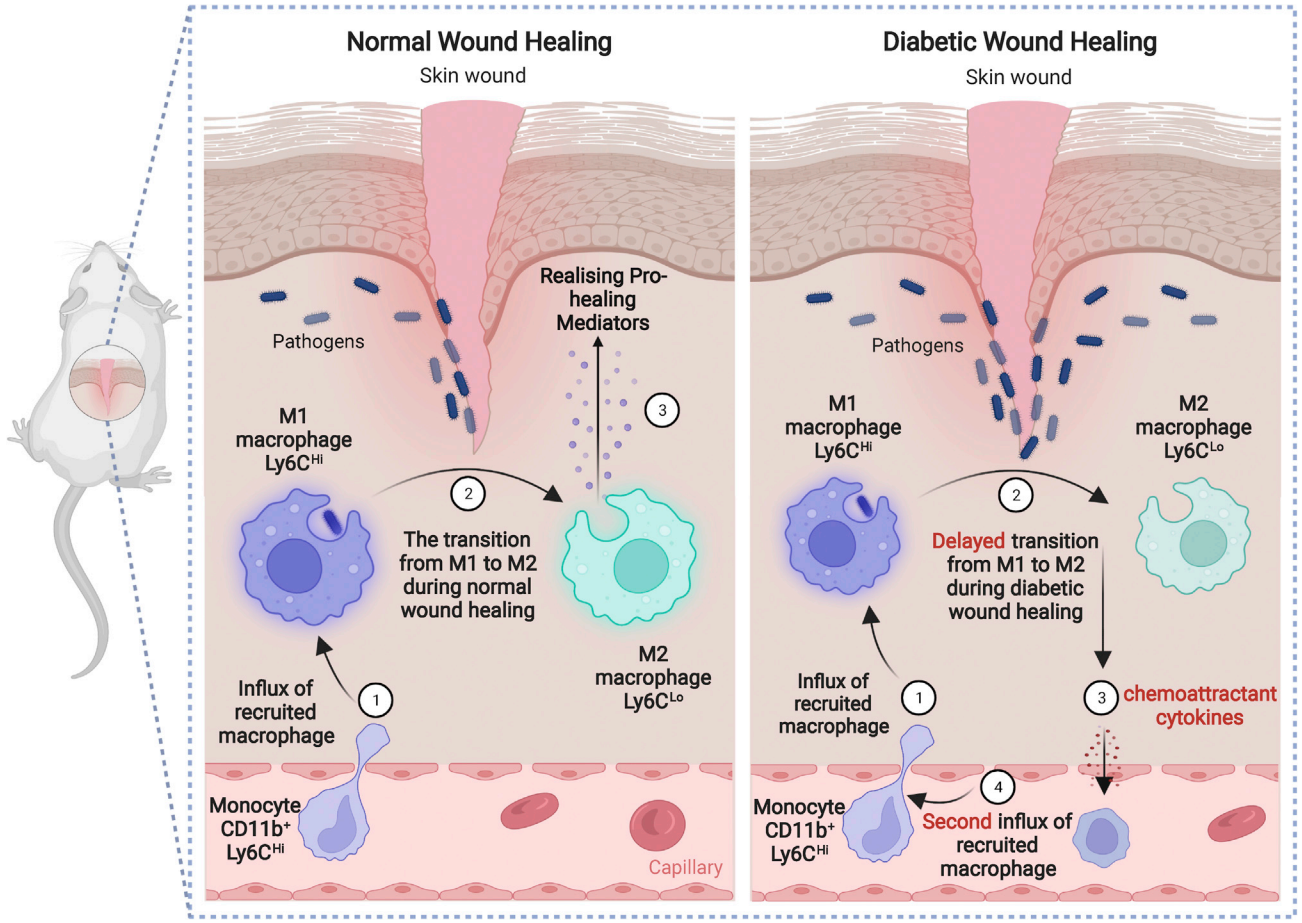


Figure 4. The influx of CD11b^{+}Ly6C^{Hi} monocytes/macrophages during wound healing under normal and diabetic conditions

During normal wound healing, monocyte-derived Ly6C^{Hi} macrophages are present within 48 h of injury and rapidly transition to Ly6C^{Lo} macrophages, releasing pro-healing mediators at the wound site. In diabetic wounds, transition of Ly6C^{Hi} to Ly6C^{Lo} macrophages is delayed, leading to a second influx of Ly6C^{Hi} monocyte/macrophages during the reparative phase. Created with BioRender.

(Figure S1B, ii and iii). Similarly, local administration of a blocking antibody against IL-17 on days 0, 1, and 2 upon wounding inhibited M1-like macrophage activity and significantly accelerated wound closure compared with the untreated group during 10 days of monitoring of diabetic mice.[78]

It has also been shown that overactivation of the Nod-like receptor protein 3 (NLRP3) inflammasome pathway in macrophages contributes to development of diabetic complications,[79] including delayed diabetic wound healing and severe cardiac dysfunction.[80,81] Therefore, inhibition of the NLRP3 inflammasome pathway was explored by local application of calcitriol (1,25-dihydroxyvitamin D3), which was proven to promote diabetic skin wound healing.[82,83] A later study indicated that topical application of calcitriol promotes corneal wound healing and reinnervation in diabetic mice by inhibiting activation of the NLRP3 inflammasome.[84] These data suggest that use of specific molecules that can inhibit the activity or effects of the pro-inflammatory M1 phenotype could be a rational approach to reduce excessive inflammation and expedite wound healing.

As explained earlier, infiltrating blood monocyte-derived M1 macrophages (Ly6C^{Hi}) are critical for the initial inflammatory phase and, under normal wound conditions, switch to Ly6C^{Lo} M2 cells. It was shown that, in diabetic wounds, not only is the Ly6C^{Hi}-to-Ly6C^{Lo} phenotype transition is delayed, but there is also a second influx of Ly6C^{Hi} monocytes/macrophages during the reparative phase that results in significantly higher numbers of M1 inflammatory macrophages and elevated levels of pro-inflammatory factors (Figure 4).[60] It was also found that the second wave of Ly6C^{Hi} cells in diabetic wounds corresponded to a spike in MCP-1 (monocyte chemoattractant protein-1). Intraperitoneal injection of anti-MCP-1 to block the second influx of Ly6C^{Hi} monocyte/macrophages in diabetic mice 24 h before the observed second wave of Ly6C^{Hi} monocyte/macrophages resulted in a significant improvement in wound healing compared with normal rabbit-serum-injected controls.[60] This study suggests

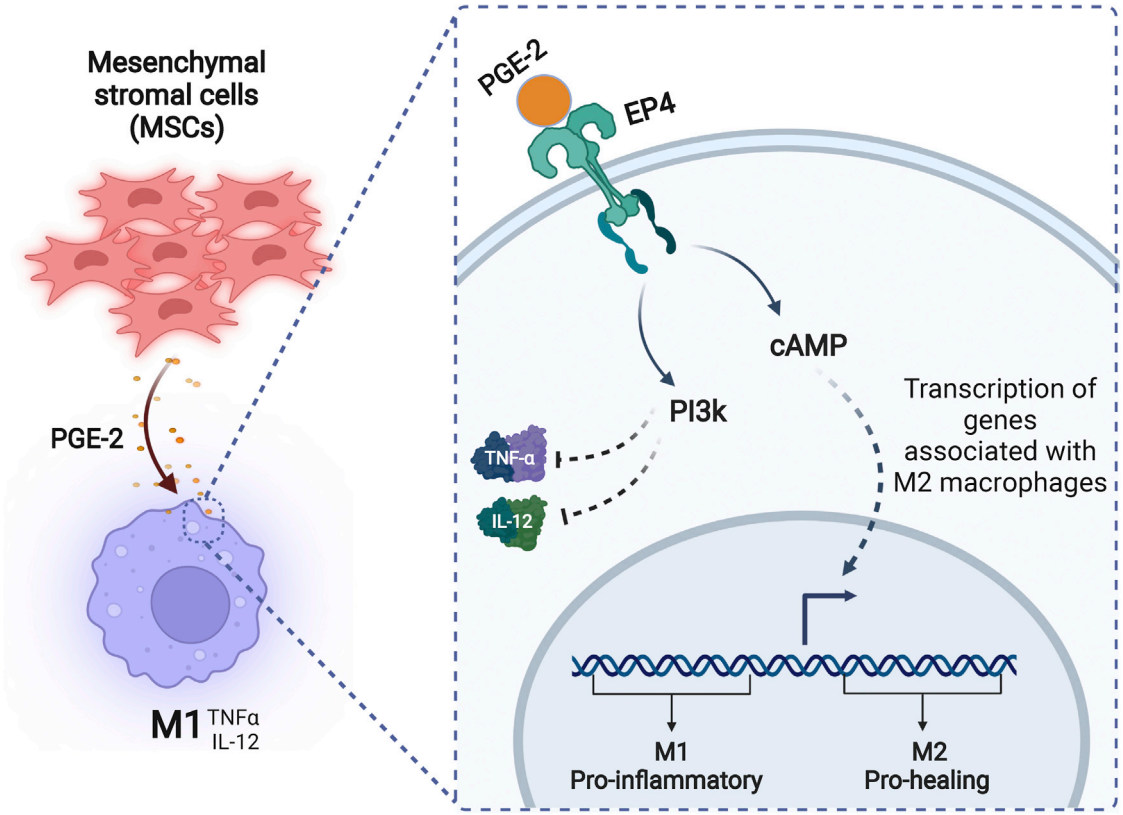


Figure 5. MSC-educated M2 macrophages

Prostaglandin E-2 (PGE-2), secreted from MSCs, can bind to EP4 receptors on M1 macrophages at the wound site and repolarize them to pro-healing M2 macrophages via two independent pathways: (1) the phosphatidylinositol 3-kinase (PI3K) pathway, which inhibits TNF-α and IL-12, and (2) the cyclic adenosine monophosphate (cAMP) pathway, which leads to activation of transcription of genes associated with the M2 macrophage phenotype. Created with BioRender.

that time-dependent inhibition of M1 macrophage accumulation may be a novel therapeutic strategy for impaired diabetic wound healing.

M2-promoting strategies

Besides inhibiting the consequences of M1 macrophage activity, it is also possible to induce a shift toward the M2 phenotype by administration of M2-promoting cells or effector molecules to enhance tissue repair. This approach is discussed next.

MSC-educated M2 macrophages. M2-promoting effector molecules can be secreted by other cell types that act as "M2 educators." Evidence has shown that mesenchymal stromal cells (MSCs), fibroblast-like cells originally isolated from bone marrow (BM), can secrete many growth factors with powerful modulating effects on macrophages with a direct effect on wound healing.[85] One *in vitro* study showed that, when cocultured with MSCs, macrophages acquired anti-inflammatory M2-like characteristics (low production of pro-inflammatory cytokines, such as IL-12 and TNF-α, and high production of pro-healing cytokines, such as IL-10).[86] It was found that MSCs secrete an important regulator, prostaglandin E-2 (PGE-2),[87,88] which is able to bind to the EP4 receptor on M1 macrophages and activate two independent pathways: (1) the phosphatidylinositol 3-kinase (PI3K) pathway, which inhibits pro-inflammatory cytokines such as TNF-α and IL12, and (2) the cyclic adenosine monophosphate (cAMP) pathway, which leads to transcription of genes that can induce up-regulation of M2-like markers (Figure 5).[89] These results were confirmed *in vivo* in a cutaneous wound model, showing that MSCs injected systemically into mice 1 day after full-thickness skin excision could concentrate at the wound site and promote the shift of M1 toward M2, resulting in better wound healing.[90]

Induction of M2 macrophages by the administration of drugs. ILs are a first example of therapeutic molecules that can be used to repolarize M1 macrophages into M2. For example, administration of IL-33, a member of the IL-1 family that potently drives production of T helper 2-associated cytokines, has shown beneficial effects in treatment of liver ischemia[91] and atherosclerosis.[92] It has been shown that intraperitoneal injection of murine recombinant IL-33 into mice subjected to incisional skin wounds once a day from day 0–3 can shift macrophages from M1 to M2, leading to accelerated collagen accumulation, wound re-epithelialization, and wound healing.[93]

IL-25, an IL-17 cytokine family member, has been shown to have an inhibitory effect on diabetic wounds, inhibiting the inflammatory

response and promoting tissue remodeling.[94] It has been reported that local intracutaneous injection of IL-25 into a full-thickness wound of diabetic mice can promote polarization of wound macrophages to M2 through the PI3K/Akt (Ak strain transforming) and mammalian target of rapamycin (mTOR) signaling pathways with a subsequent release of pro-healing cytokines at the wound site. The study showed that M2-polarized macrophages can accelerate the proliferation and migration behavior of fibroblasts in a paracrine manner.[95]

Another example of drug molecules used for macrophage repolarization are ROS-scavenging compounds. It has been shown that ROS production in the wound is usually associated with activation of M1 rather than M2 macrophages.[96] Therefore, administration of ROS-scavenging compounds, like natural or chemical antioxidants, at the wound site can promote wound closure by repolarizing macrophages to the M2 phenotype.[97] In another study, a type of ROS-scavenging hydrogel was developed, consisting of polyvinyl alcohol (PVA) cross-linked by a ROS-responsive linker.[98] The hydrogel acted as an effective ROS scavenger, promoting wound closure by decreasing ROS levels and up-regulating M2-phenotype macrophages.[98]

A final example of M2 promoting drug molecules are the natural agonists (e.g., omega-3 fatty acids) or synthetic agonists (e.g., clofibrate, fenofibrate, and bezafibrate) of the peroxisome proliferator-activated receptors (PPARs), consisting of three different isotypes: PPAR-α, PPAR-δ (PPAR-β), and PPAR-γ. They are interesting tools for pharmacological intervention to treat inflammatory conditions. PPAR-γ is a member of the superfamily of nuclear receptors,[99] and it functions as a ligand-activated transcription factor with pleiotropic effects on inflammation.[100] A study clarified the relationship between PPAR-γ activation and innate immune cells, such as macrophages.[101] The study suggested that macrophages are one of the main cellular targets for PPAR-γ activation and that its anti-inflammatory features are related to macrophage M2 polarization, reducing inflammation and promoting a return to homeostasis.[101] Previous work has shown that activated M2 macrophages express significant PPAR-γ levels[102] and that reducing its levels could result in overactivation of the immune system and elevated inflammatory responses.[103] Figure S2A shows delayed wound closing in PPARγ knockout (KO) mice in comparison with control wild-type (WT) mice during 12 days of monitoring after wounding.[104] Local administration of PPAR agonists reduced the oxidative wound microenvironment[105] and promoted a healing-associated macrophage phenotype, leading to improved wound healing.[106,107] Other studies support PPARβ/δ as a valuable pharmacologic wound healing target in macrophages. Figures S2B–S2F show the results of topical application of the polymer-encapsulated PPARβ/δ agonist GW501516 (GW) to full-thickness excisional splint wounds on the dorsal skin of diabetic mice.[105] Providing an earlier and sustained dose of GW to the wound by encapsulating GW in core-shell poly(L-lactide) and poly(D,L-lactide-co-glycolide) (PLLA:PLGA:GW) microparticles significantly reduced ROS levels close to those of WT wounds, whereas ROS levels were significantly higher in the control group treated with microparticles without encapsulated GW (PLLA:PLGA:veh [vehicle]) (Figure S2B). Treatment with PLLA:PLGA:GW accelerated diabetic wound closure (closed within 7 days) (Figures S2C and S2D), with longer and thicker wound epithelium formation (Figures S2E and S2F), suggesting that it is an appealing strategy for down-regulating inflammation and improving healing of chronic wounds.

Manipulation of macrophage phenotype

Analysis of the differential gene expression of M1 and M2 macrophages yielded a list of genes with mRNA levels differing greatly between both phenotypes.[108] A summary of molecular pathways involved in M1 and M2 macrophages is depicted in Figure 6. Of particular interest is the PI3K/AKT pathway, which mediates an inflammatory M1 type signal through tuberous sclerosis complex (TSC)/mTOR (tuberous sclerosis/mTOR) mediators.[109] Another frequent signaling pathway involved in the M1 phenotype is NOTCH1.[110] In response to inflammatory cues such as LPS, IFN-γ, or IL-1β, macrophages increase expression of NOTCH receptors that promotes M1 gene expression via complexation of intracellular domain (ICD) and recombination signal binding protein for immunoglobulin kappa J region (RBP-J) complex and recruitment of the mastermind-like (MAML) coactivator.[111,112] Pathogen recognition receptors (PRR) such as TLR are another signaling pathway that promotes inflammatory gene expression in macrophages via Janus-activated kinase 2 (Jak2) and NF-κB activation.[113] It was discovered that IFN regulatory factors (IRFs) are the intracellular signaling elements that determine gene transcription of macrophage cells in response to initial signal sensing by PRRs.[114] IRFs are responsible for mediating PRR signals to chromatin and for finally determining macrophage phenotype.[115] There are nine IRF family members: IRF1, IRF2, IRF3, IRF4, IRF5, IRF6, IRF7, IRF8/ICSBP (interferon consensus sequence binding protein), and IRF9.[116] Studies so far have shown that IRF5 and IRF8 are involved in promotion of M1 macrophage activation (M1), whereas IRF4 promotes inflammatory M2 phenotype activation.[116]

These identified genes form potential targets for alteration by gene editing tools. Arguably the most attractive approach to modify macrophage polarization is to reduce inflammatory gene expression through RNA interference (RNAi) because multiple genes can be downregulated simultaneously. Small interfering RNAs (siRNAs) are a class of short double-stranded RNA molecules (21–25 nt in length) that induce gene silencing by targeting complementary mRNA strands for degradation.[117] Unlike conventional drugs or small molecules, which have a limited range of protein targets, siRNA may be used to interfere with the expression of nearly any gene transcript in a specific manner. Despite the fact that siRNAs are capable of knocking down targets in various diseases, clinical use is hampered by the need for safe and effective delivery systems.[118,119] Naked siRNA double strands are relatively unstable in blood and serum, being prone to rapid degradation by endo- and exonucleases. Because of to its large molecular weight (13 kDa) and polyanionic nature (negative charges), a naked siRNA double strand does not freely cross the

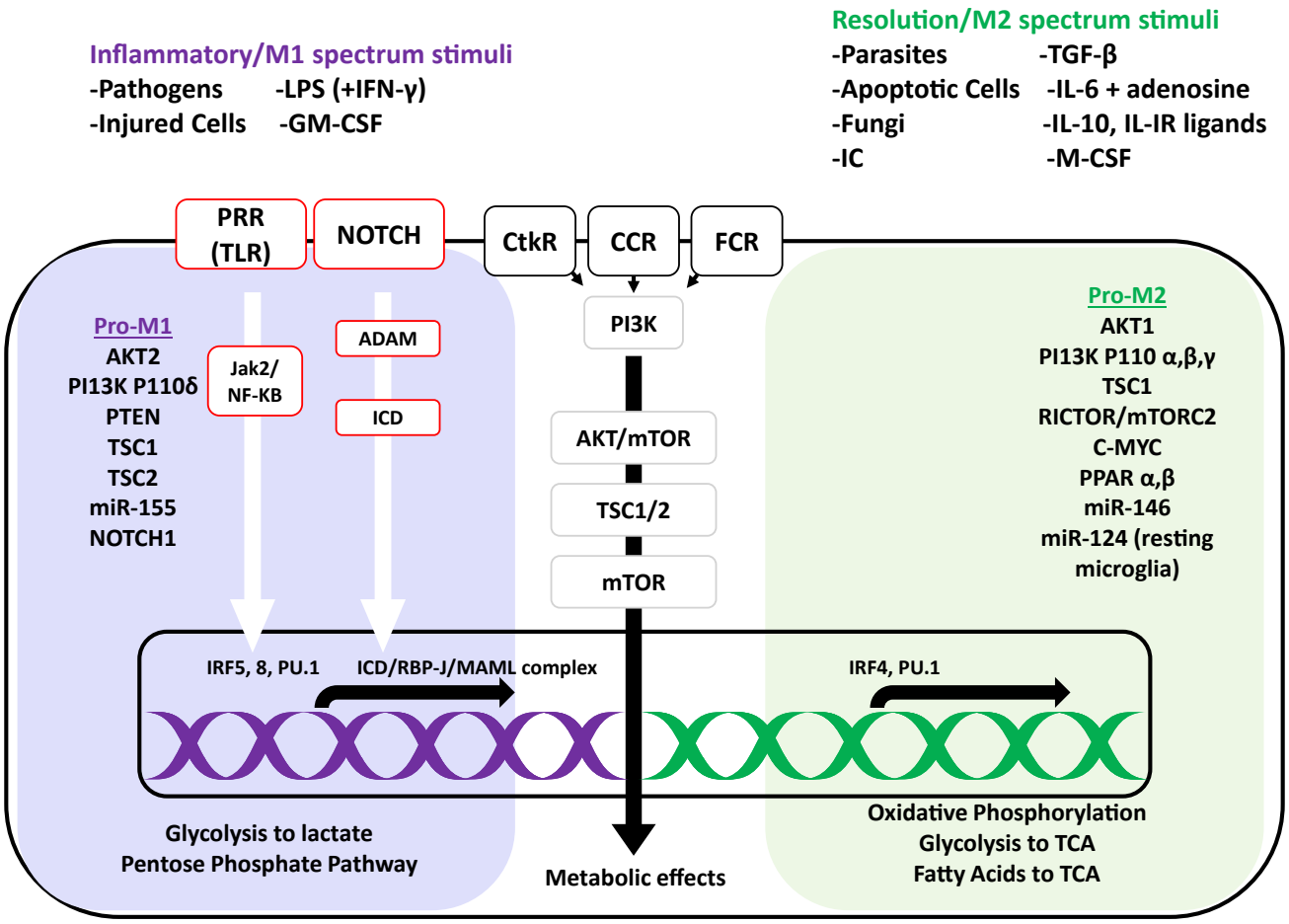


Figure 6. Key stimuli and molecular pathways in macrophages with the inflammatory (M1) versus healing phenotype (M2)

The M1 phenotype is activated by signals from pathogens, injured cells, and *in vitro* stimuli, and the M2 phenotype is activated by parasites, fungi, apoptotic cells, immune complexes, and other cytokine/growth factor stimuli. Signals that are sensed via pathogen-recognition receptors (PRRs), such as Toll-like receptors (TLRs), result in Janus-activated kinase (Jak) 2 and nuclear factor κB (NF-κB) activation. Signals received via Notch receptors, cytokine receptor (CtkR), chemokine receptor (CCR), and Fc receptor (FcR) stimulation also define gene expression and downstream metabolic reprogramming. IRF5 and IRF8 induce inflammatory M1 gene expression, whereas IRF 4 promotes M2 phenotype genes that, in turn, promote changes in nutrient uptake and metabolic pathways, depending on which part of the gene is expressed. LPS, lipopolysaccharide; GM-CSF, granulocyte monocyte colony-stimulating factor; IC, immune complex; M-CSF, monocyte colony-stimulating factor; PI3K, phosphatidylinositol 3-kinase; AKT, serine threonine kinase; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog; TSC, tuberous sclerosis complex; PPAR, peroxisome proliferator-activated receptor; ADAM, a disintegrin and metalloproteinase; RBP-J, recombination signal binding protein for immunoglobulin κJ region; MAML, mastermind-like; Rictor, rapamycin-insensitive companion of mTOR; TCA, tricarboxylic acid/Krebs cycle. Adapted from Amici et al.[113]

cell plasma membrane.[120] In this regard, specific formulations, such as non-viral delivery vectors, can contribute to enhanced siRNA delivery to target cells and improve siRNA stability.[121] For example, siRNA silencing of the genes responsible for production of TNF-α in macrophages was investigated to improve diabetic wound healing.[122–124] Chronic upregulation of TNF-α in DFUs leads to overproduced MCP-1/CCL2 in fibroblast cells, which helps with recruitment of additional macrophages to the wound bed and contributes to the positive feedback cycle of inflammation.[125]It has been demonstrated that *in vitro* silencing of TNF-α in macrophages co-cultured with fibroblasts (using lipidoid nanoparticles [LNPs] loaded with siTNF-α) leads to downregulation of MCP-1, putting an end to the paracrine feedback loop between TNF-α and MCP-1.[122] Later, it was shown that *in vivo* application of siTNF-α-loaded LNPs to diabetic wounds also reduces TNF-α expression to a similar level as in normoglycemic mice, reduces the inflammatory response, and reduces the wound area and healing time.[124] In addition, siRNA silencing of MMP-9 was tried, another secreted factor that leads to breakdown of the local ECM and delayed wound healing.[126–132] A recent study used a collagen/glycosaminoglycan scaffold to deliver siMMP-9 in a DFU wound model.[132] MMP-9 gene expression levels in M1 macrophages could be efficiently reduced by 50% in the siMMP-9-treated group compared with the non-transfected group.[132]Topical administration of siMMP-9-loaded nanoparticles (NPs) around the wound bed of diabetic rats was investigated, showing a significant reduction in expression of MMP-9 and

Table 1. Reported studies on diabetic wound healing with siRNA targeted against mediators like; Keap1, MMP2, MMP9, TNF alpha, GM3s, PHD2, HIF-1, and IRF5

Target mRNA	Wound	Study model	Delivery system	Results	Reference
MMP-2	9-mm full-thickness skin wound on the dorsal area of STZ-induced diabetic mice	● *in vitro* ● *in vivo* Female C57BL/6 mice	MMP-2 siRNA was chemically tethered to the end of multi-armed PEG and subsequently clustered to submicron particles complexed with linear PEI. MMP-cleavable peptide was used as a linker between PEG and siRNA for stimulus-responsive release of siRNA depending on local MMP concentration.	● downregulated MMP-2 gene expression ● long-term MMP-2 gene silencing ● Accelerated wound closure	133
MMP-9	10-mm full-thickness skin wound on the dorsal area of STZ-induced diabetic rats	● *in vitro* ● *in vivo* Sprague-Dawley (SD) rats (6 weeks, male)	siMMP-9 complexed with Gly-triethylenetetramine (GT) and then loaded into a thermosensitive hydrogel dressing	● downregulated MMP-9 gene expression ● biocompatible hydrogel dressing ● long-term release of GT/siMMP-9 for up to 7 days ● enhanced diabetic wound healing	127
	10-mm full-thickness skin wound on the dorsal area of STZ-induced diabetic rats	● *in vitro* ● *in vivo* Sprague-Dawley (SD) rats (5–6 weeks, male)	MMP-9 siRNA loaded in wound dressing contains hyperbranched cationic polysaccharide (HCP) derivatives.	● downregulated MMP-9 gene expression ● biocompatible dressing ● enhanced diabetic wound healing	131
	10-mm full-thickness skin wound on the dorsal area of STZ-induced diabetic rats	● *in vitro* ● *in vivo* SD rats (male)	MMP-9 siRNA encapsulated in a dressing containing bacterial cellulose (BCP)-HCP	● downregulated MMP-9 gene expression ● long-term release of MMP-9 siRNA ● enhanced diabetic wound healing	130
	full-thickness skin wound on the dorsal area of STZ-induced diabetic rats	● *in vitro* ● *in vivo* SD rats	Star-branched cationic polymer β-CD-(D3)7, which consists of β-cyclodextrin and third-generation poly(amidoamine) (PAMAM) was used as a carrier to take MMP-9 siRNA.	● downregulated MMP-9 gene expression ● enhanced diabetic wound healing ● decreased infiltration of inflammatory cells around the local wound	128
	DFU-mimicking conditions	● *in vitro*	MMP-9 siRNA loaded in collagen/glycosaminoglycan (Col/GAG) scaffolds.	● downregulated MMP-9 gene expression in fibroblast and macrophage cells	132
Keap1	10-mm full-thickness skin wound on the dorsal area of diabetic mice from The Jackson Laboratory (Bar Harbor, ME).	● *in vitro* ● *in vivo* male diabetic Lepr $^{db/db}$ mice, aged 12 weeks	liposome and protein hybrid NP delivery system	● downregulated Keap1 gene expression ● increased transfection efficacy Keap1 siRNA ● accelerated diabetic tissue regeneration	134
TNF-α	co-culture model of fibroblast and macrophage cells	● *in vitro*	LNPs to deliver TNF-α siRNA	● downregulated TNF-α gene expression ● decreased production of monocyte chemotactant protein 1 (MCP-1/CCL2) to inhibit the recruitment of additional macrophages to the wound site	122
	8-mm full-thickness skin wound on the right and left flanks	● *in vitro* ● *in vivo* genetically diabetic BKS.Cg-Dock7^{m} +/+ Lepr ^{db}/J mice	siTNF-α-loaded LNPs prepared as a potential wound treatment to combat an overzealous immune response and facilitate wound closure in a diabetic mouse model	● downregulated TNF-α gene expression ● accelerated diabetic wound healing	124
PHD2	8 mm full-thickness skin wound on the dorsal area of STZ-induced diabetic rats	● *in vitro* ● *in vivo* SD rats	PHD2 siRNA loaded in a porous poly(thioketal-urethane) scaffold	● downregulated PHD2 gene expression ● accelerated diabetic wound healing ● growth of thicker layer of skin tissue	135
GM3S	6-mm full-thickness skin wound on the dorsal area of STZ-induced diabetic mice	● *in vitro* ● *in vivo* male C57BL/6 mice	GM3S siRNA loaded on gold NPs	● downregulated GM3S gene expression ● accelerated diabetic wound healing ● increased keratinocyte migration and proliferation	136

(*Continued on next page*)

Table 1. *Continued*

Target mRNA	Wound	Study model	Delivery system	Results	Reference
HIF-1	● scratch test	● *in vitro*	HIF-1 siRNA loaded in layer-by-layer (LBL) self-assembled loaded gold NPs with poly L-arginine (AuNP@PLA) in the outer layer	● downregulated HIF-1 gene expression ● upregulated pro-angiogenic pathways ● accelerated wound healing in an *in vitro* scratch assay	137
IRF5	● scratch test simulated inflammatory condition	● *in vitro*	IRF5 siRNA loaded in selenium-based LBL NCs with polyethyleneimine (PEI) as the final polymer layer	● downregulated IRF5 gene expression. ● decreased expression level of pro-inflammatory NOS-2 and TNF-α mRNA ● increased expression level of the healing mediator Arg-1 ● accelerated wound healing in an *in vitro* scratch assay	138

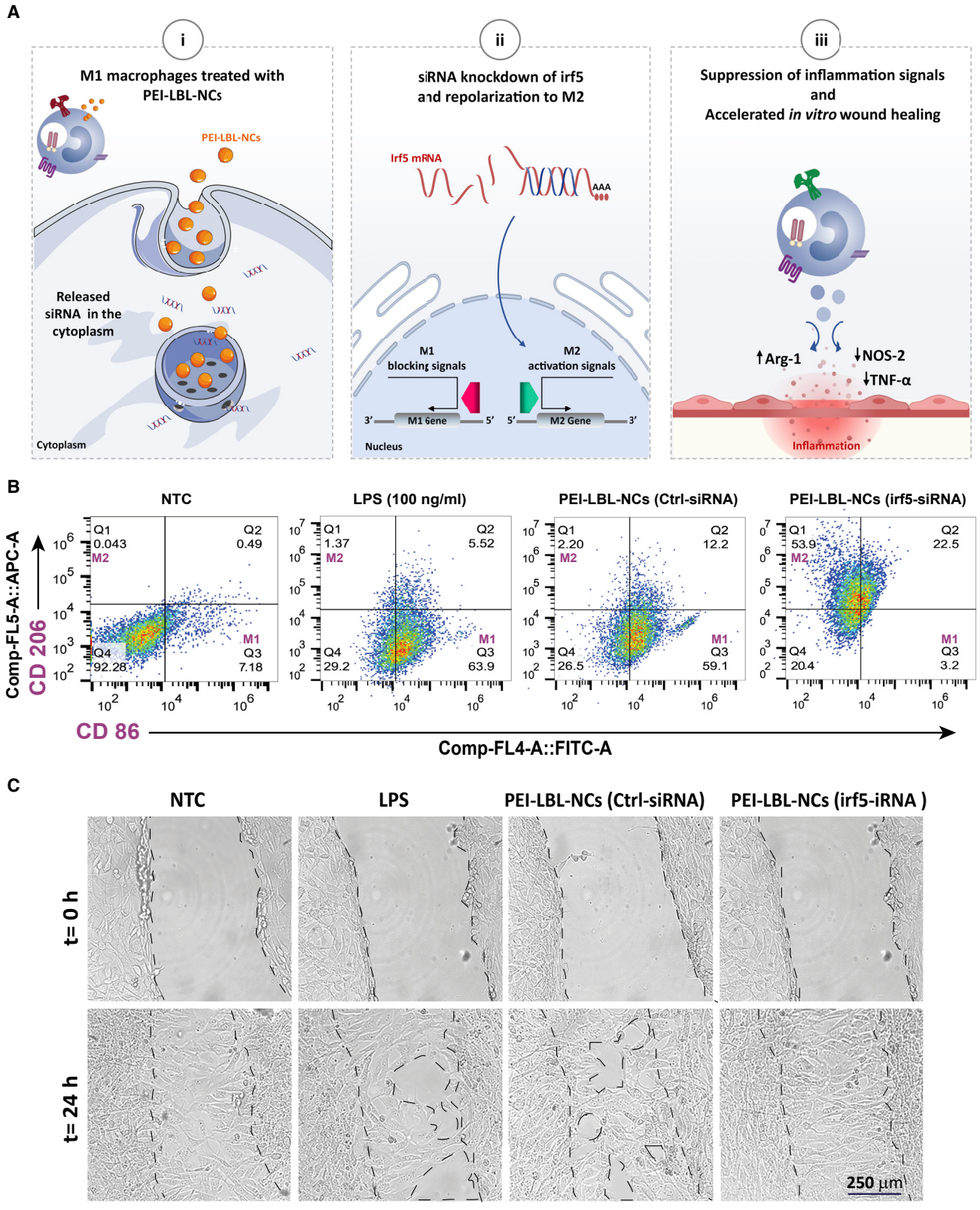
siRNA, small interfering RNA; STZ, streptozotocin or streptozocin; MMP, matrix metalloproteinase; PEG, polyethylene glycol; Keap1, kelch-like ECH-associated protein 1; TNF-α, tumor necrosis factor alpha; MCP-1, monocyte chemoattractant protein 1; CCL2, chemokine (C-C motif) ligand 2; PHD2, prolyl hydroxylase domain protein 2; GM3S, ganglioside-monosialic acid 3 synthase; HIF-1, hypoxia-inducible factor 1; IRF5, interferon regulatory factor 5.

improved wound healing.[126,128] However, because repeated injections were required to sustain long-term silencing of MMP-9, in a follow-up study, siMMP-9 was loaded in a wound dressing for sustained siRNA release.[130] A hybrid hydrogel dressing was developed for localized prolonged delivery of siMMP-9, achieving 65% release over 7 days.[127] A summary of the main studies of diabetic wound healing by siRNA silencing can be found in Table 1, from which it is apparent that NPs and scaffolds have mostly been used as delivery systems.

Recently, efforts to establish a more complete picture of the signaling pathways that define the final macrophage phenotype have uncovered the importance of the transcriptional regulator IRF5, which appears to have a central role in M1/M2 macrophage polarization.[139] Abnormal levels of IRF5 have been linked to several inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus.[140–142] This is because IRF5 is a transcription factor that can bind to the regulatory regions of inflammatory genes to modulate their expression and, therefore, regulate macrophage polarization. Krausgruber et al.[139] were the first to suggest that activation of IRF5 drives M1 macrophage polarization in such a way that IRF5 induces expression of 20 M1-specific genes and inhibits 19 M2-specific genes encoding cytokines, chemokines, costimulatory molecules, and surface receptors. This study indicated that IRF5 was indeed a major factor in defining macrophage polarization.[139] These insights led to exploration of RNAi silencing of IRF5 in macrophages as a therapeutic strategy to promote resolution of inflammation and improving healing after myocardial infarction.[143] siRNA targeting IRF5 (siIRF5) was formulated in LNPs and injected intravenously into atherosclerotic mice with myocardial infarction. The mice were injected daily for 4 days with 0.5 mg/kg siIRF5 or control siRNA (siCON). Treatment with siIRF5 resulted in efficient knockdown of IRF5 expression in macrophages and a subsequent decrease in expression of proinflammatory M1 markers, including TNF-α and IL-1β, without reducing M2 gene expression. Investigation of tissue biomarkers for infarct healing 7 days after myocardial infarction revealed a reduction in the number of neutrophils and macrophages, whereas neovascularization, the number of fibroblasts, and collagen deposition did not decline in mice treated with siIRF5.[143] There are also reports demonstrating that increasing M2 macrophages through IRF siRNA treatment attenuates neural injury and neuropathic pain after spinal cord injury (SCI).[144–146]

The effect of IRF5 silencing on skin wound healing was first demonstrated in excisional skin wounds.[143] Four days after creation of full-thickness skin wounds (6 mm in diameter), C57BL/6 mice intravenously received siIRF5 encapsulated in LNPs. The results indicated that injection of siIRF5 LNPs reduced IRF5 gene expression in macrophages isolated from the skin wounds in comparison with siCON. Wound closure was accelerated with decreased monocyte and neutrophil content accompanied by overall lower protease activity (Figures S3A–S3D). In addition, mRNA levels of TNF-α, IL-1β, and IL-6, of pro-inflammatory genes had decreased (Figure S3E). Another study found similar results in diabetic wounds, with a transition to the M2 phenotype after siIRF5 treatment. Thus, as expected, these changes in macrophage polarization were accompanied by increased expression of anti-inflammatory (CD206, CD163, IL-4, and IL-10) and reduced expression of inflammatory (CD11c, IL-1β, and TNF-α) markers.[147]

Recently, we explored M1-to-M2 macrophage repolarization using siIRF5 loaded in polyethyleneimine (PEI) layer-by-layer (LBL) nanocomplexes (NCs) (Figure 7A).[138] Reprogramming of M1 macrophages to the M2 phenotype was confirmed by a shift in CD206 (M2) and CD86 (M1) expression with a decrease in the M1 macrophage population to 3%, whereas the percentage of M2 cells increased from 1% to 54% after treatment (Figure 7B), consistent with the work performed by other groups showing that IRF5 ablation inhibits M1 macrophage polarization and suppresses progression of inflammation.[139,148,149] We demonstrated that the shift from the M1 to



(legend on next page)

M2 phenotype accelerated wound healing in an *in vitro* scratch assay on fibroblast cells that were incubated with conditioned cell medium isolated from transfected macrophages ($CD206^+$) (Figure 7C).[138]

In a recent study,[150] siIRF5 was encapsulated in fusogenic porous silicon NPs functionalized with the activated macrophage-targeting peptide CRV (F-siIRF5-CRV). It showed that effective depletion of the IRF5 gene in M1 macrophages (Figure S4A) could improve the therapeutic outcome of infected mice regardless of the bacterial strain and type. Mice with a methicillin-resistant *Staphylococcus aureus* (MRSA) muscle infection were used as a model for deep skin wound injuries. Although all treated groups had large abscess formations (purple) within the muscle tissue (pink), treatment with F-siIRF5-CRV resulted in only light and mild abscess formation on the right side of the tissue (Figure S4B). The majority of the section maintained a pink fibrous structure similar to that seen in the healthy control. The number of colony-forming units (CFUs) in the F-siIRF5-CRV treatment group was not statistically different from healthy controls (Figure S4C). The outcome of this study demonstrated that effective depletion of the IRF5 gene helped the immune system to focus on clearing of bacteria and minimizing auto-immune damage caused by excessive inflammation from M1 macrophages, improving the therapeutic outcome.[150,151] The versatility of this approach was also demonstrated using mice with antibiotic-resistant Gram-positive (MRSA) and Gram-negative (*Pseudomonas aeruginosa*) skin/muscle infections, both of which are major contributors to the morbidity and mortality of hospital-acquired infection after wounding.[152,153]

Delivery of *ex-vivo*-activated macrophages

Ex-vivo-activated macrophages as a cell therapy for treatment of cancers has been investigated extensively since the 1980s.[154] Isaiah Fidler is considered the founder of this approach because of his discovery of the anti-tumor effect of administration of *ex-vivo*-stimulated macrophages in a lung cancer mouse model,[155,156] which he later expanded to non-cancerous applications.[157] For example, one study that focused on local injection of polarized macrophages (M1) reported that, after administration, the cells promoted reperfusion recovery after femoral artery ligation in a mouse model because exogenous M1 macrophages decreased fibrosis and enhanced regeneration in lacerated muscles.[158]

It has been demonstrated in animal models that administration of *ex-vivo*-activated macrophages has anti-inflammatory functions and protect against Adriamycin-induced nephropathy in a murine model of chronic kidney injury.[159] In the case of bone regeneration, it has also been shown that local injection of *in-vitro*-activated macrophages can improve vascularization and healing in bone-related injury[160] and, in particular, that a combination of BM mononuclear cells (MNCs) and basic fibroblast growth factor (bFGF) gelatin hydrogel enhanced neovascularization and osteoinductive ability, resulting in bone regeneration. Delivery of activated M1 macrophages has also been reported for tissue healing applications. For example, it has been shown previously that administration of macrophages activated by hypo-osmotic shock promoted healing of pressure ulcers,[154] and positive effects were also reported for accelerated vascularization, tissue repair, and cardiac remodeling early after myocardial infarction.[161] The activated macrophages showed enhanced phagocytosis and augmented secretion of cytokines such as IL-1 and IL-6.[162]

A study has indicated that administration of autologous macrophages activated by TNF-α and IFN-γ (M1 phenotype) on day 1 after injury (non-chronic wound) can promote wound healing and normalize the defect in Vegf regulation associated with diabetes-induced skin repair disorders.[163] On the other hand, administration of *ex-vivo*-activated murine macrophages to M2-like phenotypes with IL-4 or IL-10 during the early inflammatory phase after wounding resulted in impaired skin healing and delayed re-epithelialization in a diabetic mouse model, pointing out the importance of the delicate balance in macrophage phenotypes that should be present in the different phases of the wound healing process.[164]

There are clear indications that delivery of activated M1 macrophages during the early inflammation phase allows positive therapeutic outcomes for tissue regeneration under normal conditions (non-chronic wounds). However, topical administration of *ex-vivo*-activated macrophages (M1 or M2) for treatment of mice with long-term dermal wounds (chronic wounds) is associated with increased wound inflammation.[165] This effect was tackled in a study that evaluated the influence of two murine macrophage subtypes on progression of chronic dermal wound healing. Macrophages were differentiated from embryonic stem cell-derived macrophages (ESDMs) and analyzed for their ability to function as non-inflammatory M2-like macrophages. ESDMs were compared with BM-derived macrophages (BMDM), which represented a more pro-inflammatory M1 macrophage subtype. The results of this study showed that ESDM- and BMDM-treated wounds displayed prolonged inflammation after administration instead of accelerating wound healing.[165] These results are also in line with the delayed wound closure observed in the genetically

Figure 7. Enforced M1-to-M2 macrophage repolarization using siIRF5-loaded polyethyleneimine (PEI) layer-by-layer (LBL) nanocomplexes (NCs)

(A) i: M1 macrophage treated by IRF5 siRNA loaded in PEI LBL (NCs. ii: reprogramming into a pro-healing phenotype by successful release of IRF5 siRNA in the cytoplasm. iii: macrophage phenotype change from the M1 to the M2 phenotype leads to faster wound healing in an *in vitro* scratch assay. (B) Representative flow cytometry results showing the $CD206^+$ (M2 marker) and $CD86^+$ (M1 marker) populations in LPS-activated RAW 264.7 cells after treatment with PEI LBL NCs loaded with IFR5 siRNA or Ctrl (control) siRNA. (C) For the *in vitro* wound healing assay, the proliferation of NIH-3T3 fibroblasts was monitored by transmission microscopy after treatment with conditioned cell culture medium (C-CCM) containing secreted factors from macrophage cells (RAW 264.7) stimulated with LPS and treated with PEI LBL NCs loaded with IRF5-siRNA or Ctrl siRNA. Transmission microscopy images show NIH 3T3cells before (0 h) and 24 h after treatment with C-CCM from LPS-stimulated RAW 264.7 cells and LPS-stimulated RAW 264.7 cells treated with PEI LBL NCs loaded with IRF5 siRNA or Ctrl siRNA, NTC, non-treated control. Adapted from Sharifiaghdam et al.[138]

diabetic mice, which was attributed to prolonged persistence of neutrophils and macrophages during the late phase of repair.[166]

Research and exploration with careful strategies that induce on-demand transitions of the local population of macrophage in the wound to generate the desired macrophage phenotype (less-inflammatory, pro-healing profile) could be more promising therapeutic approaches than administration of polarized macrophages, which can induce drastic perturbations in the wound microenvironment.

CONCLUSIONS AND OUTLOOK

Macrophages play a decisive role in the skin wound healing process. They do so through their plasticity, which allows them to shift their phenotype and, thus, their role according to environmental cues during the various stages of the healing process. Dysregulation of macrophage function in any of the wound healing steps, such as a reduced ability to switch from the inflammation-related M1 to the pro-healing M2 phenotype, leads to a delay in wound closure. This can even lead to severe consequences; for instance, in the case of DFUs with increased risk of amputation. A gradual better understanding of the factors that regulate macrophage phenotypes has led to new therapeutic interventions attempting to normalize macrophage responses in chronic inflamed wounds. In this review, macrophage-based therapeutic strategies were divided into two main categories:

1 pharmacological approaches to influence macrophages directly at the wound site.
2 transplantation of *ex-vivo*-activated macrophages.

Regarding the latter, transplantation of *ex-vivo*-activated M1 macrophages directly into wounds has been highlighted for acceleration of wound healing under normal conditions, but in the context of diabetic wounds, administration of polarized macrophages can induce drastic perturbations in the wound microenvironment and delay wound closure. The functional stability of transferred macrophages *in vivo* it is not guaranteed, and it has been reported that their phenotype can change after administration.[167] Therefore, although it is an intriguing new treatment modality, more in-depth studies of transferred macrophages over time are needed to better assess the feasibility of this approach.

Use of therapeutic molecules that can interfere with macrophage functionality directly at the wound site has certainly progressed. These molecules inhibit the activity of inflammatory M1-like macrophages and their effects or promote their transition to the M2 phenotype. Although administration of drug molecules leads to a reduction in inflammation and pro-healing wound conditions, their effect on the macrophage cell is not specific and could influence cells other than macrophages.[168] Recent advances in the field of macrophage-based therapy at the wound site have shown that genetically manipulating macrophage phenotype by siRNA delivery allows precise control of key mediators inducing switches in the phenotype of macrophages in a highly sequence-specific manner. Genetic modification of macrophages is challenging, particularly under chronic wound conditions like diabetic wounds, but the advent of siRNA delivery by novel nanotechnology-based strategies provides a solution for these delivery obstacles. Despite the increasing volume of studies that support this strategy and the demonstrated improvements in chronic wounds, clinical translation of the research performed so far will require development of wound dressings that allow sustained release and delivery of siRNA that have so far been successful in guiding wound macrophages to the pre -healing phenotype, increasing healing mediators and growth factors in addition to preventing and treating any infection that might occur in the wound.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ymthe.2022.07.016.

ACKNOWLEDGMENTS

The authors would like to acknowledge funding from the European Union's Horizon 2020 research and innovation program under grant agreements 810685 (DelNam project) and 648124 (NANOBUBBLE project). J.C.F. acknowledges the Flemish Research Foundation (FWO grants 1210120N and V407521N).

AUTHOR CONTRIBUTIONS

Conceptualization and writing – original draft, M.S.; writing – original draft, E.S.; supervision, R.F.-M. and S.D.S.; Writing – review & editing and supervision, K.B. and J.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Proksch, E., Brandner, J.M., and Jensen, J.M. (2008). The skin: an indispensable barrier. Exp. Dermatol. *17*, 1063–1072.
2. Nguyen, A.V., and Soulika, A.M. (2019). The dynamics of the skin's immune system. Int. J. Mol. Sci. *20*, 1811.
3. Rodero, M.P., and Khosrotehrani, K. (2010). Skin wound healing modulation by macrophages. Int. J. Clin. Exp. Pathol. *3*, 643–653.
4. Diegelmann, R.F., and Evans, M.C. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. Front. Biosci. *9*, 283–289.
5. Gurtner, G.C., Werner, S., Barrandon, Y., and Longaker, M.T. (2008). Wound repair and regeneration. Nature *453*, 314–321.
6. Martinez, F.O., and Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime Rep. *6*, 13.
7. Li, M., Hou, Q., Zhong, L., Zhao, Y., and Fu, X. (2021). Macrophage related chronic inflammation in non-healing wounds. Front. Immunol. *12*, 681710. https://doi.org/10.3389/fimmu.2021.681710.
8. Clark, R.A. (2003). Fibrin is a many splendored thing. J. Invest. Dermatol. *121*. xxi-xxii.
9. Vannella, K.M., Barron, L., Borthwick, L.A., Kindrachuk, K.N., Narasimhan, P.B., Hart, K.M., Thompson, R.W., White, S., Cheever, A.W., Ramalingam, T.R., and Wynn, T.A. (2014). Incomplete deletion of IL-4Rα by LysMCre reveals distinct subsets of M2 macrophages controlling inflammation and fibrosis in chronic schistosomiasis. PLoS Pathog. *10*, e1004372.
10. Raziyeva, K., Kim, Y., Zharkinbekov, Z., Kassymbek, K., Jimi, S., and Saparov, A. (2021). Immunology of acute and chronic wound healing. Biomolecules *11*, 700.

11. Kuraitis, D., Rosenthal, N., Boh, E., and McBurney, E. (2022). Macrophages in dermatology: pathogenic roles and targeted therapeutics. Arch. Dermatol. Res. *314*, 133–140.

12. Liu, C., Wu, C., Yang, Q., Gao, J., Li, L., Yang, D., and Luo, L. (2016). Macrophages mediate the repair of brain vascular rupture through direct physical adhesion and mechanical traction. Immunity *44*, 1162–1176.

13. Novak, M.L., and Koh, T.J. (2013). Phenotypic transitions of macrophages orchestrate tissue repair. Am. J. Pathol. *183*, 1352–1363.

14. Shechter, R., London, A., Varol, C., Raposo, C., Cusimano, M., Yovel, G., Rolls, A., Mack, M., Pluchino, S., Martino, G., et al. (2009). Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med. *6*, e1000113.

15. DiPietro, L.A. (2016). Angiogenesis and wound repair: when enough is enough. J. Leukoc. Biol. *100*, 979–984.

16. Sun, B.K., Siprashvili, Z., and Khavari, P.A. (2014). Advances in skin grafting and treatment of cutaneous wounds. Science *346*, 941–945.

17. Wynn, T.A., and Vannella, K.M. (2016). Macrophages in tissue repair, regeneration, and fibrosis. Immunity *44*, 450–462.

18. Olczyk, P., Mencner, Ł., and Komosinska-Vassev, K. (2014). The role of the extracellular matrix components in cutaneous wound healing. Biomed. Res. Int. *2014*, 747584.

19. Maquart, F.X., and Monboisse, J.C. (2014). Extracellular matrix and wound healing. Pathol. Biol. *62*, 91–95.

20. Goren, I., Allmann, N., Yogev, N., Schürmann, C., Linke, A., Holdener, M., Waisman, A., Pfeilschifter, J., and Frank, S. (2009). A transgenic mouse model of inducible macrophage depletion: effects of diphtheria toxin-driven lysozyme M-specific cell lineage ablation on wound inflammatory, angiogenic, and contractive processes. Am. J. Pathol. *175*, 132–147.

21. Vickers, N.J. (2017). Animal communication: when i'm calling you, will you answer too? Curr. Biol. *27*, R713–R715.

22. Barman, P.K., Urao, N., and Koh, T.J. (2019). Diabetes induces myeloid bias in bone marrow progenitors associated with enhanced wound macrophage accumulation and impaired healing. J. Pathol. *249*, 435–446.

23. Krzyszczyk, P., Schloss, R., Palmer, A., and Berthiaume, F. (2018). The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. Front. Physiol. *9*, 419.

24. Snyder, R.J., Driver, V., Fife, C.E., Lantis, J., Peirce, B., Serena, T., and Weir, D. (2011). Using a diagnostic tool to identify elevated protease activity levels in chronic and stalled wounds: a consensus panel discussion. Ostomy. Wound Manage. *57*, 36–46.

25. Khanna, S., Biswas, S., Shang, Y., Collard, E., Azad, A., Kauh, C., Bhasker, V., Gordillo, G.M., Sen, C.K., and Roy, S. (2010). Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One *5*, e9539.

26. Davies, L.C., and Taylor, P.R. (2015). Tissue-resident macrophages: then and now. Immunology *144*, 541–548.

27. Shah, J.M.Y., Omar, E., Pai, D.R., and Sood, S. (2012). Cellular events and biomarkers of wound healing. Indian J. Plast. Surg. *45*, 220–228.

28. Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., Prinz, M., Wu, B., Jacobsen, S.E.W., Pollard, J.W., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science *336*, 86–90.

29. Davies, L.C., Jenkins, S.J., Allen, J.E., and Taylor, P.R. (2013). Tissue-resident macrophages. Nat. Immunol. *14*, 986–995.

30. Mantovani, A., Biswas, S.K., Galdiero, M.R., Sica, A., and Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. J. Pathol. *229*, 176–185.

31. Chitu, V., and Stanley, E.R. (2006). Colony-stimulating factor-1 in immunity and inflammation. Curr. Opin. Immunol. *18*, 39–48.

32. Hume, D.A., and MacDonald, K.P.A. (2012). Therapeutic applications of macrophage colony-stimulating factor-1 (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling. Blood *119*, 1810–1820.

33. Minutti, C. M., Knipper, J. A., Allen, J. E. & Zaiss, D. M. (2016). Seminars in Cell & Developmental Biology. 3-11 (Elsevier).

34. Wang, Y., Szretter, K.J., Vermi, W., Gilfillan, S., Rossini, C., Cella, M., Barrow, A.D., Diamond, M.S., and Colonna, M. (2012). IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat. Immunol. *13*, 753–760.

35. Graubardt, N., Vugman, M., Mouhadeb, O., Caliari, G., Pasmanik-Chor, M., Reuveni, D., Zigmond, E., Brazowski, E., David, E., Chappell-Maor, L., et al. (2017). Ly6Chi monocytes and their macrophage descendants regulate neutrophil function and clearance in acetaminophen-induced liver injury. Front. Immunol. *8*, 626. https://doi.org/10.3389/fimmu.2017.00626.

36. Xu, Q., Choksi, S., Qu, J., Jang, J., Choe, M., Banfi, B., Engelhardt, J.F., and Liu, Z.G. (2016). NADPH oxidases are essential for macrophage differentiation. J. Biol. Chem. *291*, 20030–20041.

37. Crane, M.J., Daley, J.M., van Houtte, O., Brancato, S.K., Henry, W.L., Jr., and Albina, J.E. (2014). The monocyte to macrophage transition in the murine sterile wound. PLoS One *9*, e86660.

38. Barman, P.K., Pang, J., Urao, N., and Koh, T.J. (2019). Skin wounding–induced monocyte expansion in mice is not abrogated by IL-1 receptor 1 deficiency. J. Immunol. *202*, 2720–2727.

39. Murray, P.J., Allen, J.E., Biswas, S.K., Fisher, E.A., Gilroy, D.W., Goerdt, S., Gordon, S., Hamilton, J.A., Ivashkiv, L.B., Lawrence, T., et al. (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity *41*, 14–20.

40. Yu, H., Xiong, J., Qiu, J., He, X., Sheng, H., Dai, Q., Li, D., Xin, R., Jiang, L., Li, Q., et al. (2020). Type III secretion protein, PcrV, impairs Pseudomonas aeruginosa biofilm formation by increasing M1 macrophage-mediated anti-bacterial activities. Front. Microbiol. *11*, 1971.

41. Gallucci, S. (2016). An overview of the innate immune response to infectious and noninfectious stressors. In The Innate Immune Response to Noninfectious Stressors (Elsevier), pp. 1–24.

42. Yao, Y., Xu, X.-H., and Jin, L. (2019). Macrophage polarization in physiological and pathological pregnancy. Front. Immunol. *10*, 792.

43. Won, Y.-W., Patel, A.N., and Bull, D.A. (2014). Cell surface engineering to enhance mesenchymal stem cell migration toward an SDF-1 gradient. Biomaterials *35*, 5627–5635.

44. Wang, N., Liang, H., and Zen, K. (2014). Molecular mechanisms that influence the macrophage M1–M2 polarization balance. Front. Immunol. *5*, 614.

45. Jiang, H.R., Milovanović, M., Allan, D., Niedbala, W., Besnard, A.G., Fukada, S.Y., Alves-Filho, J.C., Togbe, D., Goodyear, C.S., Linington, C., et al. (2012). IL-33 attenuates EAE by suppressing IL-17 and IFN-γ production and inducing alternatively activated macrophages. Eur. J. Immunol. *42*, 1804–1814.

46. Daley, J.M., Brancato, S.K., Thomay, A.A., Reichner, J.S., and Albina, J.E. (2010). The phenotype of murine wound macrophages. J. Leukoc. Biol. *87*, 59–67.

47. Livingston, B., Crimi, C., Newman, M., Higashimoto, Y., Appella, E., Sidney, J., and Sette, A. (2002). A rational strategy to design multiepitope immunogens based on multiple Th lymphocyte epitopes. J. Immunol. *168*, 5499–5506.

48. Peng, H., Xian, D., Liu, J., Pan, S., Tang, R., and Zhong, J. (2020). Regulating the polarization of macrophages: a promising approach to vascular dermatosis. J. Immunol. Res. *2020*, 8148272.

49. Gan, J., Liu, C., Li, H., Wang, S., Wang, Z., Kang, Z., Huang, Z., Zhang, J., Wang, C., Lv, D., and Dong, L. (2019). Accelerated wound healing in diabetes by reprogramming the macrophages with particle-induced clustering of the mannose receptors. Biomaterials *219*, 119340.

50. Fuchs, E., and Blau, H.M. (2020). Tissue stem cells: architects of their niches. Cell Stem Cell *27*, 532–556.

51. Porta, C., Riboldi, E., Ippolito, A. & Sica, A. (2015). Seminars in Immunology. 237-248 (Elsevier).

52. Gordon, S., and Martinez, F.O. (2010). Alternative activation of macrophages: mechanism and functions. Immunity *32*, 593–604.

53. Ogle, M.E., Segar, C.E., Sridhar, S., and Botchwey, E.A. (2016). Monocytes and macrophages in tissue repair: implications for immunoregenerative biomaterial design. Exp. Biol. Med. *241*, 1084–1097.

54. Vannella, K.M., and Wynn, T.A. (2017). Mechanisms of organ injury and repair by macrophages. Annu. Rev. Physiol. *79*, 593–617.

55. Cui, N., Hu, M., and Khalil, R.A. (2017). Biochemical and biological attributes of matrix metalloproteinases. Prog. Mol. Biol. Transl. Sci. *147*, 1–73.

56. Delavary, B.M., van der Veer, W.M., van Egmond, M., Niessen, F.B., and Beelen, R.H. (2011). Niessen FB Beelen RHJ Macrophages in skin injury and repair. Immunobiology *216*, 753–762.

57. Potekaev, N.N., Borzykh, O.B., Medvedev, G.V., Pushkin, D.V., Petrova, M.M., Petrov, A.V., Dmitrenko, D.V., Karpova, E.I., Demina, O.M., and Shnayder, N.A. (2021). The role of extracellular matrix in skin wound healing. J. Clin. Med. *10*, 5947.

58. Nissinen, L.M., and Kähäri, V.M. (2015). Collagen turnover in wound repair––A macrophage connection. J. Invest. Dermatol. *135*, 2350–2352.

59. Xue, M., and Jackson, C.J. (2015). Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv. Wound Care *4*, 119–136.

60. Aitcheson, S.M., Frentiu, F.D., Hurn, S.E., Edwards, K., and Murray, R.Z. (2021). Skin wound healing: normal macrophage function and macrophage dysfunction in diabetic wounds. Molecules *26*, 4917.

61. Mirza, R., DiPietro, L.A., and Koh, T.J. (2009). Selective and specific macrophage ablation is detrimental to wound healing in mice. Am. J. Pathol. *175*, 2454–2462.

62. Lucas, T., Waisman, A., Ranjan, R., Roes, J., Krieg, T., Müller, W., Roers, A., and Eming, S.A. (2010). Differential roles of macrophages in diverse phases of skin repair. J. Immunol. *184*, 3964–3977.

63. Mirza, R., and Koh, T.J. (2011). Dysregulation of monocyte/macrophage phenotype in wounds of diabetic mice. Cytokine *56*, 256–264.

64. Huang, S.-M., Wu, C.S., Chiu, M.H., Wu, C.H., Chang, Y.T., Chen, G.S., and Lan, C.C.E. (2019). High glucose environment induces M1 macrophage polarization that impairs keratinocyte migration via TNF-α: an important mechanism to delay the diabetic wound healing. J. Dermatol. Sci. *96*, 159–167.

65. Kimball, A., Schaller, M., Joshi, A., Davis, F.M., denDekker, A., Boniakowski, A., Bermick, J., Obi, A., Moore, B., Henke, P.K., et al. (2018). Ly6CHi blood monocyte/macrophage drive chronic inflammation and impair wound healing in diabetes mellitus. Arterioscler. Thromb. Vasc. Biol. *38*, 1102–1114.

66. Geiss, L.S., Li, Y., Hora, I., Albright, A., Rolka, D., and Gregg, E.W. (2019). Resurgence of diabetes-related nontraumatic lower-extremity amputation in the young and middle-aged adult US population. Diabetes Care *42*, 50–54.

67. Davidson, M.B., Raskin, P., Tanenberg, R.J., Vlajnic, A., and Hollander, P. (2011). A stepwise approach to insulin therapy in patients with type 2 diabetes mellitus and basal insulin treatment failure. Endocr. Pract. *17*, 395–403.

68. Bus, S.A., Van Netten, J.J., Hinchliffe, R.J., Apelqvist, J., Lipsky, B.A., and Schaper, N.C.; IWGDF Editorial Board (2020). Standards for the development and methodology of the 2019 international working group on the diabetic foot guidelines. Diabetes Metab. Res. Rev. *36*, e3267.

69. Ashcroft, G.S., Jeong, M.J., Ashworth, J.J., Hardman, M., Jin, W., Moutsopoulos, N., Wild, T., McCartney-Francis, N., Sim, D., McGrady, G., et al. (2012). Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. Wound Repair Regen. *20*, 38–49.

70. Brüünsgaard, H., and Pedersen, B.K. (2003). Age-related inflammatory cytokines and disease. Immunol. Allergy Clin. North Am. *23*, 15–39.

71. Subramaniam, K., Pech, C.M., Stacey, M.C., and Wallace, H.J. (2008). Induction of MMP-1, MMP-3 and TIMP-1 in normal dermal fibroblasts by chronic venous leg ulcer wound fluid. Int. Wound J. *5*, 79–86.

72. Streit, M., Beleznay, Z., and Braathen, L.R. (2006). Topical application of the tumour necrosis factor-α antibody infliximab improves healing of chronic wounds. Int. Wound J. *3*, 171–179.

73. Van Putte, L., De Schrijver, S., and Moortgat, P. (2016). The effects of advanced glycation end products (AGEs) on dermal wound healing and scar formation: a systematic review. Scars Burn. Heal. *2*, 2059513116676828.

74. Wicks, K., Torbica, T. & Mace, K. A. (2014). Seminars in Immunology. 341-353 (Elsevier).

75. Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., van Rooijen, N., Plonquet, A., Gherardi, R.K., and Chazaud, B. (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J. Exp. Med. *204*, 1057–1069.

76. Wang, Q., Zhu, G., Cao, X., Dong, J., Song, F., and Niu, Y. (2017). Blocking AGE-RAGE signaling improved functional disorders of macrophages in diabetic wound. J. Diabetes Res. 1428537.

77. Mirza, R.E., Fang, M.M., Ennis, W.J., and Koh, T.J. (2013). Blocking interleukin-1β induces a healing-associated wound macrophage phenotype and improves healing in type 2 diabetes. Diabetes *62*, 2579–2587.

78. Rodero, M.P., Hodgson, S.S., Hollier, B., Combadiere, C., and Khosrotehrani, K. (2013). Reduced Il17a expression distinguishes a Ly6cloMHCIIhi macrophage population promoting wound healing. J. Invest. Dermatol. *133*, 783–792.

79. Yu, Z.-W., Zhang, J., Li, X., Wang, Y., Fu, Y.H., and Gao, X.Y. (2020). A new research hot spot: the role of NLRP3 inflammasome activation, a key step in pyroptosis, in diabetes and diabetic complications. Life Sci. *240*, 117138.

80. Liu, D., Yang, P., Gao, M., Yu, T., Shi, Y., Zhang, M., Yao, M., Liu, Y., and Zhang, X. (2019). NLRP3 activation induced by neutrophil extracellular traps sustains inflammatory response in the diabetic wound. Clin. Sci. *133*, 565–582.

81. Lin, H.-B., Wei, G.S., Li, F.X., Guo, W.J., Hong, P., Weng, Y.Q., Zhang, Q.Q., Xu, S.Y., Liang, W.B., You, Z.J., and Zhang, H.F. (2020). Macrophage–NLRP3 inflammasome activation exacerbates cardiac dysfunction after ischemic stroke in a mouse model of diabetes. Neurosci. Bull. *36*, 1035–1045.

82. Yuan, Y., Das, S., and Li, M. (2018). Vitamin D ameliorates impaired wound healing in streptozotocin-induced diabetic mice by suppressing NF-κB-mediated inflammatory genes. Biosci. Rep. *38*.

83. Yuan, Y.F., Das, S.K., and Li, M.Q. (2018). Vitamin D ameliorates impaired wound healing in streptozotocin-induced diabetic mice by suppressing endoplasmic reticulum stress. J. Diabetes Res. 1757925.

84. Wang, Y., Wan, L., Zhang, Z., Li, J., Qu, M., and Zhou, Q. (2021). Topical calcitriol application promotes diabetic corneal wound healing and reinnervation through inhibiting NLRP3 inflammasome activation. Exp. Eye Res. *209*, 108668.

85. Nuschke, A. (2014). Activity of mesenchymal stem cells in therapies for chronic skin wound healing. Organogenesis *10*, 29–37.

86. Kim, J., and Hematti, P. (2009). Mesenchymal stem cell–educated macrophages: a novel type of alternatively activated macrophages. Exp. Hematol. *37*, 1445–1453.

87. Németh, K., Leelahavanichkul, A., Yuen, P.S.T., Mayer, B., Parmelee, A., Doi, K., Robey, P.G., Leelahavanichkul, K., Koller, B.H., Brown, J.M., et al. (2009). Bone marrow stromal cells attenuate sepsis via prostaglandin E 2–dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat. Med. *15*, 42–49.

88. Zhang, S., Liu, Y., Zhang, X., Zhu, D., Qi, X., Cao, X., Fang, Y., Che, Y., Han, Z.C., He, Z.X., et al. (2018). Prostaglandin E2 hydrogel improves cutaneous wound healing via M2 macrophages polarization. Theranostics *8*, 5348–5361.

89. Barminko, J.A., Nativ, N.I., Schloss, R., and Yarmush, M.L. (2014). Fractional factorial design to investigate stromal cell regulation of macrophage plasticity. Biotechnol. Bioeng. *111*, 2239–2251.

90. Zhang, Q.Z., Su, W.R., Shi, S.H., Wilder-Smith, P., Xiang, A.P., Wong, A., Nguyen, A.L., Kwon, C.W., and Le, A.D. (2010). Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. Stem Cells *28*, 1856–1868.

91. Sakai, N., Van Sweringen, H.L., Quillin, R.C., Schuster, R., Blanchard, J., Burns, J.M., Tevar, A.D., Edwards, M.J., and Lentsch, A.B. (2012). Interleukin-33 Is hepatoprotective during liver ischemia/reperfusion in mice. Hepatology *56*, 1468–1478.

92. Miller, A.M., Xu, D., Asquith, D.L., Denby, L., Li, Y., Sattar, N., Baker, A.H., McInnes, I.B., and Liew, F.Y. (2008). IL-33 reduces the development of atherosclerosis. J. Exp. Med. *205*, 339–346.

93. Yin, H., Li, X., Hu, S., Liu, T., Yuan, B., Gu, H., Ni, Q., Zhang, X., and Zheng, F. (2013). IL-33 accelerates cutaneous wound healing involved in upregulation of alternatively activated macrophages. Mol. Immunol. *56*, 347–353.

94. Shao, S., He, F., Yang, Y., Yuan, G., Zhang, M., and Yu, X. (2012). Th17 cells in type 1 diabetes. Cell. Immunol. *280*, 16–21.

95. Li, S., Ding, X., Zhang, H., Ding, Y., and Tan, Q. (2022). IL-25 improves diabetic wound healing through stimulating M2 macrophage polarization and fibroblast activation. Int. Immunopharmacol. *106*, 108605.

96. Covarrubias, A., Byles, V., and Horng, T. (2013). ROS sets the stage for macrophage differentiation. Cell Res. *23*, 984–985.

97. Lurier, E., Levy, R., Barbee, K., Golomb, G., and Spiller, K.L. (2015). 41st Annual Northeast Biomedical Engineering Conference (NEBEC). IEEE *1-2*.

98. Zhao, H., Huang, J., Li, Y., Lv, X., Zhou, H., Wang, H., Xu, Y., Wang, C., Wang, J., and Liu, Z. (2020). ROS-scavenging hydrogel to promote healing of bacteria infected diabetic wounds. Biomaterials *258*, 120286.

99. Escher, P., and Wahli, W. (2000). Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat. Res. *448*, 121–138.

100. Paakinaho, V., Kaikkonen, S., Levonen, A.-L., and Palvimo, J.J. (2014). Electrophilic lipid mediator 15-deoxy-Δ12, 14-prostaglandin J2 modifies glucocorticoid signaling via receptor SUMOylation. Mol. Cell. Biol. *34*, 3202–3213.

101. Abdalla, H.B., Napimoga, M.H., Lopes, A.H., de Macedo Maganin, A.G., Cunha, T.M., Van Dyke, T.E., and Clemente Napimoga, J.T. (2020). Activation of PPAR-γ induces macrophage polarization and reduces neutrophil migration mediated by heme oxygenase 1. Int. Immunopharmacol. *84*, 106565.

102. Jabbari, P., Sadeghalvad, M., and Rezaei, N. (2021). An inflammatory triangle in Sarcoidosis: PPAR-γ, Immune microenvironment, and Inflammation. Expert Opin. Biol. Ther. *21*, 1451–1459.

103. Bouhlel, M.A., Derudas, B., Rigamonti, E., Dièvart, R., Brozek, J., Haulon, S., Zawadzki, C., Jude, B., Torpier, G., Marx, N., et al. (2007). PPARγ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. Cell Metab. *6*, 137–143.

104. Chen, H., Shi, R., Luo, B., Yang, X., Qiu, L., Xiong, J., Jiang, M., Liu, Y., Zhang, Z., and Wu, Y. (2015). Macrophage peroxisome proliferator-activated receptor γ deficiency delays skin wound healing through impairing apoptotic cell clearance in mice. Cell Death Dis. *6*, e1597.

105. Wang, X., Sng, M.K., Foo, S., Chong, H.C., Lee, W.L., Tang, M.B.Y., Ng, K.W., Luo, B., Choong, C., Wong, M.T.C., et al. (2015). Early controlled release of peroxisome proliferator-activated receptor β/δ agonist GW501516 improves diabetic wound healing through redox modulation of wound microenvironment. J. Control Release *197*, 138–147.

106. Mirza, R.E., Fang, M.M., Novak, M.L., Urao, N., Sui, A., Ennis, W.J., and Koh, T.J. (2015). Macrophage PPARγ and impaired wound healing in type 2 diabetes. J. Pathol. *236*, 433–444.

107. Silva, J.C., Pitta, M.G.R., Pitta, I.R., Koh, T.J., and Abdalla, D.S.P. (2019). New peroxisome proliferator-activated receptor agonist (GQ-11) improves wound healing in diabetic mice. Adv. Wound Care *8*, 417–428.

108. Gerrick, K.Y., Gerrick, E.R., Gupta, A., Wheelan, S.J., Yegnasubramanian, S., and Jaffee, E.M. (2018). Transcriptional profiling identifies novel regulators of macrophage polarization. PLoS One *13*, e0208602.

109. Vergadi, E., Ieronymaki, E., Lyroni, K., Vaporidi, K., and Tsatsanis, C. (2017). Akt signaling pathway in macrophage activation and M1/M2 polarization. J. Immunol. *198*, 1006–1014.

110. Fukuda, D., Aikawa, E., Swirski, F.K., Novobrantseva, T.I., Kotelianski, V., Gorgun, C.Z., Chudnovskiy, A., Yamazaki, H., Croce, K., Weissleder, R., et al. (2012). Notch ligand delta-like 4 blockade attenuates atherosclerosis and metabolic disorders. Proc. Natl. Acad. Sci. USA *109*, E1868–E1877.

111. Radtke, F., MacDonald, H.R., and Tacchini-Cottier, F. (2013). Regulation of innate and adaptive immunity by Notch. Nat. Rev. Immunol. *13*, 427–437.

112. Nakano, T., Fukuda, D., Koga, J.-i., and Aikawa, M. (2016). Delta-like ligand 4-notch signaling in macrophage activation. Arterioscler. Thromb. Vasc. Biol. *36*, 2038–2047.

113. Amici, S.A., Dong, J., and Guerau-de-Arellano, M. (2017). Molecular mechanisms modulating the phenotype of macrophages and microglia. Front. Immunol. *8*, 1520.

114. Ning, S., Pagano, J.S., and Barber, G.N. (2011). IRF7: activation, regulation, modification and function. Genes Immun. *12*, 399–414.

115. Ikushima, H., Negishi, H., and Taniguchi, T. (2013). Cold Spring Harbor Symposia on Quantitative Biology, *105-116* (Cold Spring Harbor Laboratory Press).

116. Günthner, R., and Anders, H.-J. (2013). Interferon-regulatory factors determine macrophage phenotype polarization. Mediators Inflamm. *2013*, 731023.

117. Carthew, R.W., and Sontheimer, E.J. (2009). Origins and mechanisms of miRNAs and siRNAs. Cell *136*, 642–655.

118. Gomes, M.J., Martins, S., and Sarmento, B. (2015). siRNA as a tool to improve the treatment of brain diseases: mechanism, targets and delivery. Ageing Res. Rev. *21*, 43–54.

119. Huang, C., Li, M., Chen, C., and Yao, Q. (2008). Small interfering RNA therapy in cancer: mechanism, potential targets, and clinical applications. Expert Opin. Ther. Targets *12*, 637–645.

120. Guo, P., Coban, O., Snead, N.M., Trebley, J., Hoeprich, S., Guo, S., and Shu, Y. (2010). Engineering RNA for targeted siRNA delivery and medical application. Adv. Drug Deliv. Rev. *62*, 650–666.

121. Gao, H., Cheng, R., and A Santos, H. (2021). Nanoparticle-mediated siRNA delivery systems for cancer therapy. View *2*, 20200111.

122. Kasiewicz, L.N., and Whitehead, K.A. (2016). Silencing TNFα with lipidoid nanoparticles downregulates both TNFα and MCP-1 in an in vitro co-culture model of diabetic foot ulcers. Acta Biomater. *32*, 120–128.

123. Zhang, J., Yang, X., Wang, H., Zhao, B., Wu, X., Su, L., Xie, S., Wang, Y., Li, J., Liu, J., et al. (2017). PKCζ as a promising therapeutic target for TNFα-induced inflammatory disorders in chronic cutaneous wounds. Int. J. Mol. Med. *40*, 1335–1346.

124. Kasiewicz, L.N., and Whitehead, K.A. (2019). Lipid nanoparticles silence tumor necrosis factor α to improve wound healing in diabetic mice. Bioeng. Transl. Med. *4*, 75–82.

125. Yang, J., Park, Y., Zhang, H., Gao, X., Wilson, E., Zimmer, W., Abbott, L., and Zhang, C. (2009). Role of MCP-1 in tumor necrosis factor-α-induced endothelial dysfunction in type 2 diabetic mice. Am. J. Physiol. Heart Circ. Physiol. *297*, H1208–H1216.

126. Li, N., Luo, H.C., Yang, C., Deng, J.J., Ren, M., Xie, X.Y., Lin, D.Z., Yan, L., and Zhang, L.M. (2014). Cationic star-shaped polymer as an siRNA carrier for reducing MMP-9 expression in skin fibroblast cells and promoting wound healing in diabetic rats. Int. J. Nanomed. *9*, 3377–3387.

127. Lan, B., Zhang, L., Yang, L., Wu, J., Li, N., Pan, C., Wang, X., Zeng, L., Yan, L., Yang, C., and Ren, M. (2021). Sustained delivery of MMP-9 siRNA via thermosensitive hydrogel accelerates diabetic wound healing. J. Nanobiotechnol. *19*, 130.

128. Li, N., Luo, H.C., Ren, M., Zhang, L.M., Wang, W., Pan, C.L., Yang, L.Q., Lao, G.J., Deng, J.J., Mai, K.J., et al. (2017). Efficiency and safety of β-CD-(D3) 7 as siRNA carrier for decreasing matrix metalloproteinase-9 expression and improving wound healing in diabetic rats. ACS Appl. Mater. Inter. *9*, 17417–17426.

129. Du, Y., Yang, X., Gong, Q., Xu, Z., Cheng, Y., and Su, G. (2019). Inhibitor of growth 4 affects hypoxia-induced migration and angiogenesis regulation in retinal pigment epithelial cells. J. Cell. Physiol. *234*, 15243–15256.

130. Li, N., Yang, L., Pan, C., Saw, P.E., Ren, M., Lan, B., Wu, J., Wang, X., Zeng, T., Zhou, L., et al. (2020). Naturally-occurring bacterial cellulose-hyperbranched cationic polysaccharide derivative/MMP-9 siRNA composite dressing for wound healing enhancement in diabetic rats. Acta Biomater. *102*, 298–314.

131. Lan, B., Wu, J., Li, N., Pan, C., Yan, L., Yang, C., Zhang, L., Yang, L., and Ren, M. (2020). Hyperbranched cationic polysaccharide derivatives for efficient siRNA delivery and diabetic wound healing enhancement. Int. J. Biol. Macromol. *154*, 855–865.

132. Yan, L.-P., Castaño, I.M., Sridharan, R., Kelly, D., Lemoine, M., Cavanagh, B.L., Dunne, N.J., McCarthy, H.O., and O'Brien, F.J. (2020). Collagen/GAG scaffolds activated by RALA-siMMP-9 complexes with potential for improved diabetic foot ulcer healing. Mater. Sci. Eng. C Mater. Biol. Appl. *114*, 111022.

133. Kim, H.S., Son, Y.J., and Yoo, H.S. (2016). Clustering siRNA conjugates for MMP-responsive therapeutics in chronic wounds of diabetic animals. Nanoscale *8*, 13236–13244.

134. Rabbani, P.S., Zhou, A., Borab, Z.M., Frezzo, J.A., Srivastava, N., More, H.T., Rifkin, W.J., David, J.A., Berens, S.J., Chen, R., et al. (2017). Novel lipoproteoplex delivers Keap1 siRNA based gene therapy to accelerate diabetic wound healing. Biomaterials *132*, 1–15.

135. Martin, J.R., Nelson, C.E., Gupta, M.K., Yu, F., Sarett, S.M., Hocking, K.M., Pollins, A.C., Nanney, L.B., Davidson, J.M., Guelcher, S.A., and Duvall, C.L. (2016). Local delivery of PHD2 siRNA from ROS-degradable scaffolds to promote diabetic wound healing. Adv. Healthc. Mater. *5*, 2751–2757.

136. Randeria, P.S., Seeger, M.A., Wang, X.Q., Wilson, H., Shipp, D., Mirkin, C.A., and Paller, A.S. (2015). siRNA-based spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase knockdown. Proc. Natl. Acad. Sci. USA *112*, 5573–5578.

137. Shaabani, E., Sharifiaghdam, M., Lammens, J., De Keersmaecker, H., Vervaet, C., De Beer, T., Motevaseli, E., Ghahremani, M.H., Mansouri, P., De Smedt, S., et al. (2021). Increasing angiogenesis factors in hypoxic diabetic wound conditions by siRNA delivery: additive effect of LbL-gold nanocarriers and desloratadine-induced lysosomal escape. Int. J. Mol. Sci. *22*, 9216.

138. Sharifiaghdam, M., Shaabani, E., Sharifiaghdam, Z., De Keersmaecker, H., Lucas, B., Lammens, J., Ghanbari, H., Teimoori-Toolabi, L., Vervaet, C., De Beer, T., et al. (2021). Macrophage reprogramming into a pro-healing phenotype by siRNA delivered with LBL assembled nanocomplexes for wound healing applications. Nanoscale *13*, 15445–15463.

139. Krausgruber, T., Blazek, K., Smallie, T., Alzabin, S., Lockstone, H., Sahgal, N., Hussell, T., Feldmann, M., and Udalova, I.A. (2011). IRF5 promotes inflammatory macrophage polarization and TH 1-TH 17 responses. Nat. Immunol. *12*, 231–238.

140. Weiss, M., Blazek, K., Byrne, A.J., Perocheau, D.P., and Udalova, I.A. (2013). IRF5 is a specific marker of inflammatory macrophages in vivo. Mediators Inflamm. *2013*, 245804.

141. Yang, Y., Zhang, C., Jing, D., He, H., Li, X., Wang, Y., Qin, Y., Xiao, X., Xiong, H., and Zhou, G. (2021). IRF5 acts as a potential therapeutic marker in inflammatory bowel diseases. Inflamm. Bowel Dis. *27*, 407–417.

142. Almuttaqi, H., and Udalova, I.A. (2019). Advances and challenges in targeting IRF5, a key regulator of inflammation. FEBS J. *286*, 1624–1637.

143. Courties, G., Heidt, T., Sebas, M., Iwamoto, Y., Jeon, D., Truelove, J., Tricot, B., Wojtkiewicz, G., Dutta, P., Sager, H.B., et al. (2014). In vivo silencing of the transcription factor IRF5 reprograms the macrophage phenotype and improves infarct healing. J. Am. Coll. Cardiol. *63*, 1556–1566.

144. Li, J., Liu, Y., Xu, H., and Fu, Q. (2016). Nanoparticle-delivered IRF5 siRNA facilitates M1 to M2 transition, reduces demyelination and neurofilament loss, and promotes functional recovery after spinal cord injury in mice. Inflammation *39*, 1704–1717.

145. Terashima, T., Ogawa, N., Nakae, Y., Sato, T., Katagi, M., Okano, J., Maegawa, H., and Kojima, H. (2018). Gene therapy for neuropathic pain through siRNA-IRF5 gene delivery with homing peptides to microglia. Mol. Ther. Nucleic Acids *11*, 203–215.

146. Alizadeh, A., Dyck, S.M., and Karimi-Abdolrezaee, S. (2015). Myelin damage and repair in pathologic CNS: challenges and prospects. Front. Mol. Neurosci. *8*, 35.

147. Al-Rashed, F., Sindhu, S., Arefanian, H., Al Madhoun, A., Kochumon, S., Thomas, R., Al-Kandari, S., Alghaith, A., Jacob, T., Al-Mulla, F., and Ahmad, R. (2020). Repetitive intermittent hyperglycemia drives the M1 polarization and inflammatory responses in THP-1 macrophages through the mechanism involving the TLR4-IRF5 pathway. Cells *9*, 1892.

148. Dalmas, E., Toubal, A., Alzaid, F., Blazek, K., Eames, H.L., Lebozec, K., Pini, M., Hainault, I., Montastier, E., Denis, R.G.P., et al. (2015). Irf5 deficiency in macrophages promotes beneficial adipose tissue expansion and insulin sensitivity during obesity. Nat. Med. *21*, 610–618. https://doi.org/10.1038/nm.3829.

149. Seneviratne, A.N., Edsfeldt, A., Cole, J.E., Kassiteridi, C., Swart, M., Park, I., Green, P., Khoyratty, T., Saliba, D., Goddard, M.E., et al. (2017). Interferon regulatory factor 5 controls necrotic core formation in atherosclerotic lesions by impairing efferocytosis. Circulation *136*, 1140–1154. https://doi.org/10.1161/CIRCULATIONAHA.117.027844.

150. Kim, B., Yang, Q., Chan, L.W., Bhatia, S.N., Ruoslahti, E., and Sailor, M.J. (2021). Fusogenic porous silicon nanoparticles as a broad-spectrum immunotherapy against bacterial infections. Nanoscale Horiz. *6*, 330–340.

151. Kim, B., Pang, H.B., Kang, J., Park, J.H., Ruoslahti, E., and Sailor, M.J. (2018). Immunogene therapy with fusogenic nanoparticles modulates macrophage response to Staphylococcus aureus. Nat. Commun. *9*, 1969.

152. DeLeon, S., Clinton, A., Fowler, H., Everett, J., Horswill, A.R., and Rumbaugh, K.P. (2014). Synergistic interactions of Pseudomonas aeruginosa and Staphylococcus aureus in an in vitro wound model. Infect. Immun. *82*, 4718–4728.

153. Sydnor, E.R.M., and Perl, T.M. (2011). Hospital epidemiology and infection control in acute-care settings. Clin. Microbiol. Rev. *24*, 141–173.

154. Zuloff-Shani, A., Adunsky, A., Even-Zahav, A., Semo, H., Orenstein, A., Tamir, J., Regev, E., Shinar, E., and Danon, D. (2010). Hard to heal pressure ulcers (stage III–IV): efficacy of injected activated macrophage suspension (AMS) as compared with standard of care (SOC) treatment controlled trial. Arch. Gerontol. Geriatr. *51*, 268–272.

155. Fidler, I.J. (1974). Inhibition of pulmonary metastasis by intravenous injection of specifically activated macrophages. Cancer Res. *34*, 1074–1078.

156. West, M.A., Bennet, T., Seatter, S.C., Clair, L., and Bellingham, J. (1997). LPS pretreatment reprograms macrophage LPS-stimulated TNF and IL-1 release without protein tyrosine kinase activation. J. Leukoc. Biol. *61*, 88–95.

157. Wang, Y., Wang, Y.P., Zheng, G., Lee, V.W.S., Ouyang, L., Chang, D.H.H., Mahajan, D., Coombs, J., Wang, Y.M., Alexander, S.I., and Harris, D.C.H. (2007). Ex vivo programmed macrophages ameliorate experimental chronic inflammatory renal disease. Kidney Int. *72*, 290–299.

158. Jetten, N., Donners, M.M.P.C., Wagenaar, A., Cleutjens, J.P.M., van Rooijen, N., de Winther, M.P.J., and Post, M.J. (2013). Local delivery of polarized macrophages improves reperfusion recovery in a mouse hind limb ischemia model. PLoS One *8*, e68811.

159. Lu, J., Cao, Q., Zheng, D., Sun, Y., Wang, C., Yu, X., Wang, Y., Lee, V.W.S., Zheng, G., Tan, T.K., et al. (2013). Discrete functions of M2a and M2c macrophage subsets determine their relative efficacy in treating chronic kidney disease. Kidney Int. *84*, 745–755.

160. Hisatome, T., Yasunaga, Y., Yanada, S., Tabata, Y., Ikada, Y., and Ochi, M. (2005). Neovascularization and bone regeneration by implantation of autologous bone marrow mononuclear cells. Biomaterials *26*, 4550–4556.

161. Leor, J., Rozen, L., Zuloff-Shani, A., Feinberg, M.S., Amsalem, Y., Barbash, I.M., Kachel, E., Holbova, R., Mardor, Y., Daniels, D., et al. (2006). Ex vivo activated human macrophages improve healing, remodeling, and function of the infarcted heart. Circulation *114*, I94–I100.

162. Frenkel, O., Shani, E., Ben-Bassat, I., Brok-Simoni, F., Rozenfeld-Granot, G., Kajakaro, G., Rechavi, G., Amariglio, N., Shinar, E., and Danon, D. (2002). Activated macrophages for treating skin ulceration: gene expression in human monocytes after hypo-osmotic shock. Clin. Exp. Immunol. *128*, 59–66.

163. Gu, X.-y., Shen, S.e., Huang, C.f., Liu, Y.n., Chen, Y.c., Luo, L., Zeng, Y., and Wang, A.p. (2013). Effect of activated autologous monocytes/macrophages on wound healing in a rodent model of experimental diabetes. Diabetes Res. Clin. Pract. *102*, 53–59.

164. Jetten, N., Roumans, N., Gijbels, M.J., Romano, A., Post, M.J., de Winther, M.P.J., van der Hulst, R.R.W.J., and Xanthoulea, S. (2014). Wound administration of M2-polarized macrophages does not improve murine cutaneous healing responses. PloS one *9*, e102994.

165. Dreymueller, D., Denecke, B., Ludwig, A., and Jahnen-Dechent, W. (2013). Embryonic stem cell–derived M2-like macrophages delay cutaneous wound healing. Wound Repair Regen. *21*, 44–54.

166. Wetzler, C., Kämpfer, H., Stallmeyer, B., Pfeilschifter, J., and Frank, S. (2000). Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. J. Invest. Dermatol. *115*, 245–253.

167. Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S., and Amit, I. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell *159*, 1312–1326.

168. Chamberlain, C.S., Leiferman, E.M., Frisch, K.E., Wang, S., Yang, X., Brickson, S.L., and Vanderby, R. (2011). The influence of interleukin-4 on ligament healing. Wound Repair Regen. *19*, 426–435.

YMTHE, Volume *30*

Supplemental Information

Macrophages as a therapeutic target to promote diabetic wound healing

Maryam Sharifiaghdam, Elnaz Shaabani, Reza Faridi-Majidi, Stefaan C. De Smedt, Kevin Braeckmans, and Juan C. Fraire

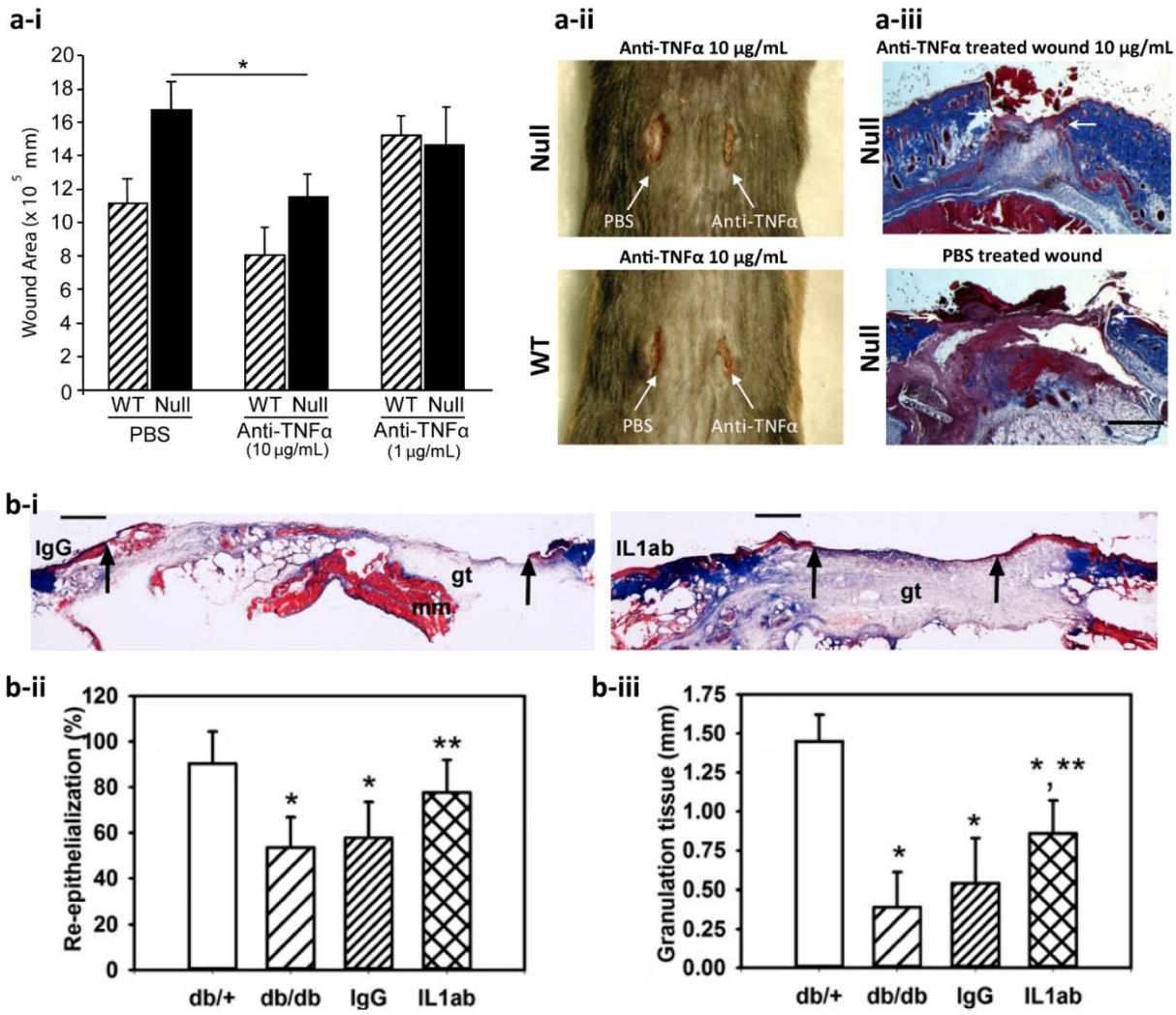


Figure S1. Inhibition of effects induced by inflammatory M1-like macrophages in the wound bed. **(a)** Accelerated wound healing by neutralization of tumor necrosis factor alpha (TNFα) in an SLPI null mice model, lacking anti-inflammatory and anti-proteolytic activity. i) Single administration of Anti-TNFα antibodies (10µg/mL) at the time of wounding significantly reduces cross-sectional wound areas compared to control (PBS treated) wounds at day 3 post-wounding. Data represent mean values and error bars the SEM. *$p < 0.05$. $n = 6$. null: secretory leukocyte protease inhibitor null mouse, WT: Wild-type. ii) The macroscopic images show accelerated healing after TNFα antibody treatment (Anti-TNFα) compared to control (PBS). iii) Masson's trichrome staining at day 3 post-wounding illustrates a marked reduction of wound size after treatment with anti-TNFα antibodies (10 µg/mL) compared to the PBS treated group. Arrows demarcate wound margins. Scale bar = 300 µm. Adopted from Ashcroft, G. S. et al.[1] Copyright (2011), with permission from Wiley. **(b)** Accelerated wound healing by Interleukin-1 beta (IL-1β) neutralization in diabetic mice. i) Accelerated re-epithelialization and granulation tissue formation is observed in the group treated by IL-1β antibody (IL1ab) in comparison to IgG-treated mice. Scale bar = 0.5 mm. Arrows indicate the ends of migrating epithelial tongues. gt, granulation tissue; mm, deep muscle. ii) Quantification of re-epithelization and iii) granulation tissue thickness of wounds in nondiabetic mice (db/+), untreated diabetic mice (db/db), IgG-treated diabetic mice (IgG), and IL1ab-treated diabetic mice (IL1ab), on day 10 postinjury. Adopted from Mirza, R. E. et al.[2]

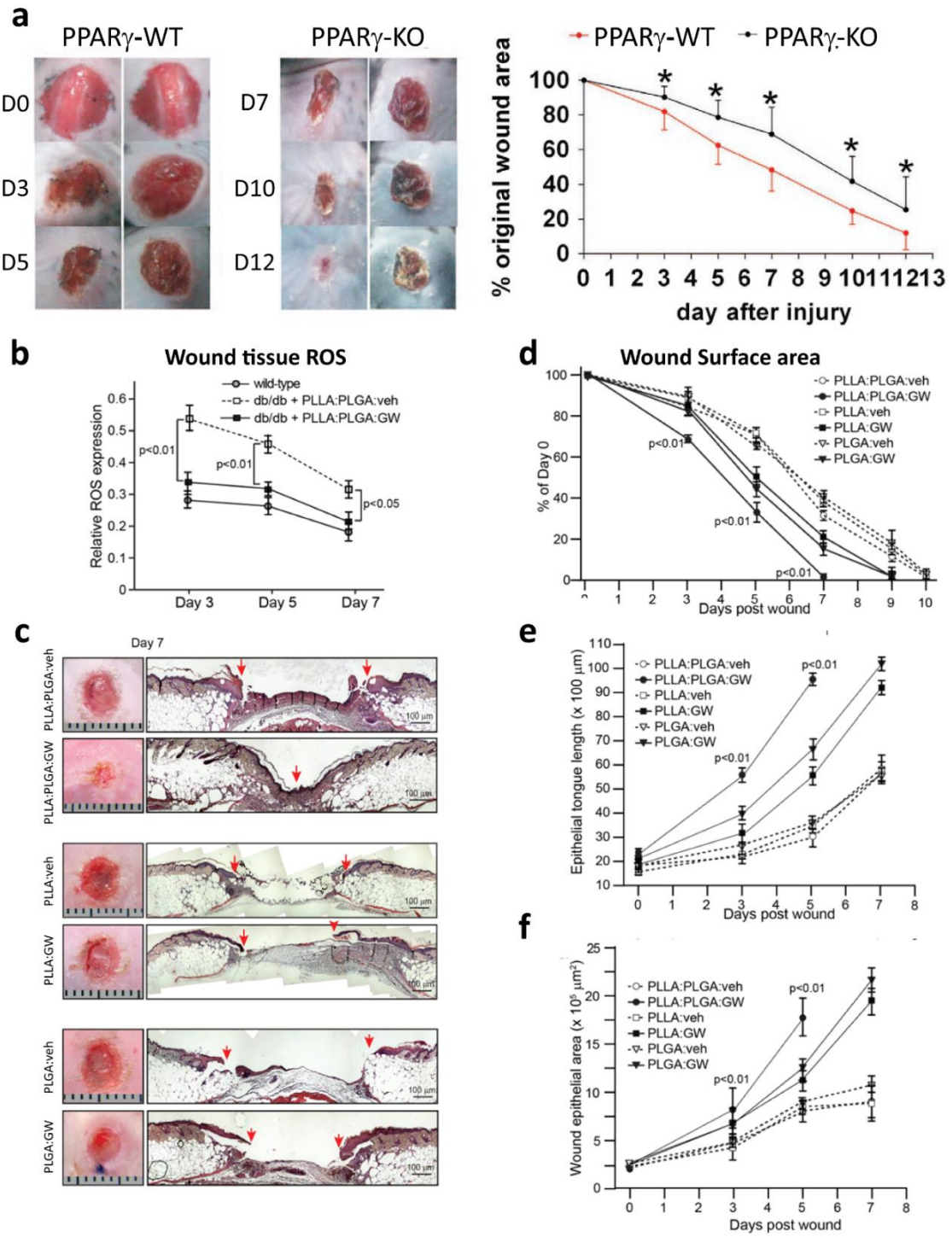


Figure S2. Diabetic wound interventions by application of peroxisome proliferator-activated receptors (PPARs) agonist. (a) Representative macroscopic images and statistical analysis of delayed wound healing in PPARγ-knock out (KO) mice versus PPARγ-wild type (WT) mice**.** Adapted from Chen, H. et al.[3] **(b)** Relative ROS levels of wounds treated with PPARβ/δ agonist GW501516 (GW) encapsulated in core-shell of Poly(L-lactide) and poly(D,L-lactide–co-glycolide) (PLLA:PLGA:GW) microparticles, and vehicle control microparticles without encapsulated GW (PLLA:PLGA:veh). **(c)** Representative macroscopic images (left) and H&E (right) sections of diabetic wounds (day 7) treated with PLLA:PLGA:veh, PLLA:PLGA:GW, PLLA:veh, PLLA:GW and PLGA:veh, PLGA:GW. Ruler units are in mm and red arrows show the epithelial wound edge. **(d)** statistical analysis of wound surface area expressed as percentage of the initial (day 0) wound size. Wound epithelial tongue length **(e)** and epithelial area **(f)** determined by histomorphometric analyses of wound biopsies treated with PLLA:PLGA:veh, PLLA:PLGA:GW, PLLA:veh, PLLA:GW and PLGA:veh, PLGA:GW at day 7 post-wounding. Data shown are mean values ± SD, P values are based on

comparisons between PLLA:PLGA:GW treatment and control PLLA:PLGA:veh. Reprinted from Wang, X. et al.[4] Copyright (2015), with permission from Elsevier.

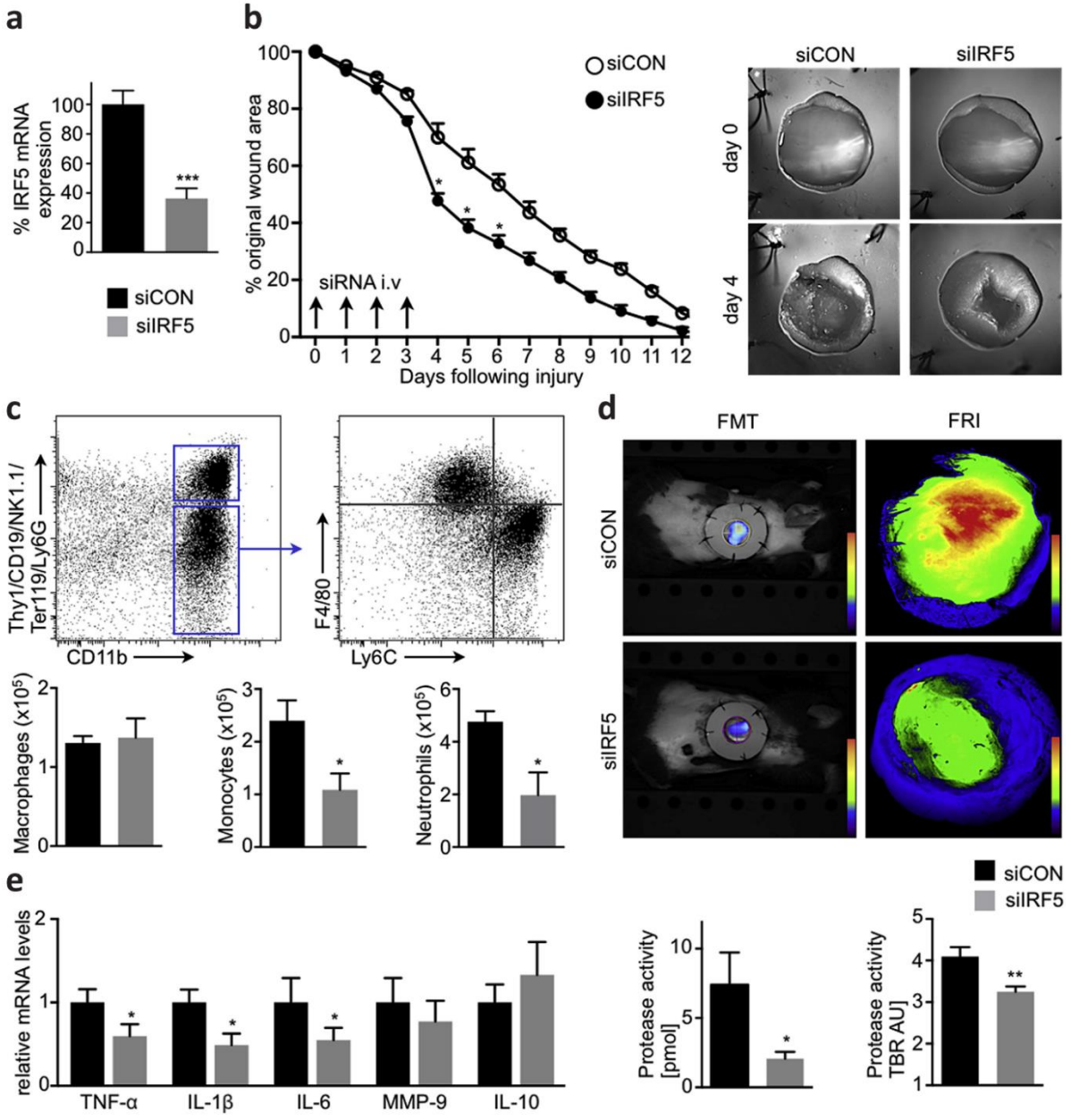


Figure S3. The effect of IRF5 silencing in skin wound healing. (a) IRF5 silencing in monocytes/macrophages isolated from excisional wounds of mice injected 4 days after wounding with siIRF5 or siCON (0.5 mg/kg) (n = 9 per group). **(b)** Wounds were imaged, and the open wound areas were measured daily in mice injected with siIRF5 and siCON (n = 7 to 8 per group). **(c)** Flowcytometry graph and analysis of wound leukocyte content on day 4 (n = 5 per group). **(d)** Wound protease activity on day 4, in mice treated i.v. with siIRF5 or siCON (0.5 mg/kg) by fluorescence molecular tomography–computed tomography (FMT-CT) and FRI (fluorescence reflectance imaging) (n = 8 per group) **(e)** Expression level of M1 and M2 mRNA in wound macrophages on day 4 (n = 10 to 12 per group). $*p < 0.05$. $**p < 0.01$. $***p < 0.001$. Reprinted from ourties, G. et al.[5] Copyright (2014), with permission from Elsevier.

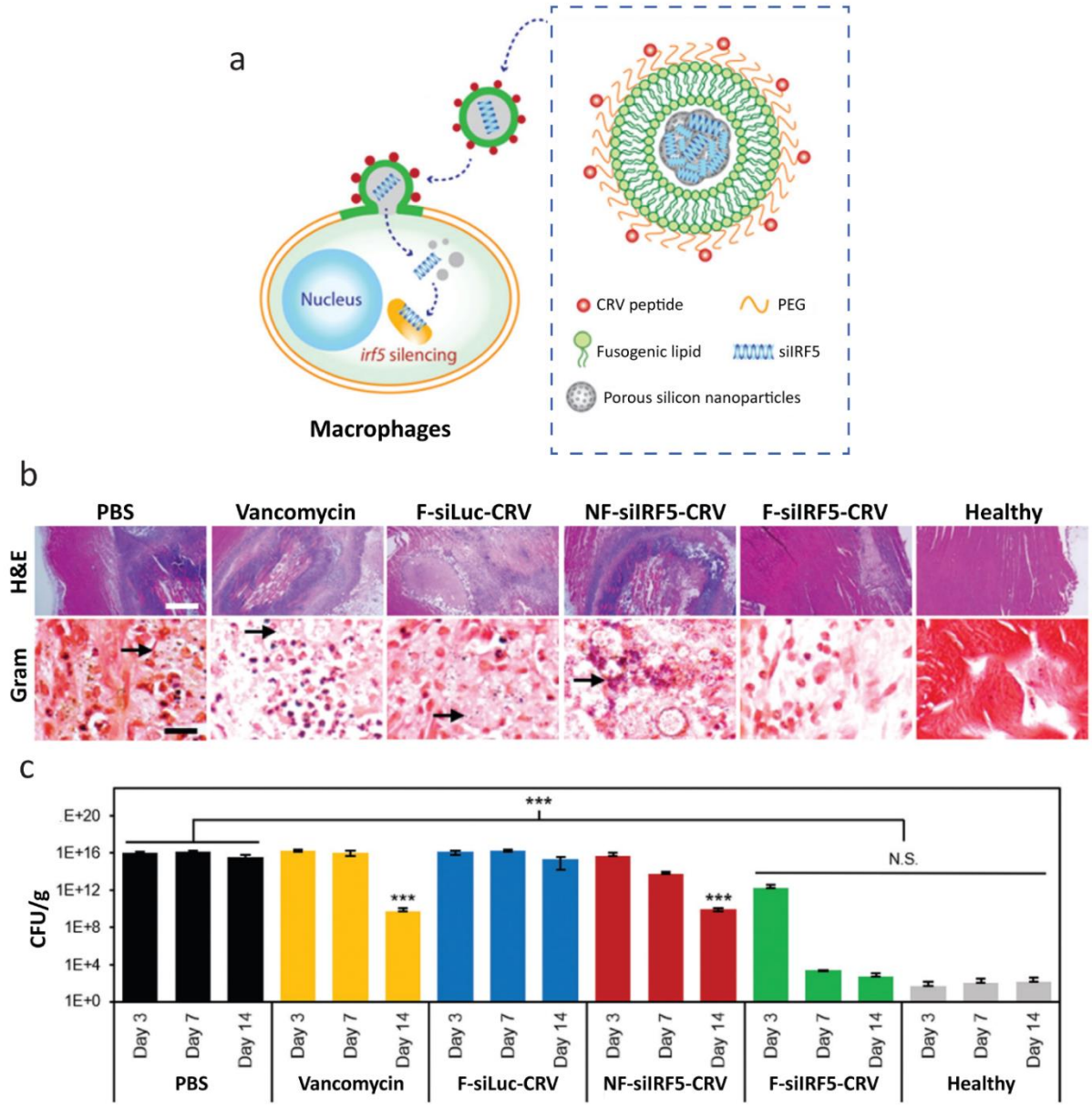


Figure S4. ***In vivo*** **therapeutic efficacy of siIRF5 in MRSA muscle infection. (a)** Schematic figure depicting the CRV peptide-targeted, fusogenic porous silicon nanoparticles (F-pSiNPs-CRV) for delivering and silencing the IRF5 gene in macrophages. **(b)** Stained infected muscle tissues (H&E and Gram) 7 days after treatment with different groups including: PBS, vancomycin, non-fusogenic, targeted pSiNPs containing siIRF5 (NF-siIRF5-CRV), fusogenic, CRV-targeted pSiNPs containing siRNA against luciferase, as a negative control for siIRF5 (F-siLuc-CRV), and fusogenic, CRV-targeted pSiNPs containing siIRF5 (F-siIRF5-CRV); scale bar represents 1 mm (top row) and 100 mm (bottom row); **(c)** Bacterial titer counts (CFU per mass of tissue) at days 3, 7, and 14 post-intravenous injection in healthy and methicillin-resistant Staphylococcus aureus (MRSA)-infected mice. Mice were infected on day 0 and injections were given on day 1. Bars indicate standard deviation with n = 6. N.S. represents no significance and ***represents $p < 0.01$ relative to the PBS group from one-way ANOVA with Tukey's HSD post hoc analyses. Reproduced from Ref.[6], with permission from the Royal Society of Chemistry.

References

1 Ashcroft, G. S. *et al.* Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. *Wound Repair and Regeneration* **20**, 38-49 (2012).

2 Mirza, R. E., Fang, M. M., Ennis, W. J. & Koh, T. J. Blocking interleukin-1β induces a healing-associated wound macrophage phenotype and improves healing in type 2 diabetes. *Diabetes* **62**, 2579-2587 (2013).

3 Chen, H. *et al.* Macrophage peroxisome proliferator-activated receptor γ deficiency delays skin wound healing through impairing apoptotic cell clearance in mice. *Cell death & disease* **6**, e1597-e1597 (2015).

4 Wang, X. *et al.* Early controlled release of peroxisome proliferator-activated receptor β/δ agonist GW501516 improves diabetic wound healing through redox modulation of wound microenvironment. *Journal of controlled release* **197**, 138-147 (2015).

5 Courties, G. *et al.* In vivo silencing of the transcription factor IRF5 reprograms the macrophage phenotype and improves infarct healing. *Journal of the American College of Cardiology* **63**, 1556-1566 (2014).

6 Kim, B. *et al.* Fusogenic porous silicon nanoparticles as a broad-spectrum immunotherapy against bacterial infections. *Nanoscale horizons* **6**, 330-340 (2021).