HHS Public Access

Author manuscript

Acta Biomater. Author manuscript; available in PMC 2022 October 01.

Published in final edited form as:

Acta Biomater. 2021 October 01; 133: 4–16. doi:10.1016/j.actbio.2021.03.038.

Macrophage phenotypes in tissue repair and the foreign body response: implications for biomaterial-based regenerative medicine strategies

Karen E Martin[a,b], **Andrés J García**[a,b,**]

[a]Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, 30332, USA

[b]Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA, 30332, USA

Abstract

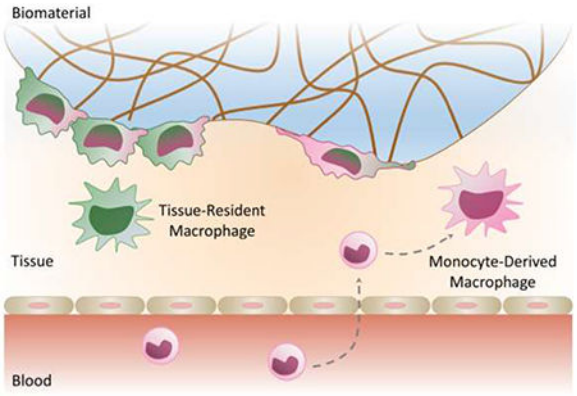
Macrophages are a highly heterogeneous and plastic population of cells that are crucial for tissue repair and regeneration. This has made macrophages a particularly attractive target for biomaterial-directed regenerative medicine strategies. However, macrophages also contribute to adverse inflammatory and fibrotic responses to implanted biomaterials, typically related to the foreign body response (FBR). The traditional model in the field asserts that the M2 macrophage phenotype is pro-regenerative and associated with positive wound healing outcomes, whereas the M1 phenotype is pro-inflammatory and associated with pathogenesis. However, recent studies indicate that both M1 and M2 macrophages play different, but equally vital, roles in promoting tissue repair. Furthermore, recent technological developments such as single-cell RNA sequencing have allowed for unprecedented insights into the heterogeneity within the myeloid compartment, related to activation state, niche, and ontogenetic origin. A better understanding of the phenotypic and functional characteristics of macrophages critical to tissue repair and FBR processes will allow for rational design of biomaterials to promote biomaterial-tissue integration and regeneration. In this review, we discuss the role of temporal and ontogenetic macrophage heterogeneity on tissue repair processes and the FBR and the potential implications for biomaterial-directed regenerative medicine applications.

Graphical abstract

** Correspondence to: andres.garcia@me.gatech.edu.

Declaration of Interest Statement

The authors have no competing interests to disclose.



Keywords

Macrophages; Tissue repair; Foreign body response; Immunomodulatory biomaterials; Regenerative medicine

1. Introduction

Macrophages are key regulators of tissue homeostasis, inflammation, and regeneration. During homeostasis, tissue resident macrophages (TRMs) serve critical supportive functions within their resident tissues. For example, alveolar macrophages in the lung are required for the normal turnover of lung surfactant, osteoclasts in bone are necessary for the continuous resorption and remodeling of osseous tissue, and Kupffer cells in the liver are responsible for the clearance of dying red blood cells and iron recycling[1]. Following tissue injury, macrophages, both tissue-resident and monocyte-derived in origin, undergo marked phenotypic changes, transiently gaining and losing functions in response to the varying microenvironmental cues present as the wound healing process progresses[2]. This phenotypic plasticity allows macrophages to play a multitude of key roles during all phases of wound healing: initiation, proliferation, and resolution[3]. Disruption of normal macrophage function can initiate a variety of pathological processes, including the uncontrolled production of inflammatory mediators, deficient generation of anti-inflammatory macrophage phenotypes, and stimulation of the overproduction of extracellular matrix (ECM) proteins by fibroblasts, all of which contribute to chronic inflammatory and/or fibrotic processes[2, 4].

As biomaterials play an ever more central role in regenerative medicine strategies, considerable effort has been made to modulate immune responses to these materials. In particular, modulating the macrophage responses is of interest due to its relationship with not only the wound repair process, but also the foreign body response (FBR)[5]. Traditional approaches focus on tuning biomaterial physical and chemical properties to mitigate the FBR. Physical approaches encompass material features such as particle size, substrate stiffness, and topography, all of which have profound effects on macrophage phenotype and the development of the FBR (reviewed[6, 7]). Traditionally, chemical approaches focused on the development of non-fouling materials that prevent serum protein adsorption in order to prevent initiation of the subsequent inflammatory cascade[8]. Material chemistries such as

poly(ethylene glycol) (PEG)[9], poly(hydroxyethylmethacrylate) (poly(HEMA))[10], and, more recently, zwitterionic materials[11, 12] are able to resist the adsorption of serum protein due to the presence of a hydration layer near the surface of the material[13]. Tuning material properties to temper this process has been shown to result in a smaller fibrotic capsule[14], mitigation of inflammatory cytokine secretion[15], and a shift towards anti-inflammatory macrophage phenotypes[16].

More recently, immunomodulatory biomaterial design has shifted towards creating biomaterial systems that actively interface with the local immune environment. A variety of bioactive approaches have been implemented to control macrophage phenotype including strategies incorporating relevant cytokines[17, 18], receptors[19–21], small molecules[22, 23], and immunomodulatory cells (e.g. mesenchymal stem cells (MSC))[24–26] (reviewed [27–30]). These bioactive strategies are impeded by a lack of understanding of macrophage phenotypes and their roles in tissue repair, especially in the biomaterial microenvironment. Furthermore, macrophages exhibit substantial cellular plasticity wherein the functional and phenotypic states of the cells fluctuate temporally throughout the duration of an immune response, suggesting that biomaterials targeting only one specific phenotype are unlikely to be successful. In this review, we discuss the role of temporal and ontogenetic macrophage heterogeneity on tissue repair processes and the FBR and the potential implications for biomaterial-directed regenerative medicine applications.

2. The role of macrophages in tissue repair

The immune system plays a central role in orchestrating the repair and regeneration of damaged tissues following infection or injury. During tissue injury, damage-associated molecular patterns (DAMPs) and/or pathogen-associated molecular patterns (PAMPs) are released by dead and dying cells or invading pathogens, respectively[31]. These molecules are recognized by pattern recognition receptors (PRRs), expressed primarily by innate immune cells, activating inflammatory signaling pathways in TRMs, neutrophils, dendritic cells, as well as other local cell populations (e.g. fibroblasts and endothelial cells)[32]. Activation of these cells leads to release of various pro-inflammatory chemokines and cytokines that induce directed chemotaxis of other innate inflammatory cells to the injured tissue.

During homeostasis, monocytes are found in the bone marrow, blood, or spleen[33]. Upon injury, they are robustly recruited from the blood stream to the site of inflammation, typically via a chemokine receptor 2 (CCR2)-dependent mechanism[33, 34]. Infiltrating monocytes secrete pro-inflammatory cytokines and chemokines such as interleukin-1 (IL-1), IL-8, monocyte chemotactic protein-1 (MCP-1) and chemokine ligand 13 (CXCL13), further stimulating the infiltration of inflammatory cell populations[35]. Once at the site of injury, monocytes either act as effectors themselves or differentiate into monocyte-derived macrophages (moMs) or monocyte-derived dendritic cells (moDCs)[36, 37]. Depending on local environmental cues, such as the local cytokine milieu, both TRMs and moMs can acquire differential activation states, traditionally classified by their relation to the classically activated (IFN-γ, LPS) and alternatively activated (IL-4, IL-13) moM phenotypes defined in *in vitro* culture[38].

During successful wound repair, macrophages (largely moMs) will initially assume a 'classically activated' phenotype (also referred to as M1) (Fig. 1) in which they promote inflammation through the release of inflammatory cytokines (e.g. IL-6, IL-12, TNFα), reactive oxygen species, and antimicrobial peptides[33, 39, 40]. M1 macrophages are also highly phagocytic allowing them to clear debris and bacteria from the wound environment[39]. Generally identified by high surface marker expression of MHC-II and co-stimulatory molecules such as CD40, CD81, CD86 as well as intracellular iNOS, M1 macrophages are efficient antigen-presenting cells and further promote a type 1 immune environment through their interactions with T helper 1 cells[41].

After the acute inflammatory phase subsides, the predominant macrophage population shifts to an 'alternatively activated' phenotype (also referred to as M2) (Fig. 1). M2 macrophages are characterized by secretion of anti-inflammatory mediators[42], especially IL-10, and growth factors (e.g. PDGF, TGF-β) that aid in tissue healing through stabilizing angiogenesis[43, 44], stimulating progenitor cell ingrowth and proliferation[45], and promoting ECM assembly and remodeling[46]. These cells are characterized by surface marker expression of scavenger receptors (e.g. CD204, CD206, CD163) and intracellular arginase-1. Interactions between M2 macrophages and the adaptive immune system, especially T helper 2 cells[47] and regulatory T cells[48–50], have been shown to be critical to the resolution of inflammatory responses in multiple tissues.

In addition to the broadly defined M2 phenotype, several subtypes have been outlined: M2a, M2b, M2c (also called M(IL-4), M(Ic), and M(IL-10) based on the cytokines used to generate the phenotype *in vitro*)[51, 52] (Table 1). Recently, M2d and $M2_{eff}$ macrophages have also been described. M2d macrophages are activated by Toll-like receptor agonists and adenosine $A_{2A}R$ agonists and respond by secreting high levels of VEGF [53]. $M2_{eff}$ macrophages are induced by efferocytosis, the process by which apoptotic cells are removed by phagocytic cells. This process suppresses the production of inflammatory mediators in these cells [54]. Of the defined M2 subtypes, M2a is the subset most commonly associated with regenerative and fibrotic processes[55]. Although this classification provides improved phenotypic stratification, it is likely still an oversimplification of macrophage phenotypes as it is generally accepted that macrophages exist on a continuum of activation states between M1 and M2[56]. Furthermore, macrophages in *in vivo* environments are not transcriptionally equivalent to their *in vitro* counterparts, and surface markers identified on *in vitro* macrophages often do not correspond to *in vivo* phenotypes[38]. Nevertheless, we will use the M1/M2 nomenclature henceforth as that is typically how results are reported in primary sources.

3. The timing and duration of M1 and M2 macrophage responses are critical to successful tissue repair

Optimal wound healing is dependent on a highly regulated transition from an M1 to an M2 macrophage response followed by a return to homeostasis. Factors such as the extent of injury, duration of inflammation, macrophage activation state, and the tissue type as well as the health state of the host can influence the course of the tissue repair response.

Taking the bone repair process as an illustrative example, studies report that the presence of macrophages during the fracture repair process is critical to successful healing, as their depletion using methods such as clodronate liposomes[57] or the murine macrophage-fas-induced apoptosis (MaFIA) model[58] severely impairs bone repair. Furthermore, the timing and duration of the M1 macrophage-mediated inflammatory response are critical determinants of bone repair outcomes. The initial inflammatory reaction, where M1 macrophages predominate, is required for optimal fracture healing[59], and suppression of the immune response (e.g. by NSAIDs or glucocorticoid medications) too early in the healing process can result in suboptimal bone regeneration[60, 61]. Additionally, depleting macrophages before or during the initial inflammatory phase has a negative impact on fracture healing[58, 62, 63]. There are several reports of M1 macrophages, but not M2 macrophages, promoting the osteogenic differentiation and mineralization of mesenchymal stem cells (MSCs) *in vitro*, suggesting an additional role for these macrophages in bone repair[64, 65]. On the other hand, sustained, chronic M1 macrophage-mediated inflammation is highly detrimental to bone repair with the continued production of pro-inflammatory cytokines resulting in bone resorption via increased osteoclast activity and suppression of bone formation by osteoblasts[59, 66].

Although M2 macrophages are typically considered “pro-healing” and are indispensable to the inflammation-resolution phase of wound healing, their presence during the acute inflammatory phase of wound healing may be detrimental to tissue repair[28]. Jetten et al. explored the role of exogenous delivery of macrophages, differentiated *ex vivo* into M2a and M2c phenotypes, on wound healing in a full-thickness cutaneous wound model[67]. In wild type mice, there was no difference in wound healing following local injection of saline, undifferentiated macrophages, M2a macrophages or M2c macrophages during the acute inflammatory phase immediately post injury. However, in diabetic db/db mice, complete re-epithelialization only occurred in mice injected with saline and undifferentiated macrophages, whereas wounds injected with M2a or M2c macrophages failed to fully close.

In soft tissues, such as the liver[68] and the lung[69], macrophages have been shown to play contrasting roles in the promotion and resolution of fibrotic processes. Macrophages are widely implicated in promoting tissue fibrosis, and their depletion in liver injury models has been shown to mitigate their fibrotic effects[68]. For example, in a thioacetamide model of liver injury in rats, macrophage depletion using gadolinium chloride decreased myofibroblast activation and overall liver fibrosis[70]. In chronic liver injury models of fibrosis, monocyte-derived pro-inflammatory macrophages predominate, suggesting that pro-fibrotic macrophages are derived from this pool. A study by Karlmark et al. identified a $Ly6C^{hi}F4/80^{+}$ iNOS-producing macrophage population, derived from a $Gr1^{+}$ inflammatory monocyte population, as the main pro-fibrotic population in a murine model of carbon tetrachloride (CCl_4)-induced hepatic fibrosis[71]. Additionally, macrophages secrete potent pro-fibrotic factors such as TGF-β and galectin-3, which can activate resident fibroblasts, promote their transdifferentiation into myofibroblasts, and stimulate myofibroblast collagen production and contractility[4, 72].

Macrophages have also been shown to play pivotal roles in fibrosis resolution, in part through their secretion of matrix metalloproteases (MMPs), a family of proteases

that degrade various ECM proteins[73]. During liver injury, macrophage-secreted MMPs are inhibited by the concurrent production of tissue inhibitors of MMPs (TIMPs) by myofibroblasts and activated hepatic stellate cells, which results in progressive ECM deposition and scar accumulation[74, 75]. Upon removal of the injury stimuli, macrophages are able to undergo a phenotypic shift, driven by the ingestion of cellular debris, towards an anti-fibrotic, pro-resolution phenotype[76, 77]. In addition to removing cellular debris, these pro-resolution macrophages secrete a variety of fibrolytic MMPs including MMP9, MMP12, and MMP13, augmenting fibrotic ECM degradation[77–79].

Several studies have demonstrated that the timing of the M2 response is critical in determining whether a soft tissue injury results in tissue regeneration and return to homeostasis or results in chronic fibrosis. For example, in a CCl_4-induced model of reversible hepatic fibrosis, Duffield et al. showed that macrophage depletion during the process of liver injury resulted in fewer myofibroblasts and reduced ECM deposition compared to control animals[80]. Interestingly, depletion of macrophages during the fibrosis resolution phase resulted in less ECM degradation compared to controls, highlighting the importance of temporal control within the macrophage response to injury[80]. In a later study by Weng et al., these macrophage depletion effects were largely attributed to IL-4Rα-expressing M2 macrophages, in that their selective depletion had a similar effect on liver fibrosis and fibrosis resolution when compared to total macrophage depletion[81].

4. M1 and M2 macrophages promote different stages of neoangiogenesis

The creation of new vasculature at the site of injury via the process of neoangiogenesis is a vital component of successful wound repair. However, failure to tightly regulate the angiogenic process can lead to aberrant vessel growth, including abnormal, excessive vasculature and insufficient vascularization or vessel regression, all associated with various pathological processes[82]. Macrophages have long been considered indispensable to the process of neoangiogenesis, and have been shown to facilitate different phases of the process, including vessel sprouting and anastomosis[83]. Pro-angiogenic characteristics have long been attributed to M2 macrophages, while M1 macrophages were typically seen as anti-angiogenic[44, 84, 85]. However, recent studies have begun to identify roles for both M1 and M2 macrophages in different phases of neoangiogenesis.

Spiller et al. showed that, *in vitro,* M1 macrophages secrete high levels proteins involved in the initiation of angiogenesis, especially the pro-angiogenic factor vascular endothelial growth factor (VEGF)[43]. This finding is further supported by *in vivo* data from Willenborg et al. that identified a VEGF-expressing macrophage population with an inflammatory gene signature at early time points during wound healing[86]. Additionally, *in vitro* interaction with M1 macrophages causes endothelial cells to upregulate genes associated with the tip cell phenotype, a phenotype associated with early vascular sprouting, as well as genes related to endothelial cell proliferation and migration[87].

A study by Gurevich et al. suggests an *in vivo* role for M1-type macrophages in early stage neoangiogenesis in a live-imaging zebrafish wound model[88]. The presence of pro-inflammatory, TNFα-expressing macrophages at the wound site was required for wound

neoangiogenesis during the early stages of tissue repair, and inhibiting their presence either through the administration of anti-inflammatory agents hydrocortisone and IL-10 or using zebrafish with mutant colony-stimulating factor 1 receptor (CSF1R) greatly reduced wound neoangiogenesis. In addition, these pro-inflammatory macrophages were observed to preferentially associate with vessel tips both *in vivo* and *in vitro*, reinforcing the role they likely play in early endothelial cell sprouting.

In contrast, Spiller and colleagues have reported that M2 macrophages in culture secrete significantly higher levels of PDGF-BB, a growth factor implicated in the recruitment of pericytes and MSCs, both cell types critical for stabilizing blood vessels[43]. Interactions between M2 macrophages and endothelial cells upregulate endothelial cell gene expression signatures associated with pericyte cell differentiation and smooth muscle differentiation, processes associated with later stages of vascular maturation[87]. Taken together, these studies support distinct roles for M1 and M2 macrophages in vascularization, where M1 macrophages promote early stages of vascularization, such as endothelial cells sprouting, while M2 macrophages play a critical role in later stages of vascularization and vessel maturation. However, the roles that these different macrophage phenotypes play during vascularization in an *in vivo* setting remain to be fully elucidated.

5. Macrophages are key players in the FBR

The early stages of the sterile inflammatory response following biomaterial implantation generally parallel that of a response to sterile tissue damage. Injury to blood vessels during biomaterial insertion initiates the blood-material interaction cascade[89]. Within minutes, plasma components including proteins, lipids, and sugars are adsorbed onto the material surface[89]. The types and quantities of molecules that are adsorbed are influenced by material properties such as the topography, roughness, chemistry, and surface energy of the implanted material[90]. Local cell interactions with DAMPs triggers the inflammatory cascade[7]. Neutrophils infiltrate the site acutely after implantation and are the primary cell type for the first 2 days, at which point macrophages, largely derived from infiltrating monocytes, become the predominant cell type[91].

Presence of a biomaterial implant often leads to a chronic inflammatory state in which the strong acute inflammatory response following implantation never resolves. Adherent macrophages will fuse to form foreign body giant cells (FBGC), large multinucleated cells, that to try to degrade the material by secreting reactive oxygen species (ROS) and degradative enzymes, a process called frustrated phagocytosis[89]. Additionally, a high concentration of cytokines, such as TGF-β, around biomaterial implants promotes the transformation of fibroblasts to myofibroblasts that secrete copious amounts of ECM proteins, forming a fibrotic capsule around the biomaterial implant[35, 92]. Fibroblasts begin to appear around 7 days post implantation and increase in number until day 28 when they represent the majority of cells in the fibrotic capsule[35, 93]. In addition to the surface properties that modulate protein adsorption to the material, other bulk material properties such as stiffness[94], adhesivity[95], porosity[96], and particle size[97] can modulate this response.

Macrophages are critical regulators of the FBR. Several macrophage depletion studies have demonstrated that depletion of macrophages from the foreign body environment significantly reduces or eliminates the FBR [35]. The depletion of macrophages using a transgenic MaFIA mouse model significantly reduced cell adhesion to implanted alginate spheres, a critical step in the initiation of the FBR [97]. Studies using clodronate liposomes as a method of depleting macrophages have also demonstrated that macrophage depletion reduces neovascularization, FBGC formation, and fibrotic capsule development [93, 98]. A recent study by Doloff et al utilizing a series of macrophage, neutrophil, and lymphocyte knockout models showed that depletion of macrophages by clodronate liposome eliminated the FBR to implanted alginate, whereas elimination of other immune populations had less significant or no effect on the FBR [99]. CSF1R, a gene specific to macrophages within the foreign body microenvironment, was identified as a key component of the FBR. Inhibiting CSF1R using the small molecule inhibitor GW2580 minimized fibrous encapsulation of the alginate gels while leaving other macrophage functions, such as VEGF and ROS production, unchanged[99].

6. Macrophage phenotype during the FBR

Although the requirement for macrophages in the FBR is well established, the phenotype of macrophages involved in the FBR and how these phenotypes may change over the course of FBR development are not well understood and reports are often conflicting. Recent intravital imaging studies have shown that M1-like macrophages are the predominant phenotype at all stages of the FBR, from initiation to FBGC formation and fibrous encapsulation in mice [98] and zebrafish[100]. In the zebrafish study, treatment with the anti-inflammatory agent hydrocortisone or using zebrafish with mutant CSF1R significantly reduced the amount of fibrosis and the number of FBGCs, indicating that a chronic inflammatory state may be responsible for progression of the FBR. This is consistent with other studies that have shown that materials and surface coatings that reduce the amount of M1 activation and increase the ratio of M2 to M1 macrophages correlate with less fibrosis and FBGC formation[101, 102].

Complicating this model, however, is the fact that *in vitro* FBGC formation is highly dependent on IL-4 and IL-13 signaling, both of which are potent M2 modulators[89, 103]. Additionally, the macrophage mannose receptor (CD206), a marker of M2 phenotype, has been shown to play a role in macrophage fusion, and inhibition of CD206 prevents multinuclear FBGC formation[104]. Furthermore, while M1-associated pro-inflammatory cytokines such as TNFα and IL-1α are described to be transiently present near implants at early time points, cytokines typically associated with M2 macrophages including TGF-β, PDGF, and IL-10 are associated with more extensive implant fibrosis, likely due to the role these cytokines play in promoting fibrosis[105–107].

In addition to the seemingly incongruent roles ascribed to M1 and M2 macrophages during FBR, several studies have also reported co-expression of M1 and M2 markers in macrophages associated with FBR[108–110]. Many of these studies analyze the biomaterial-associated macrophage phenotype by histology using a limited number of surface markers, making it difficult to ascertain whether these FBR-associated macrophages are a hybrid phenotype co-expressing M1 and M2 markers or both M1 and M2 macrophages are present

at the site at various ratios that change in time. Moreover, interpretation of studies performed using a variety of materials in a variety of different *in vitro* and *in vivo* models makes cross-study analysis challenging.

7. Macrophage heterogeneity in the biomaterial immune microenvironment – beyond M1 and M2

Recent exploration of the *in vivo* biomaterial immune microenvironment has revealed the existence of macrophage phenotypes defined by markers outside the M1/M2 paradigm, often with no clear relation to the M1/M2 activation status of the cells. Sadtler et al. identified a scaffold associated macrophage phenotype (termed SAMs) with the expression pattern $F4/80^{+}CD11c^{+/-}CD206^{hi}CD86^{+}MHCII^{+}$ in macrophages associated with both ECM-derived and synthetic matrices in subcutaneous and volumetric muscle loss injury environments[111, 112]. More recently, a SAM-type population has also been reported in the microenvironment surrounding biomaterials implemented to treat both cancer[113] and type 1 diabetes[114]. These SAMs express both the classical M1 marker CD86 and the classical M2 marker CD206, in line with the hybrid phenotypes previously described in the biomaterial immune environment[110]. A subpopulation of these cells also, interestingly, co-expresses F4/80, an established murine macrophage marker, and CD11c, which is frequently used as a dendritic cell marker, but is also canonically expressed by several TRM populations (e.g., alveolar macrophages and intestinal macrophages). Inflammatory, monocyte-derived $F4/80^{+}CD11c^{+}$ macrophages (also referred to as hybrid antigen-presenting cells) have also been identified in several tissues including intestine, lung, spleen, and vasculature, with an increased presence of this cell population during inflammatory processes such as infection, cancer, and atherosclerosis[115–117]. Whether these $F4/80^{+}CD11c^{+}$ macrophages are broadly relevant across different inflammatory conditions or represent tissue-specific or biomaterial-specific populations remains to be determined.

Using single cell RNA sequencing (scRNA-seq) techniques, Sommerfeld et al. further probed the gene expression patterns of macrophages associated with fibrotic (synthetic) or regenerative (ECM-derived) scaffold environments[118]. In the regenerative environment, phagocytic $F4/80^{+}CD301b^{+}CD9^{-}CD206^{+}$ and non-phagocytic $F4/80^{+}CD301b^{+}CD9^{+}CD11c^{+}$ phenotypes were identified. Macrophages associated with the fibrotic environment were $CD301b^{-}CD9^{hi}$ and expressed genes associated with autoimmunity including IL-36γ, a cytokine associated with a type 17 immune response, and Trem1. Type 17 inflammation has been shown to play a critical role in fibrosis associated with the FBR[119]. $CD9^{+}$ macrophages have also been associated with fibrotic processes related to liver cirrhosis[120] and atherosclerosis[121]. Indeed, when examining other data sets, Sommerfeld et al. also found $CD301b^{-}CD9^{hi}$ macrophages populations within human sarcoma and idiopathic pulmonary fibrosis samples, indicating a possible broader relevance of these markers for identifying a subpopulation of macrophages associated with fibrosis and inflammation.

8. Macrophage ontogeny – tissue-resident macrophages vs. monocyte-derived macrophages

In addition to the different roles that macrophages play based on their activation state, their ontogenetic origin can heavily influence their behavior (Fig. 2). There are two main ontogenetic classes of macrophages: TRMs and moMs. TRMs perform tissue-specific functions to maintain homeostasis within their local niche (reviewed [1, 122, 123]). Although it was originally assumed that TRMs arose from the differentiation of circulating monocytes, recent studies have demonstrated that most TRMs originate from the early yolk sac or fetal liver and populate their resident tissue during fetal development[124–126]. Under homeostatic conditions, many TRM populations are able to self-maintain within their niche via longevity and limited self-renewal, independently of adult hematopoiesis[127], with the notable exception of intestinal TRMs[128]. The niche is critical in driving the maturation of functional, tissue-specific TRMs, irrespective of ontogenetic origin (e.g. yolk sac, fetal liver, or adult monocytes)[129, 130]. Additionally, TRMs themselves are sensitive to the niche in which they reside, and they readily change their transcriptional program in response to new environments[131, 132]. When designing biomaterials for regenerative medicine, it is therefore critical to think about the tissue-specific functions that macrophages must fulfill in order to return to tissue-specific homeostasis and also the niche-specific factors required to promote the maturation of tissue-specific macrophage phenotypes.

Inflammation requires the rapid recruitment of myeloid cells to the site of injury. For this reason, acutely following injury, there is a large influx of $Ly6C^{hi}CCR2^{+}$ monocytes, where their egress from the bone marrow and entry into the inflamed tissue are both CCR2-dependent processes[133, 134]. Monocytes and moMs rapidly become the major class of mononuclear phagocytes at the injury site, where they perform a variety of functions from antigen-presentation to promoting and resolving inflammation to repopulating empty TRM niches (reviewed [135, 136]). A continuum of phenotypic states from monocyte to moM has been demonstrated to exist as monocytes progressively lose monocyte characteristics and gain those of macrophages through a process termed the “monocyte waterfall[137].” In the classical monocyte waterfall model, originally outlined during the process of intestinal macrophage repopulation, monocytes transition through four phases: (P1) $Ly6C^{hi}MHC^{-}II^{-}CCR2^{+}$ monocytes that are phenotypically similar to blood monocytes, (P2) $Ly6C^{+}MHC^{-}II^{+}CCR2^{+}$ monocytes upregulate MHC-II, (P3) $Ly6C^{-}MHC\text{-}II^{+}CCR2^{-}$ intermediates downregulate Ly6C and markers of extravasation such as CCR2, and finally (P4) $Ly6C^{-}$ $MHC\text{-}II^{+}CCR2^{-}$ ($CX3CR1^{hi}CD64^{hi}$) cells upregulate markers such as CX3CR1 giving rise to mature macrophages[138, 139]. A recent study has also shown that, upon arrival in the tissue, $Ly6C^{hi}$ monocytes give rise to two distinct interstitial macrophage populations, one associated with nerve bundles and one with blood vessels, a dichotomy observed across several tissues[140]. In addition to the phenotypic heterogeneity that arises as a result of the monocyte to moM maturation process, monocytes themselves are comprised of multiple subpopulations, most notably the $Ly6C^{hi}$ classical monocytes and $Ly6C^{lo}$ non-classical monocytes in mice ($CD14^{+}CD16^{-}$ and $CD14^{lo}CD16^{+}$ respectively in humans), which have been shown to assume divergent functions during homeostasis and periods of inflammation[37, 135, 141, 142].

9. Outlook

Macrophages are highly heterogeneous, a feature that allows them to play many key roles during homeostasis, inflammation, and wound healing processes. This makes them an attractive target for biomaterial-directed immunomodulatory strategies. However, when it comes to macrophage phenotypes in the biomaterial immune microenvironment, there are many outstanding questions.

Analysis of macrophages present in the FBR has conventionally been done using histology, reporter genes, or flow cytometry techniques, which are all limited in the number of markers they are able to analyze. While useful for gaining a general understanding of the FBR as a whole, defining macrophage populations based off a limited number of protein markers hinders finer understanding of the subpopulations present. Out of these studies has arisen the question of whether 'hybrid' macrophage phenotypes, which express both M1 and M2 macrophage markers, have a role in the FBR[110, 143]. While many behaviors are attributed to macrophages at both ends of the M1/M2 spectrum, the function of intermediary populations remains to be defined[144]. There is the additional question of whether these M1/M2 hybrid cells represent a stable population or are a transient population on their way to one end of the spectrum. A recent study showed that *in vitro* co-stimulation of macrophages with both M1 (LPS and IFN-γ) and M2 (IL-4) stimuli results in a heterogeneous population with a distinct global transcriptional program from M1 and M2 macrophages[145]. Nevertheless, how 'hybrid' macrophages generated using this *in vitro* method relate to *in vivo* populations remains to be explored.

Further confounding *in vivo* application of the M1/M2 phenotype paradigm is the presence of both TRMs and moMs acutely following tissue injury [34, 146]. Immature moMs express higher levels of M1-type markers, whereas mature TRMs express more M2-type markers[131, 147]. During the course of tissue repair, moMs mature and increasingly phenotypically resemble TRMs, a process that can take weeks[147]. Thus, initially, when immature moMs predominate, local macrophage population will appear to be M1 polarized and as the moM population matures the mixed moM/TRM population will appear to switch to an M2 phenotype[34]. Ultimately, this raises the question of whether these markers are solely indicative of macrophage activation, or more reflective of macrophage differentiation and maturity. Additionally, the relative contributions of TRMs and moMs to inflammatory and reparative processes remain to be studied in detail in many disease contexts, and this subject has not been broached at all in the biomaterial environment. However, recently researchers have shown that selective depletion of moMs, but not TRMs ameliorates asbestos-induced lung fibrosis[148]. Thus, exploring the role of macrophage ontogeny in the context of FBR, another fibrotic process, could be illuminating.

More research is needed to further understand the specific roles of macrophage phenotypes in wound healing and fibrotic processes, including in response to implanted biomaterials. Even small differences in macrophage activities may be important in orchestrating these processes, and these differences will likely be appreciated at both the transcriptional and post-transcriptional level (e.g. microRNAs, protein turnover)[149]. Furthermore, it is likely that these differences will be tissue-dependent, time-dependent, and material-dependent, in

cases where biomaterials are implanted. Understanding of macrophage phenotypes in all of these contexts will be required to optimize biomaterial strategies for tissue integration and wound healing.

Traditional immunology techniques such as flow cytometry and quantitative polymerase chain reaction (qPCR) make studying macrophage heterogeneity challenging. Flow cytometry is limited to probing a small number of pre-characterized proteins due to the relatively small number of concurrent markers that can be run (~17 markers) and can be further biased when performing analysis by hand using bivariate dot plots[150]. qPCR is limited by the need for pre-determined primer selection and design as well as the fact that, traditionally, it is a measure of the gene expression of a population of cells and thus not suitable for appreciating cell heterogeneity within this population. With advances in cytometry techniques (e.g. mass cytometry (CyTOF) and spectral flow cytometry), lineage tracing systems, and single-cell transcriptomics, rapid progress is being made in identifying distinct macrophage subpopulations in a variety of contexts. CyTOF allows for the simultaneous measurement of up to 40 markers on a single cell[151]. scRNA-seq allows for transcriptomic analysis through techniques based on next generation sequencing, giving information on the expression of thousands of genes at the single cell level[152]. An additional benefit of scRNA-seq is its unbiased nature. Combined with novel bioinformatics and dimensionality reduction algorithms (e.g. tSNE[153], UMAP[154], FlowSOM[155], SPADE[156]), scRNA-seq can lead to the identification of novel immune cell subpopulations without being biased by the selection of markers. Emerging next-generating adaptions of single-cell transcriptomics (e.g. CITE-seq[157], INs-seq[158]) are being developed that incorporate features of both flow cytometry and scRNA-seq, integrating protein-level and transcriptome-level data from the same cell. CyTOF and scRNA-seq, either alone or in combination, have been used to probe myeloid cell heterogeneity and plasticity within a variety of immunological systems including identification of macrophage subpopulations that contribute to pathological fibrosis in the lung[159] and liver[120], identification of myeloid populations present during kidney injury and repair[160], characterization of myeloid populations in a mouse model of multiple sclerosis[161] and in human MS patients[162], and exploring aortic macrophage heterogeneity in a mouse model of atherosclerosis[121].

When studying immune responses to biomaterials, consideration must also be given to differences in immune system organization between animal models and humans. Due to the difficulty of acquiring biomaterial explants from human patients, the vast majority of biomaterial-related (immunological) studies occur in small animal models. Mice, in particular, are used for this purpose due to their reproducibility, scalability, low cost, and the range of transgenic models readily available for mechanistic studies. Although there is appreciable homology amongst many human and murine immune cell subsets[163], there is a significant body of literature highlighting immunological differences between the two species [164, 165]. These include differences in cellular phenotypes[166], the ratio of myeloid to lymphoid cells in the blood[167], signaling responses to molecules such as IFN-γ[168] and lipopolysaccharide (LPS)[169], macrophage gene expression *in vitro*[170], and transcriptional responses to inflammation[171]. Even considering high-level marker gene/protein expression for general immune populations (e.g. monocytes, neutrophils), it is easy

to identify discrepancies between organisms. For example, murine monocytes, neutrophils, and macrophages can be identified by surface expression of Ly6C, Ly6G, and F4/80 respectively [163]. However, Ly6C and Ly6G have no know human orthologs [172] and the human ortholog for F4/80, EMR1, is an eosinophil-specific receptor in humans[173]. These immunological differences raise concerns over the ability to fully model human immune responses to biomaterials and other immunomodulatory agents in mice. Thus, as we continue to grow a more nuanced and complete understanding of macrophage phenotypes, it will be critical to relate subpopulations identified in humans and mice to avoid designing materials around macrophage subpopulations not present in human patients. One strategy being explored is the development of methods for aligning transcriptomics data across disease states[174], individuals[175], and species[175–177]. Using these methods, two recent studies comparing human and murine mononuclear phagocyte populations reaffirmed the existence of conserved broad immune populations between the two species[175, 176]. However, differences among subtype-specific gene sets[176] and significant variability in macrophage subtype complexity between species[175] caution against blindly extrapolating more specific immune cell states and genes from murine data to human immunology.

Exploration of the biomaterial immune microenvironment using high-dimensional data analysis techniques is in its infancy. Insights gained from their application towards understanding biomaterial-immune-tissue interactions will inform biomaterial design by identifying novel macrophage phenotypes important in biomaterial-driven tissue regeneration and fibrotic processes (Fig. 3). To capitalize on this new knowledge, novel biomaterial strategies will likely also need to be developed. Due to the complexity and plasticity of macrophage responses to tissue injury, it is unlikely that static biomaterial systems will be able to overcome the challenge of integrating with the immune microenvironment to promote biomaterial-tissue integration and tissue repair. Thus, designing biomaterial systems to target one particular macrophage phenotype (typically M2 in past literature) may have limited impact, as even current knowledge indicates that M2 macrophages are not the only ones performing beneficial functions. Instead, novel strategies involving dynamic systems, such as sequential drug delivery platforms[17, 178, 179] or biomaterial delivery of immunomodulatory cells that can actively interact with local immune cells[25, 180, 181], could potentially result in more productive biomaterial-driven macrophage immunomodulation.

Additionally, scRNA-seq is being used to identify novel transcription factors (TFs) and receptors associated with specific macrophage subpopulations. Using biomaterials to delivery molecules that directly target these TFs and receptors could allow localized and prolonged modulation of specific macrophage phenotypes. For example, the transcription factor interferon regulatory factor 5 (IRF5) has been shown to promote monocyte differentiation into inflammatory macrophages[117]. The receptor TREM-1 has been shown to have elevated expression in macrophages associated with fibrosis-inducing biomaterials[118]. Inhibitory peptides have been previously developed for both IRF5[182] and TREM-1[183] that could be potentially incorporated into biomaterials. scRNA-seq data has also demonstrated that macrophage heterogeneity varies between natural and synthetic materials[118]. Given that, it is possible that as we broaden our understanding of macrophage phenotypes related to other materials/material properties (e.g. chemistry,

stiffness, surface topology, adhesivity), we could rationally design materials that elicit certain macrophage phenotypes without the need for additional modulatory proteins/drugs.

Acknowledgements

We thank María Coronel for feedback on the manuscript. The authors acknowledge funding from the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health (R01AR062368 [A.J.G.]) and a National Science Foundation Graduate Research Fellowship [K.E.M.]). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or National Science Foundation.

References

[1]. Kierdorf K, Prinz M, Geissmann F, Gomez Perdiguero E, Development and function of tissue resident macrophages in mice, Semin Immunol 27(6) (2015) 369–378. [PubMed: 27036090]

[2]. Wynn TA, Vannella KM, Macrophages in Tissue Repair, Regeneration, and Fibrosis, Immunity 44(3) (2016) 450–462. [PubMed: 26982353]

[3]. Lech M, Anders H-J, Macrophages and fibrosis: How resident and infiltrating mononuclear phagocytes orchestrate all phases of tissue injury and repair, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1832(7) (2013) 989–997. [PubMed: 23246690]

[4]. Braga TT, Agudelo JSH, Camara NOS, Macrophages During the Fibrotic Process: M2 as Friend and Foe, Frontiers in immunology 6(2015) 602–602. [PubMed: 26635814]

[5]. Sheikh Z, Brooks PJ, Barzilay O, Fine N, Glogauer M, Macrophages, Foreign Body Giant Cells and Their Response to Implantable Biomaterials, Materials (Basel) 8(9) (2015) 5671–5701. [PubMed: 28793529]

[6]. McWhorter FY, Davis CT, Liu WF, Physical and mechanical regulation of macrophage phenotype and function, Cell Mol Life Sci 72(7) (2015) 1303–16. [PubMed: 25504084]

[7]. Vishwakarma A, Bhise NS,Evangelista MB, Rouwkema J, Dokmeci MR, Ghaemmaghami AM, Vrana NE, Khademhosseini A, Engineering immunomodulatory biomaterials to tune the inflammatory response,Trends in biotechnology 34(6) (2016) 470–482. [PubMed: 27138899]

[8]. Wilson CJ, Clegg RE, Leavesley DI, Pearcy MJ, Mediation of Biomaterial—Cell Interactions by Adsorbed Proteins: A Review, Tissue Engineering 11(1-2) (2005) 1–18. [PubMed: 15738657]

[9]. Zhang M, Desai T, Ferrari M, Proteins and cells on PEG immobilized silicon surfaces, Biomaterials 19(10) (1998) 953–960. [PubMed: 9690837]

[10]. Wang C, Yu B, Knudsen B, Harmon J, Moussy F, Moussy Y, Synthesis and Performance of Novel Hydrogels Coatings for lmplantable Glucose Sensors, Biomacromolecules 9(2) (2008) 561–567. [PubMed: 18166014]

[11]. Zhang L, Cao Z, Bai T, Carr L, Ella-Menye J-R, Irvin C, Ratner BD, Jiang S, Zwitterionic hydrogels implanted in mice resist the foreign-body reaction, Nature Biotechnology 31(6) (2013) 553–556.

[12]. Jiang S, Cao Z, Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications, Advanced Materials 22(9) (2010) 920–932. [PubMed: 20217815]

[13]. Schlenoff JB, Zwitteration: Coating Surfaces with Zwitterionic Functionality to Reduce Nonspecific Adsorption, Langmuir 30(32) (2014) 9625–9636. [PubMed: 24754399]

[14]. Jansen LE, Amer LD, Chen EYT,Nguyen TV, Saleh LS,Emrick T, Liu WF, Bryant SJ, Peyton SR, Zwitterionic PEG-PC Hydrogels Modulate the Foreign Body Response in a Modulus-Dependent Manner, Biomacromolecules 19(7) (2018) 2880–2888. [PubMed: 29698603]

[15]. Bridges AW, Singh N, Burns KL, Babensee JE, Andrew Lyon L, García AJ, Reduced acute inflammatory responses to microgel conformal coatings, Biomaterials 29(35) (2008) 4605–4615. [PubMed: 18804859]

[16]. Rostam HM, Fisher LE, Hook AL, Burroughs L, Luckett JC, Figueredo GP, Mbadugha C, Teo ACK, Latif A, Kämmerling L, Day M, Lawler K, Barrett D, Elsheikh S, Ilyas M, Winkler

DA, Alexander MR, Ghaemmaghami AM, Immune-Instructive Polymers Control Macrophage Phenotype and Modulate the Foreign Body Response In Vivo, Matter 2(6) (2020) 1564–1581.

[17]. Spiller KL, Nassiri S, Witherel CE, Anfang RR, Ng J, Nakazawa KR,Yu T, Vunjak-Novakovic G, Sequential delivery of immunomodulatory cytokines to facilitate the M1-to-M2 transition of macrophages and enhance vascularization of bone scaffolds, Biomaterials 37 (2015) 194–207. [PubMed: 25453950]

[18]. Chen J, Li M, Yang C, Yin X, Duan K, Wang J, Feng B, Macrophage phenotype switch by sequential action of immunomodulatory cytokines from hydrogel layers on titani a nanotubes, Colloids and Surfaces B: Biointerfaces 163 (2018) 336–345. [PubMed: 29331905]

[19]. Kim YK, Chu S-H, Hsieh JY, Kamoku CM, Tenner AJ, Liu WF, Wang S-W, Incorporation of a Ligand Peptide for Immune Inhibitory Receptor LAIR-1 on Biomaterial Surfaces Inhibits Macrophage Inflammatory Responses, Advanced Healthcare Materials 6(24) (2017) 1700707.

[20]. Zaveri TD,Lewis JS, Dolgova NV, Clare-Salzler MJ, Keselowsky BG, Integrin-directed modulation of macrophage responses to biomaterials, Biomaterials 35(11) (2014) 3504–3515. [PubMed: 24462356]

[21]. Cha B-H, Shin SR, Leijten J, Li Y-C, Singh S,Liu JC, Annabi N, Abdi R, Dokmeci MR, Vrana NE, Ghaemmaghami AM, Khademhosseini A, Integrin-Mediated Interactions Control Macrophage Polarization in 3D Hydrogels, Advanced Healthcare Materials 6(21) (2017) 1700289.

[22]. Sok MCP, Tria MC, Olingy CE, San Emeterio CL, Botchwey EA, Aspirin-Triggered Resolvin D1-modified materials promote the accumulation of pro-regenerative immune cell subsets and enhance vascular remodeling, Acta Biomaterialia 53 (2017) 109–122. [PubMed: 28213094]

[23]. Sok MCP, Baker N, McClain C, Lim HS,Turner T, Hymel L, Ogle M, Olingy C, Palacios JI, Garcia JR, Srithar K, García AJ, Qiu P, Botchwey EA, Dual delivery of IL-10 and AT-RvD1 from PEG hydrogels polarize immune cells towards pro-regenerative phenotypes, Biomaterials 268(2021) 120475. [PubMed: 33321293]

[24]. García JR, Quirós M, Han WM, O'Leary MN, Cox GN, Nusrat A, García AJ, IFN-γ-tethered hydrogels enhance mesenchymal stem cell-based immunomodulation and promote tissue repair, Biomaterials 220 (2019) 119403. [PubMed: 31401468]

[25]. Clark AY, Martin KE, García JR, Johnson CT, Theriault HS, Han WM, Zhou DW, Botchwey EA, Garcia AJ, Integrin-specific hydrogels modulate transplanted human bone marrow-derived mesenchymal stem cell survival, engraftment, and reparative activities, Nature Communications 11(1) (2020) 114.

[26]. Swartzlander MD, Blakney AK, Amer LD, Hankenson KD,Kyriakides TR,Bryant SJ, Immunomodulation by mesenchymal stem cells combats the foreign body response to cell-laden synthetic hydrogels, Biomaterials 41(2015) 79–88. [PubMed: 25522967]

[27]. Kim YK, Chen EY, Liu WF, Biomolecular strategies to modulate the macrophage response to implanted materials, Journal of Materials Chemistry B 4(9) (2016) 1600–1609. [PubMed: 32263014]

[28]. O'Brien EM, Risser GE, Spiller KL, Sequential drug delivery to modulate macrophage behavior and enhance implant integration, Advanced Drug Delivery Reviews 149(2019) 85–94. [PubMed: 31103451]

[29]. Mukherjee S,Darzi S, Paul K, Werkmeister JA, Gargett CE, Mesenchymal stem cell-based bioengineered constructs: foreign body response, cross-talk with macrophages and impact of biomaterial design strategies for pelvic floor disorders, Interface Focus 9(4) (2019) 20180089. [PubMed: 31263531]

[30]. Alvarez MM,Liu JC, Trujillo-de Santiago G, Cha B-H, Vishwakarma A, Ghaemmaghami AM, Khademhosseini A, Delivery strategies to control inflammatory response: Modulating M1–M2 polarization in tissue engineering applications, Journal of Controlled Release 240 (2016) 349–363. [PubMed: 26778695]

[31]. Zhang Q, Raoof M, Chen Y, Sumi Y,Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ, Circulating mitochondrial DAMPs cause inflammatory responses to injury, Nature 464(7285) (2010) 104–107. [PubMed: 20203610]

[32]. Gong T, Liu L, Jiang W, Zhou R, DAMP-sensing receptors in sterile inflammation and inflammatory diseases, Nature Reviews Immunology 20(2) (2020) 95–112.

[33]. Ogle ME, Segar CE, Sridhar S, Botchwey EA, Monocytes and macrophages in tissue repair: Implications for immunoregenerative biomaterial design, Experimental Biology and Medicine 241(10) (2016) 1084–1097. [PubMed: 27229903]

[34]. Watanabe S, Alexander M, Misharin AV, Budinger GS,The role of macrophages in the resolution of inflammation, J Clin Invest 129(7) (2019) 2619–2628. [PubMed: 31107246]

[35]. Veiseh O, Vegas AJ, Domesticating the foreign body response: recent advances and applications, Advanced Drug Delivery Reviews 144 (2019) 148–161. [PubMed: 31491445]

[36]. Menezes S, Melandri D, Anselmi G,Perchet T, Loschko J, Dubrot J, Patel R, Gautier EL, Hugues S, Longhi MP, Henry JY, Quezada SA, Lauvau G, Lennon-Duménil A-M, Gutiérrez-Martínez E, Bessis A, Gomez-Perdiguero E, Jacome-Galarza CE, Garner H, Geissmann F, Golub R, Nussenzweig MC, Guermonprez P,The Heterogeneity of Ly6C(hi) Monocytes Controls Their Differentiation into iNOS(+) Macrophages or Monocyte-Derived Dendritic Cells, Immunity 45(6) (2016) 1205–1218. [PubMed: 28002729]

[37]. Olingy CE,San Emeterio CL, Ogle ME, Krieger JR, Bruce AC, Pfau DD, Jordan BT, Peirce SM, Botchwey EA, Non-classical monocytes are biased progenitors of wound healing macrophages during soft tissue injury, Scientific Reports 7(1) (2017) 447. [PubMed: 28348370]

[38]. Orecchioni M, Ghosheh Y, Pramod AB, Ley K, Macrophage polarization: different gene signatures in M1 (LPS+) vs. classically and M2 (LPS−) vs. alternatively activated macrophages, Frontiers in immunology 10 (2019) 1084. [PubMed: 31178859]

[39]. Hesketh M, Sahin KB,West ZE, Murray RZ, Macrophage phenotypes regulate scar formation and chronic wound healing, International journal of molecular sciences 18(7) (2017) 1545.

[40]. Mosser DM, Edwards JP, Exploring the full spectrum of macrophage activation, Nature reviews immunology 8(12) (2008) 958–969.

[41]. Biswas SK, Mantovani A, Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm, Nature Immunology 11(10) (2010) 889–896. [PubMed: 20856220]

[42]. Kiss M, Van Gassen S, Movahedi K,Saeys Y, Laoui D, Myeloid cell heterogeneity in cancer: not a single cell aIike, CeIlular Immunology 330 (2018) 188–201.

[43]. Spiller KL, Anfang RR, Spiller KJ, Ng J, Nakazawa KR, Daulton JW, Vunjak-Novakovic G, The role of macrophage phenotype in vascularization of tissue engineering scaffolds, Biomaterials 35(15) (2014) 4477–4488. [PubMed: 24589361]

[44]. Jetten N, Verbruggen S, Gijbels MJ, Post MJ, De Winther MP, Donners MM, Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo, Angiogenesis 17(1) (2014) 109–118. [PubMed: 24013945]

[45]. Pinto AR, Godwin JW, Rosenthal NA, Macrophages in cardiac homeostasis, injury responses and progenitor cell mobilisation, Stem cell research 13(3) (2014) 705–714. [PubMed: 25087895]

[46]. Madsen DH, Leonard D, Masedunskas A, Moyer A, Jürgensen HJ, Peters DE, Amornphimoltham P, Selvaraj A, Yamada SS, Brenner DA, M2-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway, Journal of Cell Biology 202(6) (2013) 951–966.

[47]. Sadtler K, Estrellas K, Allen BW, Wolf MT, Fan H, Tam AJ, Patel CH, Luber BS, Wang H, Wagner KR,Powell JD, Housseau F, Pardoll DM,Elisseeff JH, Developing a pro-regenerative biomaterial scaffold microenvironment requires Thelper 2 cells, Science 352(6283) (2016) 366–370. [PubMed: 27081073]

[48]. Cao Q, Wang Y, Zheng D, Sun Y, Wang Y, Lee VWS,Zheng G,Tan TK, Ince J, Alexander SI, Harris DCH, IL-10/TGF-β-Modified Macrophages Induce Regulatory T Cells and Protect against Adriamycin Nephrosis, Journal of the American Society of Nephrology 21(6) (2010) 933–942. [PubMed: 20299353]

[49]. Soroosh P,Doherty TA, Duan W, Mehta AK, Choi H, Adams YF,Mikulski Z, Khorram N, Rosenthal P, Broide DH, Croft M, Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance, Journal of Experimental Medicine 210(4) (2013) 775–788.

[50]. Haribhai D, Ziegelbauer J, Jia S, Upchurch K, Yan K, Schmitt EG, Salzman NH, Simpson P, Hessner MJ,Chatila TA, Williams CB, Alternatively Activated Macrophages Boost Induced

Regulatory T and Th17 Cell Responses during Immunotherapy for Colitis, The Journal of Immunology 196(8) (2016) 3305–3317. [PubMed: 26927797]

[51]. Hams E, Bermingham R, Fallon PG, Macrophage and Innate Lymphoid Cell Interplay in the Genesis of Fibrosis, Frontiers in Immunology 6(597) (2015).

[52]. Klopfleisch R, Macrophage reaction against biomaterials in the mouse model—Phenotypes, functions and markers, Acta biomaterialia 43 (2016) 3–13. [PubMed: 27395828]

[53]. Ferrante CJ, Pinhal-Enfield G, Elson G, Cronstein BN, Hasko G, Outram S, Leibovich SJ,The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of interleukin-4 receptor alpha (IL-4Rα) signaling, Inflammation 36(4) (2013) 921–931. [PubMed: 23504259]

[54]. Fadok VA, Bratton DL, Konowal A, Freed PW,Westcott JY, Henson PM, Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF,J Clin Invest 101(4) (1998) 890–898. [PubMed: 9466984]

[55]. Novak ML,Koh TJ, Macrophage phenotypes during tissue repair, Journal of leukocyte biology 93(6) (2013) 875–881. [PubMed: 23505314]

[56]. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, De Nardo D,Gohel TD, Emde M, Schmidleithner L, Ganesan H, Nino-Castro A, Mallmann MR, Labzin L, Theis H, Kraut M, Beyer M, Latz E, Freeman TC,Ulas T, Schultze JL,Transcriptome-based network analysis reveals a spectrum model of human macrophage activation, Immunity 40(2) (2014) 274–288. [PubMed: 24530056]

[57]. Davison NL, Gamblin A-L, Layrolle P, Yuan H, de Bruijn JD, Barrère-de Groot F, Liposomal clodronate inhibition of osteoclastogenesis and osteoinduction by submicrostructured beta-tricalcium phosphate, Biomaterials 35(19) (2014) 5088–5097. [PubMed: 24698521]

[58]. Alexander KA, Chang MK, Maylin ER,Kohler T, Müller R, Wu AC, Van Rooijen N, Sweet MJ, Hume DA, Raggatt LJ, Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model, Journal of Bone and Mineral Research 26(7) (2011) 1517–1532. [PubMed: 21305607]

[59]. Claes L, Recknagel S, Ignatius A, Fracture healing under healthy and inflammatory conditions, Nature Reviews Rheumatology 8(3) (2012) 133–143. [PubMed: 22293759]

[60]. Høgevold HE, Grøgaard B, Reikerås O, Effects of short-term treatment with corticosteroids and indomethacin on bone healing: a mechanical study of osteotomies in rats, Acta Orthopaedica Scandinavica 63(6) (1992) 607–611. [PubMed: 1471505]

[61]. Pountos I,Georgouli T,Calori GM, Giannoudis PV, Do nonsteroidal anti-inflammatory drugs affect bone healing? A critical analysis, The Scientific World Journal 2012 (2012).

[62]. Raggatt LJ, Wullschleger ME, Alexander KA, Wu AC, Millard SM, Kaur S, Maugham ML, Gregory LS, Steck R, Pettit AR, Fracture healing via periosteal call us formation requires macrophages for both initiation and progression of early endochondral ossification, The American journal of pathology 184(12) (2014) 3192–3204. [PubMed: 25285719]

[63]. Sinder BP, Pettit AR, McCauley LK, Macrophages: their emerging roles in bone, Journal of Bone and Mineral Research 30(12) (2015) 2140–2149. [PubMed: 26531055]

[64]. Guihard P, Danger Y, Brounais B, David E, Brion R, Delecrin J,Richards CD, Chevalier S, Rédini F, Heymann D, Induction of osteogenesis in mesenchymal stem cells by activated monocytes/macrophages depends on oncostatin M signaling, Stem cells 30(4) (2012) 762–772. [PubMed: 22267310]

[65]. Lu LY, Loi F, Nathan K,Lin T.h., Pajarinen J, Gibon E, Nabeshima A, Cordova L, Jämsen E,Yao Z, Pro-inflammatory M1 macrophages promote Osteogenesis by mesenchymal stem cells via the COX-2-prostaglandin E2 pathway, Journal of Orthopaedic Research 35(11) (2017) 2378–2385. [PubMed: 28248001]

[66]. Schmidt-Bleek K, Schell H, Schulz N, Hoff P, Perka C, Buttgereit F, Volk H-D,Lienau J,Duda GN, Inflammatory phase of bone healing initiates the regenerative healing cascade, Cell and tissue research 347(3) (2012) 567–573. [PubMed: 21789579]

[67]. Jetten N, Roumans N, Gijbels MJ, Romano A, Post MJ, de Winther MP, van der Hulst RR, Xanthoulea S, Wound administration of M2-polarized macrophages does not improve murine cutaneous healing responses, PLoS One 9(7) (2014) e102994. [PubMed: 25068282]

[68]. Pellicoro A, Ramachandran P,Iredale JP, Fallowfield JA, Liver fibrosis and repair: immune regulation of wound healing in a solid organ, Nature Reviews Immunology 14(3) (2014) 181–194.

[69]. Pechkovsky DV, Prasse A, Kollert F, Engel KM, Dentler J, Luttmann W, Friedrich K, Müller-Quernheim J, Zissel G, Alternatively activated alveolar macrophages in pulmonary fibrosis—mediator production and intracellular signal transduction, Clinical immunology 137(1) (2010) 89–101. [PubMed: 20674506]

[70]. Ide M,Kuwamura M,Kotani T, Sawamoto O,Yamate J, Effects of Gadolinium Chloride (GdCI3) on the Appearance of Macrophage Populations and Fibrogenesis in Thioacetamide-Induced Rat Hepatic Lesions, Journal of Comparative Pathology 133(2) (2005) 92–102. [PubMed: 15964588]

[71]. Karlmark KR, Weiskirchen R, Zimmermann HW, Gassier N, Ginhoux F, Weber C, Merad M, Luedde T,Trautwein C, Tacke F, Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis, Hepatology 50(1) (2009) 261–274. [PubMed: 19554540]

[72]. Shook BA, Wasko RR,Rivera-Gonzalez GC, Salazar-Gatzimas E, López-Giráldez F, Dash BC, Muñoz-Rojas AR, Aultman KD, Zwick RK, Lei V, Arbiser JL, Miller-Jensen K, Clark DA, Hsia HC, Horsley V, Myofibroblast proliferation and heterogeneity are supported by macrophages during skin repair, Science 362(6417) (2018) eaar2971. [PubMed: 30467144]

[73]. Geervliet E, Bansal R, Matrix Metalloproteinases as Potential Biomarkers and Therapeutic Targets in Liver Diseases, Cells 9(5) (2020) 1212.

[74]. Roderfeld M, Matrix metalloproteinase functions in hepatic injury and fibrosis, Matrix Biology 68–69 (2018) 452–462.

[75]. Ruiz V, Ordóñez RM, Berumen J, Ramírez R, Uhal B, Becerril C, Pardo A, Selman M, Unbalanced collagenases/TIMP-1expression and epithelial apoptosis in experimental lung fibrosis, American Journal of Physiology-Lung Cellular and Molecular Physiology 285(5) (2003) L1026–L1036. [PubMed: 12882763]

[76]. Popov Y, Sverdlov DY, Bhaskar KR, Sharma AK, Millonig G, Patsenker E, Krahenbuhl S, Krahenbuhl L, Schuppan D, Macrophage-mediated phagocytosis of apoptotic cholangiocytes contributes to reversal of experimental biliary fibrosis, American Journal of Physiology-Gastrointestinal and Liver Physiology 298(3) (2010) G323–G334. [PubMed: 20056896]

[77]. Ramachandran P, Pellicoro A, Vernon MA, Boulter L, Aucott RL, Ali A, Hartland SN, Snowdon VK, Cappon A,Gordon-Walker TT, Williams MJ, Dunbar DR,Manning JR, van Rooijen N, Fallowfield JA, Forbes SJ, Iredale JP, Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis, Proceedings of the National Academy of Sciences 109(46) (2012) E3186–E3195.

[78]. Fallowfield JA, Mizuno M,Kendall TJ, Constandinou CM, Benyon RC,Duffield JS, Iredale JP, Scar-Associated Macrophages Are a Major Source of Hepatic Matrix Metalloproteinase-13 and Facilitate the Resolution of Murine Hepatic Fibrosis, The Journal of Immunology 178(8) (2007) 5288–5295. [PubMed: 17404313]

[79]. Hironaka K, Sakaida I,Matsumura Y, Kaino S, Miyamoto K, Okita K, Enhanced Interstitial Collagenase (Matrix Metalloproteinase-13) Production of Kupffer Cell by Gadolinium Chloride Prevents Pig Serum-induced Rat Liver Fibrosis, Biochemical and Biophysical Research Communications 267(1) (2000) 290–295. [PubMed: 10623612]

[80]. Duffield JS, Forbes SJ,Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP, Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair, J Clin Invest 115(1) (2005) 56–65. [PubMed: 15630444]

[81]. Weng S-Y, Wang X, Vijayan S,Tang Y, Kim YO, Padberg K,Regen T, Molokanova O,Chen T, Bopp T, IL-4 receptor alpha signaling through macrophages differentially regulates liver fibrosis progression and reversal, EBioMedicine 29(2018) 92–103. [PubMed: 29463471]

[82]. Carmeliet P, Angiogenesis in health and disease, Nature Medicine 9(6) (2003) 653–660.

[83]. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C, Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction, Blood 116(5) (2010) 829–840. [PubMed: 20404134]

[84]. Chambers SE, 0'Neill CL, O'Doherty TM, Medina RJ, Stitt AW, The role of immune-related myeloid cells in angiogenesis, Immunobiology 218(11) (2013) 1370–1375. [PubMed: 23932437]

[85]. Moore EM, Suresh V, Ying G, West JL, M0 and M2 Macrophages Enhance Vascularization of Tissue Engineering Scaffolds, Regenerative Engineering and Translational Medicine 4(2) (2018) 51–61.

[86]. Willenborg S,Lucas T, Van Loo G, Knipper JA,Krieg T, Haase I, Brachvogel B, Hammerschmidt M, Nagy A, Ferrara N, CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair, Blood 120(3) (2012) 613–625. [PubMed: 22577176]

[87]. Graney P, Ben-Shaul S, Landau S, Bajpai A, Singh B, Eager J, Cohen A, Levenberg S, Spiller K, Macrophages of diverse phenotypes drive vascularization of engineered tissues, Science Advances 6(18) (2020) eaay6391. [PubMed: 32494664]

[88]. Gurevich DB, Severn CE, Twomey C, Greenhough A, Cash J, Toye AM, MeIlor H, Martin P, Live imaging of wound angiogenesis reveals macrophage orchestrated vessel sprouting and regression,The EMBO journal 37(13) (2018) e97786. [PubMed: 29866703]

[89]. Anderson JM, Rodriguez A, Chang DT, Foreign body reaction to biomaterials, Semin Immunol 20(2) (2008) 86–100. [PubMed: 18162407]

[90]. Rahmati M, Silva EA, Reseland JE,Heyward CA, Haugen HJ, Biological responses to physicochemical properties of biomaterial surface, Chemical Society Reviews 49(15) (2020) 5178–5224. [PubMed: 32642749]

[91]. Selders GS, Fetz AE, Radic MZ, Bowlin GL, An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration, Regen Biomater 4(1) (2017) 55–68. [PubMed: 28149530]

[92]. Li AG, Quinn MJ, Siddiqui Y, Wood MD, Federiuk IF, Duman HM, Ward WK, Elevation of transforming growth factor beta (TGFβ) and its downstream mediators in subcutaneous foreign body capsule tissue, Journal of Biomedical Materials Research Part A 82A(2) (2007) 498–508.

[93]. Mooney JE, Rolfe BE,Osborne GW, Sester DP, van Rooijen N, Campbell GR, Hume DA, Campbell JH, Cellular Plasticity of Inflammatory Myeloid Cells in the Peritoneal Foreign Body Response, The American Journal of Pathology 176(1) (2010) 369–380. [PubMed: 20008135]

[94]. Blakney AK, Swartzlander MD, Bryant SJ, The effects of substrate stiffness on the in vitro activation of macrophages and in vivo host response to poly(ethylene glycol)-based hydrogels, J Biomed Mater Res A 100(6) (2012) 1375–1386. [PubMed: 22407522]

[95]. Lee TT, García JR, Paez JI, Singh A, Phelps EA, Weis S,Shafiq Z, Shekaran A, del Campo A, García AJ, Light-triggered in vivo activation of adhesive peptides regulates cell adhesion, inflammation and vascularization of biomaterials, Nature Materials 14(3) (2015) 352–360. [PubMed: 25502097]

[96]. Sussman EM, Halpin MC, Muster J, Moon RT, Ratner BD, Porous implants modulate healing and induce shifts in local macrophage polarization in the foreign body reaction, Ann Biomed Eng 42(7) (2014) 1508–16. [PubMed: 24248559]

[97]. Veiseh O, Doloff JC, Ma M, Vegas AJ, Tam HH, Bader AR, Li J, Langan E, Wyckoff J, Loo WS, Jhunjhunwala S, Chiu A,Siebert S, Tang K, Hollister-Lock J, Aresta-Dasilva S, Bochenek M,Mendoza-Elias J, Wang Y, Qi M, Lavin DM, Chen M, Dholakia N, Thakrar R, Lacík I, Weir GC, Oberholzer J, Greiner DL, Langer R, Anderson DG, Size- and shape-dependent foreign body immune response to materials implanted in rodents and non-human primates, Nature materials 14(6) (2015) 643–651. [PubMed: 25985456]

[98]. Dondossola E, Holzapfel BM, Alexander S, Filippini S, Hutmacher DW, Friedl P, Examination of the foreign body response to biomaterials by nonlinear intravital microscopy, Nature Biomedical Engineering 1(1) (2016) 0007.

[99]. Doloff JC, Veiseh O, Vegas AJ, Tam HH, Farah S, Ma M, Li J, Bader A, Chiu A, Sadraei A, Aresta-Dasilva S, Griffin M,Jhunjhunwala S, Webber M,Siebert S, Tang K, Chen M, Langan E, Dholokia N, Thakrar R, Qi M, Oberholzer J, Greiner DL, Langer R, Anderson DG, Colony

stimulating factor-1 receptor is a central component of the foreign body response to biomaterial implants in rodents and non-human primates, Nature Materials 16(6) (2017) 671–680. [PubMed: 28319612]

[100]. Gurevich DB, French KE, Collin JD, Cross SJ, Martin P, Live imaging the foreign body response in zebrafish reveals how dampening inflammation reduces fibrosis, Journal of Cell Science 133(5) (2020) jcs236075.

[101]. Brown BN, Londono R, Tottey S, Zhang L, Kukla KA, Wolf MT, Daly KA,Reing JE,Badylak SF, Macrophage phenotype as a predictor of constructive remodeling following the implantation of biologically derived surgical mesh materials, Acta biomaterialia 8(3) (2012) 978–987. [PubMed: 22166681]

[102]. Wolf MT, Dearth CL, Ranallo CA, LoPresti ST, Carey LE, Daly KA, Brown BN, Badylak SF, Macrophage polarization in response to ECM coated polypropylene mesh, Biomaterials 35(25) (2014) 6838–6849. [PubMed: 24856104]

[103]. McNally AK, Anderson JM, Phenotypic expression in human monocyte-derived interleukin-4-induced foreign body giant cells and macrophages in vitro: Dependence on material surface properties, Journal of Biomedical Materials Research Part A 103(4) (2015) 1380–1390. [PubMed: 25045023]

[104]. McNally AK, De Fife KM, Anderson JM, Interleukin-4-induced macrophage fusion is prevented by inhibitors of mannose receptor activity, The American journal of pathology 149(3) (1996) 975–985. [PubMed: 8780401]

[105]. Higgins DM, Basaraba RJ, Hohnbaum AC, Lee EJ, Grainger DW, Gonzalez-Juarrero M, Localized immunosuppressive environment in the foreign body response to implanted biomaterials, The American journal of pathology 175(1) (2009) 161–170. [PubMed: 19528351]

[106]. Hernandez-Pando R, Bornstein Q, Aguilar Leon D, Orozco E, Madrigal V, Martinez Cordero E, Inflammatory cytokine production by immunological and foreign body multinucleated giant cells, Immunology 100(3) (2000) 352–358. [PubMed: 10929057]

[107]. Anderson JM, Jones JA, Phenotypic dichotomies in the foreign body reaction, Biomaterials 28(34) (2007) 5114–5120. [PubMed: 17706278]

[108]. Palmer JA, Abberton KM, Mitchell GM, Morrison WA, Macrophage phenotype in response to implanted synthetic scaffolds: an immunohistochemical study in the rat, Cells Tissues Organs 199(2-3) (2014) 169–183. [PubMed: 25412799]

[109]. Badylak SF, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM, Macrophage phenotype as a determinant of biologic scaffold remodeling,Tissue Engineering Part A 14(11) (2008) 1835–1842. [PubMed: 18950271]

[110]. Witherel CE, Abebayehu D,Barker TH, Spiller KL, Macrophage and Fibroblast Interactions in Biomaterial-Mediated Fibrosis, Advanced healthcare materials 8(4) (2019) 1801451.

[111]. Sadtler K, Allen BW, Estrellas K, Housseau F, Pardoll DM, Elisseeff JH, The Scaffold Immune Microenvironment: Biomaterial-Mediated Immune Polarization in Traumatic and Nontraumatic Applications<sup/>, Tissue Eng Part A 23(19-20) (2017) 1044–1053. [PubMed: 27736323]

[112]. Sadtler K, Wolf MT,Ganguly S, Moad CA, Chung L,Majumdar S, Housseau F, Pardoll DM, Elisseeff JH, Divergent immune responses to synthetic and biological scaffolds, Biomaterials 192 (2019) 405–415. [PubMed: 30500722]

[113]. Wolf MT, Ganguly S,Wang TL, Anderson CW, Sadtler K, Narain R, Cherry C, Parrillo AJ, Park BV,Wang G, Pan F,Sukumar S, Pardoll DM, Elisseeff JH, A biologic scaffold—associated type 2 immune microenvironment inhibits tumor formation and synergizes with checkpoint immunotherapy, Science Translational Medicine 11(477) (2019) eaat7973. [PubMed: 30700576]

[114]. Coronel MM, Martin KE, Hunckler MD, Barber G, O'Neill EB, Medina JD, Opri E,McClain CA, Batra L, Weaver JD, Lim HS, Qiu P, Botchwey EA, Yolcu ES, Shirwan H, García AJ, Immunotherapy via PD-L1-presenting biomaterials leads to long-term islet graft survival, Science Advances 6(35) (2020) eaba5573. [PubMed: 32923626]

[115]. Sheng J, Chen Q, Soncin I, Ng SL, Karjalainen K,Ruedl C, A discrete subset of monocyte-derived cells among typical conventional type 2 dendritic cells can efficiently cross-present, Cell reports 21(5) (2017) 1203–1214. [PubMed: 29091760]

[116]. Cole JE, Park I, Abeam DJ, Kassiteridi C, Danso Abeam D, Goddard ME, Green P, Maffia P, Monaco C, Immune cell census in murine atherosclerosis: cytometry by time of flight illuminates vascular myeloid cell diversity, Cardiovascular research 114(10) (2018) 1360–1371. [PubMed: 29726984]

[117]. Corbin AL, Gomez-Vazquez M, Berthold DL, Attar M, Arnold IC, Powrie FM, Sansom SN, Udalova IA, IRF5 guides monocytes toward an inflammatory CD11c^{+} macrophage phenotype and promotes intestinal inflammation, Science Immunology 5(47) (2020) eaax6085. [PubMed: 32444476]

[118]. Sommerfeld SD, Cherry C, Schwab RM, Chung L, Maestas DR, Laffont P, Stein JE, Tam A, Ganguly S, Housseau F,Taube JM, Pardoll DM, Cahan P,Elisseeff JH, Interleukin-36γ —producing macrophages drive IL-17-mediated fibrosis, Science Immunology 4(40) (2019) eaax4783. [PubMed: 31604843]

[119]. Chung L, Maestas DR, Lebid A, Mageau A,Rosson GD,Wu X, Wolf MT, Tam AJ, Vanderzee I,Wang X, Andorko JI, Zhang H, Narain R, Sadtler K, Fan H, Čiháková D, Le Saux CJ, Housseau F, Pardoll DM, Elisseeff JH, Interleukin 17 and senescent cells regulate the foreign body response to synthetic material implants in mice and humans, Science Translational Medicine 12(539) (2020) eaax3799. [PubMed: 32295900]

[120]. Ramachandran P, Dobie R,Wilson-Kanamori JR, Dora EF, Henderson BEP, Luu NT, Portman JR, Matchett KP, Brice M,Marwick JA, Taylor RS, Efremova M, Vento-Tormo R, Carragher NO,Kendall TJ, Fallowfield JA, Harrison EM, Mole DJ,Wigmore SJ, Newsome PN, Weston CJ,Iredale JP, Tacke F,Pollard JW,Ponting CP, Marioni JC, Teichmann SA, Henderson NC, Resolving the fibrotic niche of human liver cirrhosis at single-cell level, Nature 575(7783) (2019) 512–518. [PubMed: 31597160]

[121]. Cochain C, Vafadarnejad E, Arampatzi P, Pelisek J, Winkels H, Ley K, Wolf D, Saliba A-E, Zernecke A, Single-cell RNA-seq reveals the transcriptional landscape and heterogeneity of aortic macrophages in murine atherosclerosis, Circulation research 122(12) (2018) 1661–1674. [PubMed: 29545365]

[122]. Gordon S, Plüddemann A, Tissue macrophages: heterogeneity and functions, BMC Biology 15(1) (2017) 53. [PubMed: 28662662]

[123]. Davies LC, Jenkins SJ, Allen JE, Taylor PR, Tissue-resident macrophages, Nature Immunology 14(10) (2013) 986–995. [PubMed: 24048120]

[124]. Bian Z,Gong Y,Huang T,Lee CZW, Bian L,Bai Z, Shi H,Zeng Y,Liu C,He J, Zhou J,Li X, Li Z, Ni Y,Ma C, Cui L, Zhang R, Chan JKY, Ng LG, Lan Y, Ginhoux F, Liu B, Deciphering human macrophage development at single-cell resolution, Nature 582(7813) (2020) 571–576. [PubMed: 32499656]

[125]. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, Garner H, Trouillet C, de Bruijn MF, Geissmann F, Rodewald H-R, Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors, Nature 518(7540) (2015) 547–551. [PubMed: 25470051]

[126]. Yona S, Kim K-W, Wolf Y, Mildner A, Varol D, Breker M, Strauss-Ayali D, Viukov S, Guilliams M, Misharin A, Hume David A., Perlman H, Malissen B, Zelzer E, Jung S, Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis, Immunity 38(1) (2013) 79–91. [PubMed: 23273845]

[127]. Hashimoto D, Chow A, Noizat C, Teo P, Mary B Beasley, Leboeuf M, Becker Christian D., See P, Price J, Lucas D, Greter M,Mortha A, Boyer Scott W., Forsberg EC, Tanaka M, van Rooijen N, García-Sastre A, Stanley ER, Ginhoux F, Frenette Paul S., Merad M, Tissue-Resident Macrophages Self-Maintain Locally throughout Adult Life with Minimal Contribution from Circulating Monocytes, Immunity 38(4) (2013) 792–804. [PubMed: 23601688]

[128]. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, Malissen B, Osborne LC, Artis D, Mowat AM, Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice, Nature Immunology 15(10) (2014) 929–937. [PubMed: 25151491]

[129]. Guilliams M, Scott CL, Does niche competition determine the origin of tissue-resident macrophages?, Nature Reviews Immunology 17(7) (2017) 451–460.

[130]. van de Laar L, Saelens W, De Prijck S, Martens L, Charlotte L Scott, Van Isterdael G, Hoffmann E, Beyaert R,Saeys Y, Lambrecht Bart N., Guilliams M,Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages, Immunity44(4) (2016) 755–768. [PubMed: 26992565]

[131]. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, Jung S, Amit I, Tissue-Resident Macrophage Enhancer Landscapes Are Shaped by the Local Microenvironment, Cell 159(6) (2014) 1312–1326. [PubMed: 25480296]

[132]. Gosselin D, Link VM, Casey E Romanoski, Fonseca GregoryJ., Eichenfield DawnZ., Spann Nathanael J., Stender Joshua D., Chun Hyun B., Garner H, Geissmann F, Glass Christopher K., Environment Drives Selection and Function of Enhancers Controlling Tissue-Specific Macrophage Identities, Cell 159(6) (2014) 1327–1340. [PubMed: 25480297]

[133]. Tsou C-L, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, Mack M, Charo IF, Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites, J Clin Invest 117(4) (2007) 902–909. [PubMed: 17364026]

[134]. Tacke F, Alvarez D,Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, Garin A, Liu J, Mack M, van Rooijen N,Lira SA, Habenicht AJ,Randolph GJ, Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques, J Clin Invest 117(1) (2007) 185–194. [PubMed: 17200718]

[135]. Guilliams M, Mildner A, Yona S, Developmental and Functional Heterogeneity of Monocytes, Immunity 49(4) (2018) 595–613. [PubMed: 30332628]

[136]. Jakubzick CV, Randolph GJ, Henson PM, Monocyte differentiation and antigen-presenting functions, Nature Reviews Immunology 17(6) (2017) 349–362.

[137]. Bain CC, Schridde A, Origin, Differentiation, and Function of Intestinal Macrophages, Frontiers in Immunology 9(2733) (2018).

[138]. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O,Grip O, Guilliams M, Malissen B, Agace WW, Mowat AM, Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors, Mucosal Immunology 6(3) (2013) 498–510. [PubMed: 22990622]

[139]. Tamoutounour S, Henri S, Lelouard H, de Bovis B, de Haar C, van der Woude CJ, Woltman AM,Reyal Y, Bonnet D, Sichien D,Bain CC, Mowat AM, Reis e Sousa C, Poulin LF, Malissen B, Guilliams M, CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis, European Journal of Immunology 42(12) (2012) 3150–3166. [PubMed: 22936024]

[140]. Chakarov S, Lim HY, Tan L, Lim SY, See P, Lum J, Zhang X-M, Foo S, Nakamizo S, Duan K, Kong WT, Gentek R, Balachander A, Carbajo D, Bleriot C, Malleret B,Tam JKC, Baig S, Shabeer M,Toh S-AES, Schlitzer A, Larbi A,Marichal T, Malissen B, Chen J, Poidinger M, Kabashima K, Bajenoff M, Ng LG, Angeli V, Ginhoux F,Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches, Science 363(6432) (2019) eaau0964. [PubMed: 30872492]

[141]. Geissmann F, Jung S, Littman DR, Blood Monocytes Consist of Two Principal Subsets with Distinct Migratory Properties, Immunity 19(1) (2003) 71–82. [PubMed: 12871640]

[142]. Jakubzick C, Gautier Emmanuel L. , Gibbings Sophie L., Sojka Dorothy K., Schlitzer A, Johnson Theodore E.,Ivanov S,Duan Q, Bala S,Condon T, van Rooijen N,Grainger John R.,Belkaid Y, Ma[ISP CHK]'ayan A, Riches David W.H., Yokoyama Wayne M., Ginhoux F, Henson Peter M., Randolph Gwendalyn J., Minimal Differentiation of Classical Monocytes as They Survey Steady-State Tissues and Transport Antigen to Lymph Nodes, Immunity39(3) (2013) 599–610. [PubMed: 24012416]

[143]. Witherel CE, Sao K, Brisson BK, Han B, Volk SW, Petrie RJ, Han L, Spiller KL, Regulation of extracellular matrix assembly and structure by hybrid M1/M2 macrophages, Biomaterials 269 (2021) 120667. [PubMed: 33450585]

[144]. Mills CD, Ley K, Ml and M2 macrophages: the chicken and the egg of immunity, Journal of innate immunity 6(6) (2014) 716–726. [PubMed: 25138714]

[145]. Muñoz-Rojas AR, Kelsey I, Pappalardo JL, Chen M, Miller-Jensen K, Co-stimulation with opposing macrophage polarization cues leads to orthogonal secretion programs in individual cells, Nature Communications 12(1) (2021) 301.

[146]. Davenport Huyer L, Pascual-Gil S, Wang Y, Mandla S, Yee B, Radisic M, Advanced strategies for modulation of the material-macrophage interface, Advanced Functional Materials 30(44) (2020) 1909331.

[147]. Misharin AV, Morales-Nebreda L, Reyfman PA,Cuda CM, Walter JM, McQuattie-Pimentel AC, Chen C-I, Anekalla KR, Joshi N, Williams KJN, Abdala-Valencia H,Yacoub TJ, Chi M, Chiu S, Gonzalez-Gonzalez FJ, Gates K, Lam AP,Nicholson TT, Homan PJ, Soberanes S, Dominguez S, Morgan VK, Saber R, Shaffer A, Hinchcliff M, Marshall SA, Bharat A, Berdnikovs S, Bhorade SM, Bartom ET, Morimoto RI, Balch WE, Sznajder JI, Chandel NS, Mutlu GM, Jain M, Gottardi CJ, Singer BD, Ridge KM, Bagheri N, Shilatifard A, Budinger GRS, Perlman H, Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span, Journal of Experimental Medicine214(8) (2017) 2387–2404.

[148]. Joshi N, Watanabe S, Verma R, Jablonski RP, Chen C-I, Cheresh P, Markov NS, Reyfman PA, McQuattie-Pimentel AC, Sichizya L, Lu Z, Piseaux-Aillon R, Kirchenbuechler D, Flozak AS, Gottardi CJ, Cuda CM, Perlman H, Jain M, Kamp DW, Budinger GRS, Misharin AV, A spatially restricted fibrotic niche in pulmonary fibrosis is sustained by M-CSF/M-CSFR signalling in monocyte-derived alveolar macrophages, European Respiratory Journal 55(1) (2020) 1900646.

[149]. Williams JW,Giannarelli C, Rahman A, Randolph GJ,Kovacic JC, Macrophage Biology, Classification, and Phenotype in Cardiovascular Disease: JACC Macrophage in CVD Series (Part 1), J Am Coll Cardiol 72(18) (2018) 2166–2180. [PubMed: 30360826]

[150]. Palit S, Heuser C, de Almeida GP, Theis FJ, Zielinski CE, Meeting the Challenges of High-Dimensional Single-Cell Data Analysis in Immunology, Frontiers in immunology 10(2019) 1515–1515. [PubMed: 31354705]

[151]. Spitzer MH, Nolan GP, Mass Cytometry: Single Cells, Many Features, Cell 165(4) (2016) 780–791. [PubMed: 27153492]

[152]. Tang F, Barbacioru C,Wang Y, Nordman E, Lee C, Xu N,Wang X,Bodeau J, Tuch BB, Siddiqui A, Lao K, Surani MA, mRNA-Seq whole-transcriptome analysis of a single cell, Nature Methods 6(5) (2009) 377–382. [PubMed: 19349980]

[153]. Maaten Lv.d., Hinton G, Visualizing data using t-SNE, Journal of machine learning research 9(Nov) (2008) 2579–2605.

[154]. Becht E, McInnes L, Healy J, Dutertre C-A, Kwok IWH, Ng LG, Ginhoux F, Newell EW, Dimensionality reduction for visualizing single-cell data using UMAP, Nature Biotechnology 37(1) (2019) 38–44.

[155]. Van Gassen S, Callebaut B, Van Helden MJ, Lambrecht BN, Demeester P,Dhaene T, Saeys Y, FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data, Cytometry Part A 87(7) (2015) 636–645.

[156]. Qiu P, Simonds EF, Bendall SC, Gibbs KD, Bruggner RV, Linderman MD, Sachs K, Nolan GP, Plevritis SK, Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE, Nature Biotechnology 29(10) (2011) 886–891.

[157]. Stoeckius M, Hafemeister C, Stephenson W, Houck-Loomis B, Chattopadhyay PK, Swerdlow H, Satija R, Smibert P, Simultaneous epitope and transcriptome measurement in single cells, Nature Methods 14(9) (2017) 865–868. [PubMed: 28759029]

[158]. Katzenelenbogen Y, Sheban F, Yalin A, Yofe I, Svetlichnyy D, Jaitin DA, Bornstein C, Moshe A, Keren-Shaul H, Cohen M, Wang S-Y, Li B, David E,Salame T-M, Weiner A, Amit I, Coupled scRNA-Seq and Intracellular Protein Activity Reveal an Immunosuppressive Role of TREM2 in Cancer , Cell 182(4) (2020) 872–885.el9. [PubMed: 32783915]

[159]. Aran D, Looney AP, Liu L, Wu E, Fong V, Hsu A, Chak S, Naikawadi RP, Wolters PJ, Abate AR, Butte AJ, Bhattacharya M, Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage, Nature Immunology 20(2) (2019) 163–172. [PubMed: 30643263]

[160]. Conway BR, O'Sullivan ED, Cairns C, O'Sullivan J,Simpson DJ, Salzano A, Connor K, Ding P, Humphries D, Stewart K, Teenan O, Pius R, Henderson NC, Bénézech C, Ramachandran P, Ferenbach D, Hughes J, Chandra T, Denby L, Kidney Single-Cell Atlas Reveals Myeloid Heterogeneity in Progression and Regression of Kidney Disease, Journal of the American Society of Nephrology (2020) ASN.2020060806.

[161]. Jordão MJC, Sankowski R,Brendecke SM, Sagar G, Locatelli, Tai Y-H, Tay TL, Schramm E, Armbruster S, Hagemeyer N, Groß O, Mai D, Çiçek Ö, Falk T, Kerschensteiner M, Grün D, Prinz M, Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation, Science 363(6425) (2019) eaat7554. [PubMed: 30679343]

[162]. Schafflick D, Xu CA, Hartlehnert M, Cole M, Schulte-Mecklenbeck A,Lautwein T, Wolbert J, Heming M, Meuth SG,Kuhlmann T, Gross CC, Wiendl H, Yosef N, Meyerzu Horste G, Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis, Nature Communications 11(1) (2020) 247.

[163]. Reynolds G, Haniffa M, Human and mouse mononuclear phagocyte networks: a tale of two species?, Frontiers in immunology 6(2015) 330. [PubMed: 26124761]

[164]. Zschaler J, Schlorke D,Arnhold J, Differences in innate immune response between man and mouse, Critical Reviews™ in Immunology 34(5) (2014).

[165]. Mestas J, Hughes CC, Of mice and not men: differences between mouse and human immunology, The Journal of Immunology 172(5) (2004) 2731–2738. [PubMed: 14978070]

[166]. Bjornson-Hooper ZB, Fragiadakis GK, Spitzer MH, Madhireddy D, McIlwain D, Nolan GP, A comprehensive atlas of immunological differences between humans, mice and non-human primates, biorxiv (2019) 574160.

[167]. Rongvaux A, Takizawa H,Strowig T,Willinger T, Eynon EE, Flavell RA, Manz MG, Human hemato-lymphoid system mice: current use and future potential for medicine, Annual review of immunology 31(2013) 635–674.

[168]. Coers J, Starnbach MN, Howard JC, Modeling infectious disease in mice: co-adaptation and the role of host-specific IFNγ responses, PLoS Pathog 5(5) (2009) e1000333. [PubMed: 19478881]

[169]. Schneemann M,Schoedon G, Hofer S, Blau N, Guerrero L, Schaffner A, Nitric oxide synthase is not a constituent of the antimicrobial armature of human mononuclear phagocytes, Journal of Infectious Diseases 167(6) (1993) 1358–1363.

[170]. Spiller KL, Wrona EA,Romero-Torres S, Pallotta I, Graney PL,Witherel CE, Panicker LM, Feldman RA, Urbanska AM, Santambrogio L, Differential gene expression in human, murine, and cell line-derived macrophages upon polarization, Experimental cell research 347(1) (2016) 1–13. [PubMed: 26500109]

[171]. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Genomic responses in mouse models poorly mimic human inflammatory diseases, Proceedings of the National Academy of Sciences 110(9) (2013) 3507–3512.

[172]. Upadhyay G, Emerging role of lymphocyte antigen-6 family of genes in cancer and immune cells, Frontiers in immunology 10 (2019) 819. [PubMed: 31068932]

[173]. Hamann J, Koning N, Pouwels W, Ulfman LH, van Eijk M, Stacey M, Lin HH, Gordon S, Kwakkenbos MJ, EMR1, the human homolog of F4/80, is an eosinophil-specific receptor, European journal of immunology 37(10) (2007) 2797–2802. [PubMed: 17823986]

[174]. Peters JM, Blainey PC, Bryson BD, Consensus transcriptional states describe human mononuclear phagocyte diversity in the lung across health and disease, bioRxiv (2020).

[175]. Zilionis R, Engblom C, Pfirschke C, Savova V, Zemmour D, Saatcioglu HD, Krishnan I,Maroni G,Meyerovitz CV, Kerwin CM,Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species, Immunity 50(5) (2019) 1317–1334.e10. [PubMed: 30979687]

[176]. Leach SM, Gibbings SL, Tewari AD, Atif SM, Vestal B, Danhorn T, Janssen WJ, Wager TD, Jakubzick CV, Human and mouse transcriptome profiling identifies cross-species homology in pulmonary and lymph node mononuclear phagocytes, Cell reports 33(5) (2020) 108337. [PubMed: 33147458]

[177]. Geirsdottir L, David E, Keren-Shaul H, Weiner A, Bohlen SC, Neuber J, Balic A, Giladi A, Sheban F, Dutertre C-A, Cross-species single-cell analysis reveals divergence of the primate microglia program, Cell 179(7) (2019) 1609–1622.e16. [PubMed: 31835035]

[178]. Li T, Peng M,Yang Z, Zhou X,Deng Y, Jiang C, Xiao M, Wang J, 3D-printed IFN-γ-loading calcium silicate-β-tricalcium phosphate scaffold sequentially activates M1 and M2 polarization

of macrophages to promote vascularization of tissue engineering bone, Acta Biomater 71 (2018) 96–107. [PubMed: 29549051]

[179]. Alhamdi JR,Peng T, Al-Naggar IM, Hawley KL, Spiller KL, Kuhn LT,Controlled M1-to-M2 transition of aged macrophages by calcium phosphate coatings, Biomaterials 196 (2019) 90–99. [PubMed: 30075952]

[180]. Su N, Gao P-L, Wang K, Wang J-Y, Zhong Y,Luo Y, Fibrous scaffolds potentiate the paracrine function of mesenchymal stem cells: A new dimension in cell-material interaction, Biomaterials 141 (2017) 74–85. [PubMed: 28667901]

[181]. Barminko J, Kim JH,Otsuka S, Gray A, Schloss R, Grumet M, Yarmush ML, Encapsulated mesenchymal stromal cells for in vivo transplantation, Biotechnol Bioeng 108(11) (2011) 2747–2758. [PubMed: 21656712]

[182]. Thompson CD, Matta B, Barnes BJ, Therapeutic Targeting of IRFs: Pathway-Dependence or Structure-Based?, Frontiers in Immunology 9(2622) (2018).

[183]. Tammaro A, Derive M, Gibot S,Leemans JC, Florquin S, Dessing MC,TREM-1and its potential ligands in non-infectious diseases: from biology to clinical perspectives, Pharmacology & Therapeutics 177 (2017) 81–95. [PubMed: 28245991]

[184]. Martinez FO, Gordon S, The M1 and M2 paradigm of macrophage activation: time for reassessment, Fl000Prime Rep 6 (2014) 13–13.

[185]. Raes G, Van den Bergh R, De Baetselier P, Ghassabeh GH, Scotton C, Locati M, Mantovani A, Sozzani S, Arginase-1and Ym1 are markers for murine, but not human, alternatively activated myeloid cells, J Immunol 174(11) (2005) 6561; author reply 6561-2. [PubMed: 15905489]

[186]. Wang L-x., Zhang S.-x., Wu H.-j., Rong X-I, Guo J, M2b macrophage polarization and its roles in diseases, Journal of Leukocyte Biology 106(2) (2019) 345–358. [PubMed: 30576000]

[187]. Lurier EB, Dalton D, Dampier W, Raman P, Nassiri S, Ferraro NM, Rajagopalan R, Sarmady M, Spiller KL, Transcriptome analysis of IL-10-stimulated (M2c) macrophages by next-generation sequencing, Immunobiology 222(7) (2017) 847–856. [PubMed: 28318799]

[188]. Sindrilaru A, Scharffetter-Kochanek K, Disclosure of the Culprits: Macrophages—Versatile Regulators of Wound Healing, Advances in Wound Care 2(7) (2013) 357–368. [PubMed: 24587973]

[189]. Gharib SA, McMahan RS, Eddy WE, Long ME, Parks WC, Aitken ML, Manicone AM, Transcriptional and functional diversity of human macrophage repolarization, Journal of Allergy and Clinical Immunology 143(4) (2019) 1536–1548.

[190]. Lai YS, Wahyuningtyas R, Aui SP, Chang KT, Autocrine VEGF signalling on M2 macrophages regulates P D-L1 expression for immunomodulation of T cells, Journal of cellular and molecular medicine 23(2) (2019) 1257–1267. [PubMed: 30456891]

[191]. Grinberg S, Hasko G, Wu D, Leibovich SJ, Suppression of PLCβ2 by endotoxin plays a role in the adenosine A2A receptor-mediated switch of macrophages from an inflammatory to an angiogenic phenotype, The American journal of pathology 175(6) (2009) 2439–2453. [PubMed: 19850892]

[192]. Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, Bhasker V, Gordillo GM, Sen CK, Roy S, Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice, PloSone 5(3) (2010) e9539.

[193]. Brecht K, Weigert A, Hu J, Popp R, Fisslthaler B,Korff T, Fleming I, Geisslinger G, Brüne B, Macrophages programmed by apoptotic cells promote angiogenesis via prostaglandin E2,The FASEB Journal 25(7) (2011) 2408–2417. [PubMed: 21450910]

[194]. Van Loon S, Smits A, Driessen-Mol A, Baaijens F, Bouten C,The immune response in in situ tissue engineering of aortic heart valves, Calcific aortic valve disease (2013) 207–245.

[195]. Shafer ME, Cross-species analysis of single-cell transcriptomic data, Frontiers in cell and developmental biology 7(2019) 175. [PubMed: 31552245]

Statement of Significance

This review outlines the contributions of different macrophage phenotypes to different phases of wound healing and angiogenesis. Pathological outcomes, such as chronic inflammation, fibrosis, and the foreign body response, related to disruption of the macrophage inflammation-resolution process are also discussed. We summarize recent insights into the vast heterogeneity of myeloid cells related to their niche, especially the biomaterial microenvironment, and ontogenetic origin. Additionally, we present a discussion on novel tools that allow for resolution of cellular heterogeneity at the single-cell level and how these can be used to build a better understanding of macrophage heterogeneity in the biomaterial immune microenvironment to better inform immunomodulatory biomaterial design.

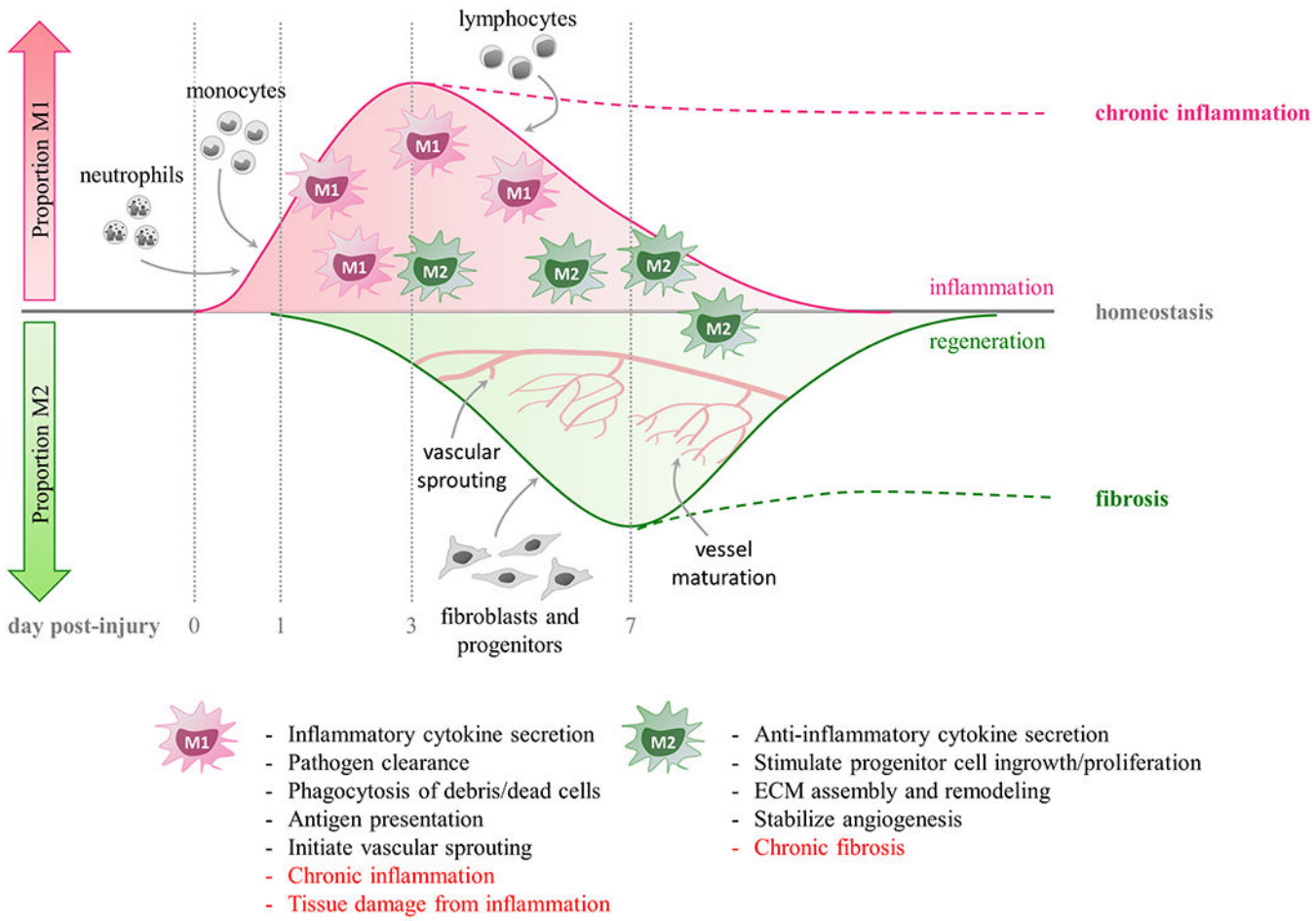


Figure 1. Time course of immune cell recruitment and macrophage phenotype following tissue injury.

Neutrophils and monocytes predominate during the early inflammatory phase. Upon arrival at the injury site, infiltrating monocytes differentiate into macrophages, which at first take on an M1-type phenotype. Around days 4-7, the primary macrophage phenotype switches from M1 to M2. Lymphocytes, fibroblasts, and tissue progenitor cells are also readily recruited to the injury site during this period. For successful wound healing to occur, both the inflammatory and regenerative phases of wound healing must terminate, resulting in a return to homeostasis. If the inflammatory phase does not resolve, chronic inflammation will result. Conversely, if the regenerative phase does not resolve, chronic fibrosis will result. Tissue repair functions ascribed to M1 and M2 macrophages are listed in black. Pathological processes associated with M1 and M2 macrophages are listed in red. Figure adapted from [194].

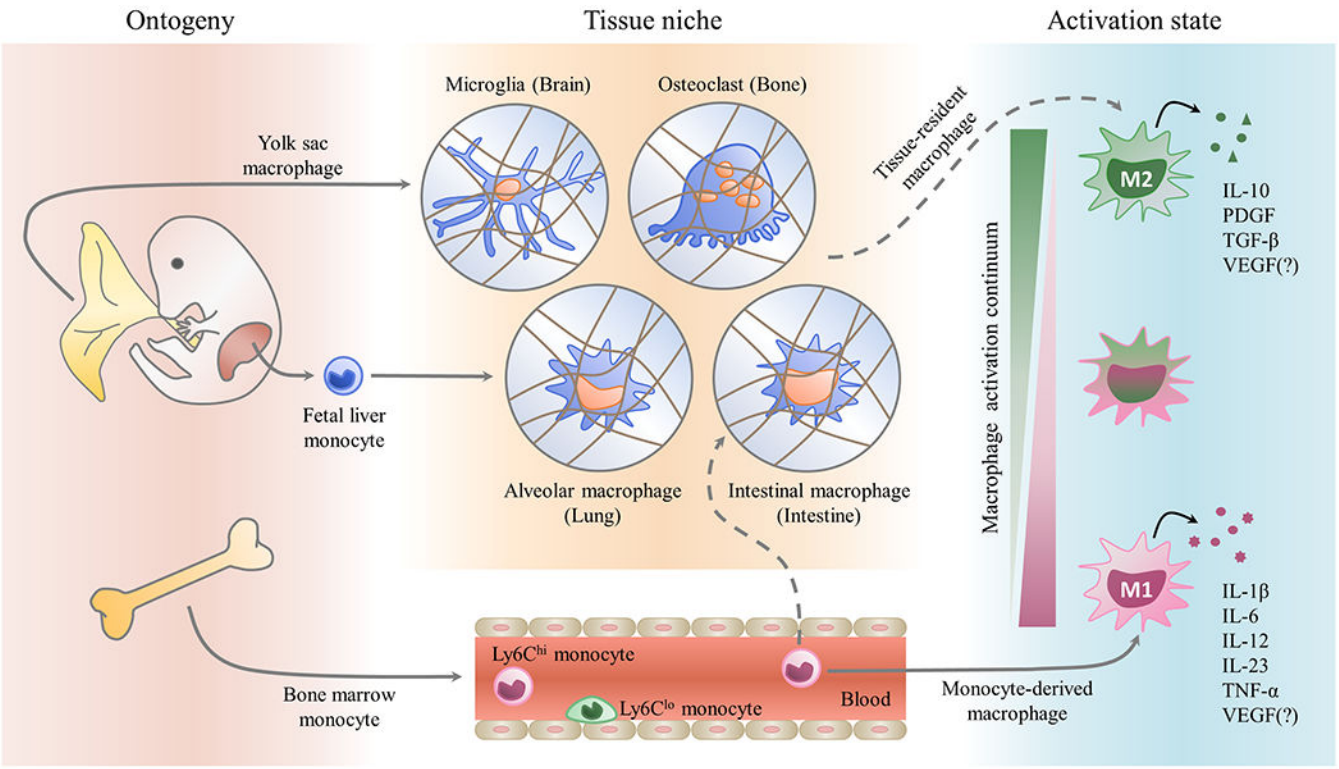


Figure 2. Factors contributing to macrophage heterogeneity.

During embryogenesis, tissue-resident macrophages (TRMs) are derived from the embryonic yolk sac and fetal liver progenitors. Distinct macrophage phenotypes arise as a result of the tissue niche in which the macrophage resides. Microglia (brain and central nervous system), osteoclasts (bone), alveolar macrophages (lung), and intestinal macrophages (intestine) are four examples of TRMs, and each organ system in the body has its own unique TRMs. In the post-natal period and adulthood, monocyte-derived macrophages arise from bone marrow progenitors and typically traffic to their destination via the circulatory system. Ly6C^{hi} monocytes are typically the early responders to inflammation and differentiate into M1-type macrophages upon arrival at the injury site. Additionally, Ly6C^{hi} monocytes can fill empty TRM niches, such as in the intestine. Ly6C^{lo} monocytes patrol the vasculature during homeostasis and have been indicated to preferentially differentiate into M2-type macrophages during inflammation. Tissue resident macrophages have also been shown to have a more M2-like phenotype. During periods of inflammation, macrophages are polarized along a spectrum of activation states from a pro-inflammatory, M1-type phenotype to a pro-regenerative, M2-type phenotype, with most *in vivo* macrophages acquiring a phenotype somewhere in between the two extremes.

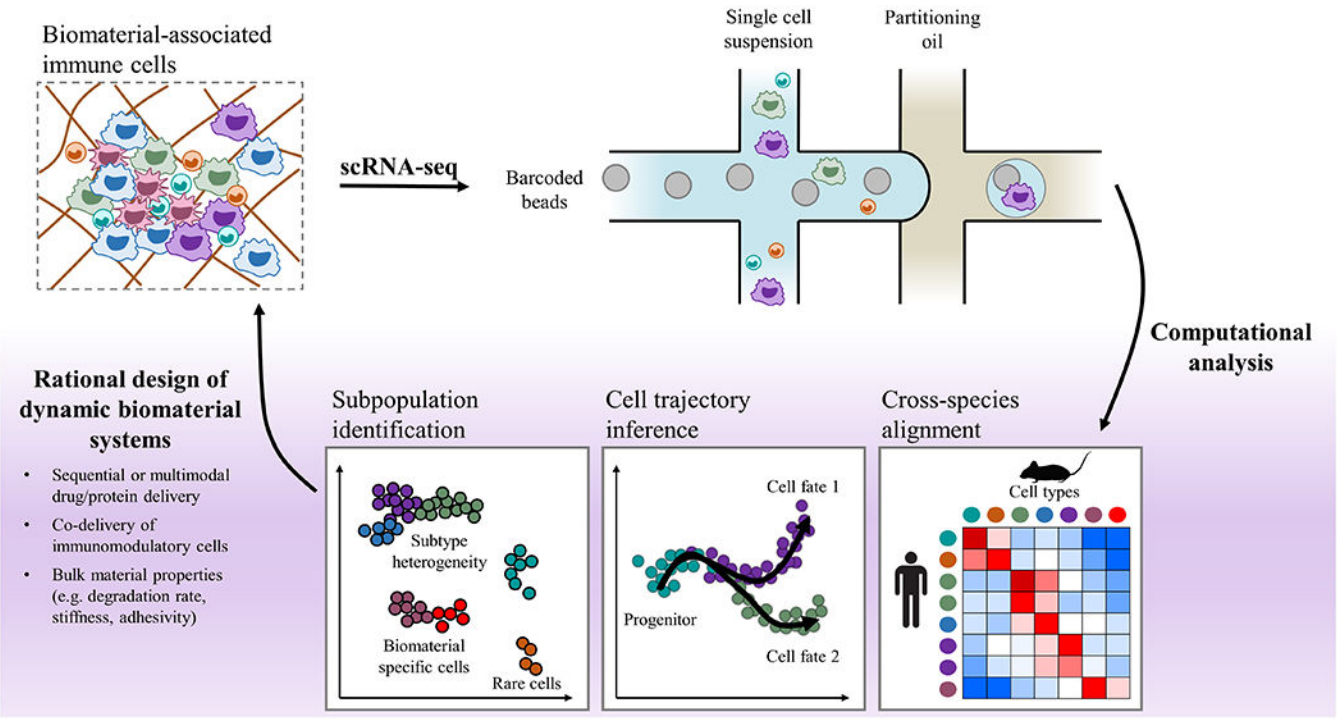


Figure 3. Outlook.

Single-cell techniques for generating high-dimensional data, such as CyTOF and scRNA-seq, can be combined with a variety of novel computational methods to achieve a more nuanced understanding of biomaterial-associated macrophage heterogeneity. Subpopulation identification: clustering cells by single-cell transcriptomic profiles can enable the identification of subtypes and underrepresented or rare cell states, as well as biomaterial- or disease-specific cells states when compared to the appropriate controls. Cell trajectory inference: scRNA-seq data can be analyzed using pseudotime diffusion mapping or RNA velocity algorithms to map cell trajectories over the course of a dynamic process, such as differentiation, clonal evolution, or cell state transition, of a specific cell type or between related cell types (e.g. from monocyte to macrophage). Cross-species analysis: single-cell data can either be analyzed and annotated separately, then cross-annotated by hand or combined into a single analysis/annotation step using unbiased, computationally intensive methodologies[195]. Results of such studies can then be used to iterate on biomaterial design.

Table 1

Classifications of *in vitro* monocyte-derived macrophage subtypes.

Macrophages can be classified by their *in vitro* differentiation stimuli into a classically activated (M1) phenotype or one of several alternatively activated (M2) phenotypes. These phenotypes are characterized by their surface markers (receptors), gene expression, cytokine secretion, and resulting associated functions. Genes denoted (mo) are markers for macrophage subtypes in murine, but not human, cells. IFN-γ: interferon gamma, TLR: toll-like receptor, LPS: lipopolysaccharide, TNF-α: tumor necrosis factor alpha, IL: interleukin, TGF-β: transforming growth factor beta, NOS2: nitric oxide synthase 2, Arg1: Arginase-1, Fizz1: resistin-like

molecule alpha, Yml: chitinase-like protein, PGE2: prostaglandin E2, PAF: platelet-activating factor, MMP: matrix metalloproteinase, VEGF: vascular endothelial growth factor.

	M1	M2a	M2b	M2c	M2d	$M2_{eff}$
						
***In vitro* stimulation:**	IFN-γ, TLR agonists (e.g. LPS), TNF-α	IL-4, IL-13	Immune complexes (ICs), TLR agonists (e.g. LPS), IL-1β	IL-10, TGF-β1	IL-6, TLR agonists, Adenosine A_{2A}Ragonists	Efferocytosis
Surface markers:	CD86, CD80, MHC-II, TLR2, TLR4	CD206, CD163, MHC-II	MHC-II, CD86	CD163, CCR2, TLR1, TLR8	?	?
Marker genes:	*NOS2* (mo)	*Arg1* (mo), *Fizz1* (mo), *Ym1* (mo)	*NOS2* (mo)			
Associated cytokine secretion:	TNF-α, IL-1, IL-6, IL-12, IL-23	IL-10, TGF-β, IL-1Ra	TNF-α, IL-1, IL-6, IL-10	IL-10, TGF-β	IL-10, TGF-β	TGF-β, PGE2, PAF
Associated functions:	Type 1 inflammatory response, Phagocytosis of debris/dead cells, Antigen presentation, Initiation of vascular sprouting	Type 2 inflammatory response, ECM deposition, Fibrosis, Angiogenesis	Immune regulation, T helper 2 activation	MMP secretion, Matrix remodeling, Fibrolysis, Angiogenesis, Phagocytosis	High VEGF secretion, Angiogenesis, Immune suppression	Anti-inflammatory, Angiogenesis
References:	[39, 52, 87, 184]	[52, 69, 87, 184, 185]	[52, 184, 186]	[52, 184, 187-189]	[52, 53, 190, 191]	[54, 87, 192, 193]