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Macrophage polarization: An opportunity for improved outcomes in biomaterials and regenerative medicine☆

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Abstract

The host response to biomaterials has been studied for decades. Largely, the interaction of host immune cells, macrophages in particular, with implanted materials has been considered to be a precursor to granulation tissue formation, the classic foreign body reaction, and eventual encapsulation with associated negative impacts upon device functionality. However, more recently, it has been shown that macrophages, depending upon context dependent polarization profiles, are capable of affecting both detrimental and beneficial outcomes in a number of disease processes and in tissue remodeling following injury. Herein, the diverse roles played by macrophages in these processes are discussed in addition to the potential manipulation of macrophage effector mechanisms as a strategy for promoting site-appropriate and constructive tissue remodeling as opposed to deleterious persistent inflammation and scar tissue formation.

Keywords

Foreign body response; Leukocyte; Macrophage; Biocompatibility

1. Introduction

Materials used in the fabrication of implantable medical devices, including those targeted for regenerative medicine applications, are typically characterized by their mechanical and material properties including tensile strength, modulus, density, porosity, surface chemistry

☆ *Editor's Note:* This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

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and degradability, among others. The clinical acceptance of such materials also depends upon factors such as suture retention strength, physical properties and handling characteristics with respect to the intended clinical application, and resistance to infection. However, the ultimate functional success or failure of such materials is invariably a function of the host tissue response to the presence of the materials and the associated remodeling process at each anatomical site.

It is well recognized that the material–tissue interface represents the point at which the first responding cells initiate intra-cellular events that drive subsequent paracrine and autocrine processes of the host tissue response. Therefore, surface structure, both topographical and chemical, are critically important determinants of downstream events. Indeed, a wide variety of surface modification techniques have been explored as potential strategies for mitigating components of the host immune response and improving tissue specific cell–material interactions.

A number of cell types, plasma proteins, and extracellular fluid constituents are involved in these early, interactive events. Platelets, neutrophils, mononuclear phagocytes, and parenchymal cells all play important roles in the tissue response. Largely, the interaction of these host immune cells, both innate and adaptive, with biomaterials is considered to have negative implications for downstream functionality or tissue remodeling outcomes. For example, large numbers of mononuclear cells within newly deposited extracellular matrix accompanied by an angiogenic response is generally interpreted as a precursor of granulation tissue formation with negative implications for downstream outcomes. This interpretation has led to a number of strategies which seek to avoid activation of components of the host immune response altogether.

Recently however, one component of the innate immune system, the macrophage, has received considerable attention as an important, and in many cases beneficial, modulator of disease and tissue remodeling following injury. These studies indicate that macrophages possess remarkable functional plasticity and, therefore, can play both positive and negative roles in the pathogenesis of disease and tissue remodeling. As such, while it should not be inferred that other components of the inflammatory response and adaptive immunity are insignificant, the subject matter herein focuses upon the role of the mononuclear phagocyte component of the innate immune response, its role in modulating disease and tissue remodeling, and its potential to direct inflammatory versus constructive tissue remodeling events following the implantation of biomaterials.

2. Macrophages

Macrophages are monocyte-derived myeloid cells that develop from a common myeloid progenitor cell residing within the bone marrow of adult mammals [1,2]. This same progenitor cell also gives rise to other cells of the myeloid lineage including neutrophils, eosinophils, basophils and dendritic cells depending upon the cytokines to which the cell is exposed during its maturation. Monocytes are derived from the common myeloid progenitor cell through a cascade that includes the cytokines granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), and macrophage colony stimulating factor (M-CSF). These signals induce differentiation of the common myeloid progenitor to monoblasts, pro-monocytes, and finally monocytes. Mature monocytes then leave the bone marrow and enter the bloodstream where they may reside for several days before entering tissues to become macrophages, either in a process of steady state turnover or due to chemoattractant factors that are produced as part of a local inflammatory process.

Prior to entering tissues and differentiating into macrophages, circulating blood monocytes are known to be heterogeneous with at least two general populations having been identified [1,3–5]. These two phenotypes consist of cluster of differentiation (CD) 14^{hi}CD16[−] and CD14⁺CD16⁺ populations in humans and Ly-6c^{high} (Gr1⁺) and Ly-6c^{low} (Gr1[−]) subsets in mice, and have been grouped into two basic categories termed ‘inflammatory’ and ‘resident’, respectively. Inflammatory (CD14^{hi}CD16[−]/Ly-6c^{high}) monocytes are characterized by their ability to rapidly migrate to sites of injury or infection through chemokine C-C motif receptor (CCR)2-chemokine C-C ligand (CCL)2 mediated pathways and to propagate chronic inflammatory diseases. Resident monocytes lack CCR2 expression and are characterized by their ability to patrol the vasculature, populate normal tissues, and act as regulators of the inflammatory response.

Monocytes differentiate into macrophages upon emigration from the vasculature into the tissue. Once within the tissue, macrophages may undergo differentiation into a number of distinct phenotypes depending on the tissue type, microenvironmental conditions, and the immunologic milieu [1]. However, the specific factors and exact mechanisms which control macrophage differentiation upon entering tissues have not yet been fully identified. Native tissue-resident macrophage populations include those of bone (osteoclast), lung (alveolar macrophage), nervous system (microglia), connective tissues (histiocytes), the gut, liver (Kupffer cells), the spleen, and the peritoneum. Each tissue specific macrophage population shows a functional profile that is associated with distinct patterns of gene expression related to the tissue specific microenvironment in which they reside [1]. For example, macrophages present within the gut perform a specialized function that requires high levels of phagocytosis and low production of proinflammatory cytokines, a phenotype and function that is distinct from the other macrophage populations listed above [6]. It has been shown that this “gut” phenotype can be induced in other macrophage populations through exposure to intestinal stromal cell products, suggesting that the local microenvironment plays a large role in determining the phenotype and activity of various macrophage populations [7]. This phenotypic transformation is also an example of the remarkable heterogeneity and plasticity inherent within macrophages, the causes and consequences of which are further examined in the following sections.

2.1. Macrophage polarization

Macrophages, like the monocytes from which they derive, are a heterogeneous cell population each with different markers and functions [1,8–12]. Upon leaving the bloodstream and migration into sites of inflammation, macrophages become activated in response to signals present in damaged tissue or associated with pathogens. In response, macrophages increase the production of cytokines, chemokines, and other molecules that contribute to the local milieu. Macrophages assume diverse and context dependent phenotypes, which are defined by their functional properties and patterns of gene expression. Broadly, polarized macrophages are referred to as having either an M1 or an M2 phenotype, mimicking the Th1/Th2 nomenclature which has been described for T helper cells [10]. M1, or “classically activated” pro-inflammatory macrophages are induced by interferon (IFN) γ alone or in combination with lipopolysaccharide (LPS, endotoxin) or tumor necrosis factor (TNF) α . In general, M1 activated macrophages express interleukin (IL)-12^{high}, IL-23^{high}, IL-10^{low}; metabolize arginine, produce high levels of inducible nitric oxide synthase (iNOS); secrete toxic reactive oxygen and nitric oxygen intermediates and inflammatory cytokines such as IL-1 β , IL-6, and TNF α ; and are inducer and effector cells in Th1 type inflammatory responses. In contrast, M2, “alternatively activated”, macrophages are induced by exposure to a variety of signals including the cytokines IL-4, IL-13, and IL-10, immune complexes, and glucocorticoid or secosteroid (vitamin D3) hormones. M2 activated macrophages express IL-12^{low}, IL-23^{low}, and IL-10^{high}; have high levels of

scavenger, mannose, and galactose receptors; produce arginase in the place of arginine, subsequently producing ornithine and polyamines; and are involved in polarized Th2 reactions. While the term 'M2' encompasses all non-classically activated macrophages, M2 macrophages have been shown to include three well recognized sub-populations (M2a, M2b, and M2c), each with its own distinct inducers, markers, and functions. A discussion of each M2 subtype and its role in myriad disease and tissue remodeling processes is beyond the scope of this review. However, the inducers and characteristics of M1 and M2 (including M2a, M2b, M2c) macrophages have been reviewed in depth elsewhere [8,12,13].

While macrophages can be broadly described as having an M1 or an M2 phenotype, this description and segregation of macrophage phenotype into two distinct subcategories represents a simplistic depiction of the *in vivo* scenario. Rather, macrophage phenotype has been described as occurring along a continuum between the M1 and M2 extremes [12]. However, the M1/M2 paradigm provides a simplified framework from which to begin deciphering the complex and context dependent roles of macrophages in disease and tissue remodeling.

2.2. Macrophage plasticity

It has been shown that unlike lymphocytes, where cellular phenotype is relatively fixed upon differentiation, macrophages have dynamic and plastic phenotypes that change with local microenvironmental signals [5,14–16]. These changes are dependent upon the type, concentration and duration of the polarizing signals. For example, *in vitro* experiments have shown that LPS stimulated, classically activated macrophages, become Toll-like receptor (TLR)-tolerant and unable to produce similarly elevated levels of pro-inflammatory cytokines upon second exposure to LPS [16–18]. These cells do, however, retain the ability to produce anti-inflammatory cytokines such as IL-10. Similarly, cells exposed to M2 polarizing signals can be readily induced to express genes characteristic of an M1 phenotype by exposure to LPS or IFN γ . Therefore, it is not surprising that interrogation of the macrophage population at any given point in time may show a mixture of macrophage phenotypes and/or macrophages in a state of transition that express markers of both M1 and M2 phenotypes.

While the mechanisms that underlie this observed plasticity are not well understood, recent studies have shown that a number of mutually exclusive and feed forward signaling pathways may play a role [19]. All activated macrophages, regardless of polarization state, express high levels of the transcription factors runt-related transcription factor 1 (RUNX1) and PU.1, which are thought to represent global regulators of macrophage phenotypic expression [20–22]. These regulators, in concert with activation, repression, and interplay between the signal transducer and activator of transcription (STAT), interferon-regulatory factor (IRF), cAMP-responsive element binding protein-CCAAT/enhancer-binding protein- β (CREB-C/EBP β), and peroxisome proliferator-activated receptor- γ (PPAR γ) transcription factors downstream of surface receptor ligation are thought to play an important role in the polarization and maintenance of specific macrophage phenotypes [19]. Specifically, STAT1 and IRF5 have been shown to be involved in polarization and maintenance of an M1 phenotype while STAT6, IRF4, CREB-C/EBP β , and PPAR γ are involved in polarization and maintenance of an M2 phenotype [23–30]. The role of transcription factors in macrophage polarization has been reviewed recently [19].

This highly complex and highly regulated plasticity likely represents a protective mechanism that allows the host to mount an appropriate response to a pathogen, but also rapidly and effectively restore tissue homeostasis following injury. As will be discussed in the section which follows, dysregulation of macrophage phenotype and the inability to resolve polarized M1 or M2 states and return to tissue specific baselines is increasingly

recognized to have an important role in chronic inflammatory and disease states, as well as adverse tissue remodeling following injury [31].

3. The role of macrophage polarization in disease and tissue remodeling

The concepts and paradigms of macrophage polarization into M1 and M2 subtypes as described above have emerged largely in the contexts of the host response to pathogens and cancer. These concepts have now been applied to a broader scope of host response scenarios including disease pathogenesis, tissue injury, the implantation of biomaterials, and the use of biomaterials in regenerative medicine, which will be discussed in more depth in the following sections.

3.1. The role of macrophage polarization in the pathogenesis of disease

Macrophage polarization and plasticity have been shown to play important roles in a diverse spectrum of disease processes [32,33]. While consideration of the roles of the macrophage populations which participate in each of these disease processes is too expansive to be within the scope of this manuscript, four disease states and the role of the macrophage populations which drive them are briefly discussed to illustrate important concepts which underlie many macrophage mediated disease processes. It should be noted that a number of these disease states also represent an opportunity for biomaterial-based therapies and regenerative medicine strategies which are capable of modulation of macrophage phenotype.

3.1.1. Cancer—The participation of large numbers of inflammatory cells in tumor development and progression has been observed since the nineteenth century. Now commonly described as “smoldering inflammation”, these observations have led to a well-established link between inflammatory cells, macrophages in particular, and cancer [34–36]. In healthy conditions, macrophages serve a protective role, producing inflammatory mediators (cytokines and reactive oxygen species) and activating adaptive immune cells, leading to the destruction of transformed cells. It was initially thought that development of oncogenes resulted in the hallmark microenvironment of cancer, in which transformed cells secrete cytokines and chemokines that promote tissue development and prevent apoptosis as well as suppress cytotoxic immune responses (termed the “intrinsic pathway”). It is now recognized that another pathway leading to tumorigenesis exists. This “extrinsic pathway” is initially characterized by a chronic pro-inflammatory environment resulting from a persistent microbial infection, autoimmune disease, or other etiology of unknown origin [37,38]. The chronic production of large quantities of inflammatory mediators in these cases can lead, through a number of pathways, to tumor cell proliferation and survival or to the induction of genetic instabilities in normal cells, with resultant expression of oncogenes and production of immune suppressive cytokines [37,38]. Thus, early tumor development is, in many cases, characterized by a polarized inflammatory, M1-like, environment.

Mature tumors are also characterized by significant infiltration of mononuclear cells; however, the cells commonly observed in mature tumors are of a more suppressive, M2-like, phenotype [36,39]. These cells, termed “tumor associated macrophages” (TAM), are characterized by production of large amounts of IL-10, low production of inflammatory cytokines and reactive oxygen intermediates, and poor antigen presenting capabilities. This profile leads to an inability to mount a tumor-destructive Th1 type response while also driving the recruitment and development of T-regulatory cells (T-regs) and leading to a more tumor-permissive environment. TAMs also drive tumor development via more direct mechanisms. Specifically, TAMs produce cytokines that drive cellular recruitment and proliferation, angiogenesis, matrix production and remodeling. These cytokines include epidermal growth factor (EGF), basic fibroblast growth factor (b-FGF), transforming growth factor (TGF)- β 1, vascular endothelial growth factor (VEGF), and matrix metalloproteinases

(MMPs), among others. Interestingly, TAMs are found largely within hypoxic regions of tumors and have been shown drive the angiogenic response that is required for tumor survival and metastasis [40,41]. Indeed, TAM presence and number has been directly correlated with prognosis in a number of cancer types and model systems [42–44].

Thus, cancer can be thought to represent a disease in which an inability to resolve macrophage polarization, first M1 and then M2, results in pathologic outcomes. The specific mechanisms that underlie the M1 to M2 switch in tumor development remain unknown and are, along with methods of re-polarization of TAMs towards an M1-like phenotype, an ongoing target of anti-cancer drug development.

3.1.2. Atherosclerosis—Similar to cancer, atherosclerosis has an etiology that has, for many years, been recognized to include the involvement an inflammatory cell population, in particular macrophages [45–47]. Also similar to cancer, a macrophage phenotypic switch is observed to occur with disease progression, albeit with significantly different pathologic sequelae [45–48]. Briefly, in atherosclerosis, circulating monocytes are recruited to sites of fatty deposit accumulation within the vascular intima and subintima via CCR2 and endothelial adhesion mediated mechanisms [49–52]. Upon arrival these cells become activated and differentiate into macrophages. The fatty deposits then begin to mature into plaques with continued recruitment of inflammatory cells, smooth muscle cells, and the production of extracellular matrix.

A number of recent studies have begun to assess the polarization profile of plaque infiltrating macrophages, suggesting that the initial infiltrating population in early atherosclerosis is heterogeneous, but possesses a predominantly M2-like phenotype [28,45]. This accumulation of cells with a pro-remodeling and proangiogenic phenotypic profile likely contributes to the formation, expansion, and stabilization of the plaque. However, concurrent with lesion progression and expansion, a switch to a predominantly M1 phenotype has been observed [53]. It is thought that this phenotypic switch is due to the phagocytosis of excess oxidized low-density lipoproteins (LDL) within the plaque by macrophages and the production of IFN γ by local Th1 cells, resulting in the development of foam cell macrophages [54,55]. Foam cell macrophages exhibit a highly activated phenotype leading to production of pro-inflammatory mediators and MMPs that destabilize the plaques, potentially leading to thromboembolism [56].

These observations suggest that therapies which prevent the M2 to M1 switch or deplete M1 macrophages selectively may be of clinical utility for the stabilization of atherosclerotic plaques. However, it is not clear if the phenotypic switch observed within the lesion site represents polarization of newly recruited monocytes or transition of local M2 polarized cells to M1. Additionally, macrophages are thought to have a primarily pathogenic role in the formation of atherosclerotic plaques, with some studies suggesting that plaque regression correlates with the continued emigration of cells from the plaque [57]. Therefore, strategies that prevent the recruitment of additional monocytes and their differentiation into macrophages or that promote emigration of cells from the plaques may also be effective in slowing or preventing plaque formation. Indeed, depletion of circulating monocytes via injection of clodronate laden liposomes have been shown to reduce the formation of atherosclerotic plaques in rabbits [58]. However, clodronate treatment was not shown to reduce the size of plaques that were already established prior to treatment, suggesting that such a treatment may be most effective in the early stages of disease progression [59].

3.1.3. Obesity and insulin resistance—Adipose tissue macrophages (ATM) have been shown to comprise a significant proportion of the cellular component of adipose tissue in both lean and obese states [60–63]. In normal rodents and humans, ATMs make up as much

as ten percent of the cellular constituents of the tissue [62]. In comparison, it has been shown that in obese subjects that number rises to as much as 40% [62]. In normal, non-obese subjects ATMs have a polarized M2 phenotype characterized by increased baseline STAT6 and PPAR γ expression [29]. These cells are thought to play an important and beneficial role in nutrient metabolism [63,64]. Studies in PPAR γ deficient mice exhibit impaired M2 macrophage function and susceptibility to diet-induced inflammation and insulin resistance [29,65–67]. It has been suggested that local IL-4 producing eosinophils may be required for maintenance of this polarized M2 state [68].

While the ATMs present in healthy lean adipose tissue are primarily of an M2 phenotype, those that accumulate in the adipose tissue during obesity have a strongly polarized pro-inflammatory, M1 phenotype [63,69–71]. These cells produce high levels of TNF α , IL-6, and IL-1 β , all of which are also observed in increased levels of adipose tissue from insulin resistant individuals. It seems likely that high levels of pro-inflammatory mediators locally impair the function of resident insulin processing cells. Additional studies suggest that, while systemic levels of these same pro-inflammatory mediators are lower than those observed locally, these tissue cytokines may “leak” into the circulation and affect other tissues and organs. Indeed, in obese individuals, similar accumulations of M1 activated macrophages are observed in both muscle and the liver [63].

The exact mechanisms driving the accumulation and polarization of macrophages in obese adipose tissue are not fully elucidated. However, macrophage accumulation in obese adipose tissue has been observed to be localized around necrotic or apoptotic adipocytes [72]. A number of free fatty acid products released from necrotic adipocytes have been shown to cause pro-inflammatory activation of macrophages through TLR-2 and TLR-4 signaling pathways, suggesting a potential mechanism by which obesity-induced inflammation may occur [73,74]. Additionally, damage associated molecular pattern molecules, such as heat shock proteins, released from dead or dying adipocytes may signal through TLR pathways, producing activation of the NLRP3 inflammasome and promotion of a pro-inflammatory phenotype [75]. A number of studies have shown that TLR-4 deficient mice are not protected from diet-induced obesity, but are protected from obesity-induced insulin resistance [74,76,77]. These studies suggest that inhibition of the recruitment of M1 macrophages may hold promise. However, the ability to target these interventions to specific locales without impacting essential systemic immune function remains difficult.

3.1.4. Periodontal disease—Periodontal disease is an inflammatory condition which presents with a wide range of clinical variability and severity. However, research in the past decade has shed substantial light on the initiating infectious agents and the role of the host immune response in periodontal disease. Overall, a complex multifactorial etiology of periodontal disease including the host immune response and environmental factors has been suggested likely involving multiple cell types [78]. However, several lines of evidence suggest the macrophage as a critical determinant of the periodontal immune response to infection: both because macrophages represent a significantly higher proportion of the cells within periodontitis sites compared to clinically healthy sites and because recent data demonstrate that macrophages, but not other myeloid cells, are the dominant source of pro-inflammatory cytokines important to the resolution of periodontal disease [79–81]. Additional studies have suggested dysregulation of innate immunity as a key event in the progression of periodontal disease [82]. Specifically, if the host innate immune response becomes suppressed or tolerant (i.e. unable to mount an appropriate M1 type macrophage response) following low-level stimulation of critical pattern recognition receptors, this may lead to a muted local immune response, thus enabling periodontal disease-associated bacteria such as *Porphyromonas gingivalis* (*P. gingivalis*) to evade the host immune system [83,84]. Although M1 and M2 macrophage dichotomy has been hypothesized to play a role

in periodontal pathogenesis, further studies are needed to better define their respective involvement. Importantly, periodontal disease has been shown to have many mechanistic parallels and links to the pathogenic processes of atherosclerosis and obesity [69].

3.2. The role of macrophage polarization in tissue remodeling

Each of the above examples highlights the context dependent role of macrophage polarization in diverse disease processes. In each case, the pathogenesis of disease results from inappropriate macrophage polarization, an inhibition of macrophage polarization, or an inability to resolve a chronic polarization towards the M1 or M2 extreme. Additionally, each of these scenarios involves some form of phenotypic switch from M1 to M2 or vice versa. An increasing number of studies in multiple animal models and organ systems have shown similar phenomena also occur during the course of remodeling which occurs following tissue injury. That is, macrophages can play both beneficial and detrimental roles in the process of tissue remodeling and, in many cases, an efficient and timely phenotypic switch is essential for appropriate and functional remodeling as opposed to a deleterious or scar tissue outcome with a loss of function. A brief overview of the default sequence of events which occur following tissue injury are provided and three additional examples that explore the tissue specific role of macrophage polarization in tissue remodeling following injury are provided below. A fourth example, fibrosis, is provided to illustrate the consequences of dysregulation of macrophage phenotype during the course of tissue remodeling. In the section that follows, the role of the macrophage in the tissue remodeling response which occurs following the implantation of biomaterials is explored.

3.2.1. The default mammalian response to tissue injury—The default mammalian host response following tissue injury is a well-documented series of events that typically result in the deposition of dense fibrous connective tissue (i.e., scar tissue) within the site of injury [85–87]. Very few tissues in adult mammals have the ability for true regeneration; among them are the bone marrow, liver, intestinal epithelium, and epidermis of the skin. The default response to tissue injury has been described as occurring in four stages: hemostasis, inflammation, proliferation, and remodeling [86]. Each of these states can be observed following injury in almost every tissue of the body and are, therefore, reviewed briefly below.

3.2.1.1. Hemostasis: Following tissue injury and resultant damage to the vasculature, platelets are activated by tissue factor from damaged tissues resulting in the release of clotting factors that initiate hemostasis. A provisional matrix forms consisting largely of fibrin and entrapped erythrocytes. The provisional matrix provides a substrate for further cell migration into the site of injury and a medium for cell signaling [88]. In addition to their role in hemostasis and provisional matrix formation, platelets also release cytokines including platelet derived growth factor (PDGF), TGF- β , chemokine C-X-C ligand 4 (C-X-C L4), and IL-1 β [89–91]. These factors, among others, contribute to the initial repair process via recruitment of multiple cell types including neutrophils, macrophages, fibroblasts, and other tissue specific cells to the injury site [91].

3.2.1.2. Inflammation: Neutrophils are the first inflammatory cell type to arrive at the wound site. Neutrophils phagocytose and destroy foreign material, bacteria, or dead cells that may have entered the wound site as a result of the injury and also provide further signaling molecules that recruit macrophages to the injury site [89]. Mast cells also participate in the early stages of wound healing by releasing granules containing enzymes, histamine, and other factors that modulate the inflammatory response [86,92]. By 48–72 h post-injury, however, macrophages begin to dominate the cell population at the site of injury [93]. These cells are of a predominantly pro-inflammatory phenotype and secrete cytokines

and chemokines that promote the further recruitment of leukocytes to the site of injury [89,91]. Macrophages also remove apoptotic neutrophils, the phagocytosis of which may lead to a change toward a more reparative (M2) macrophage phenotype and the resolution of the inflammatory phase of wound healing [94–96]. The T lymphocyte population also plays an important late regulatory role in the resolution of the inflammatory process through local secretion of cytokines and chemokines, many of which are known to affect macrophage polarization [97].

3.2.1.3. Proliferative phase: The proliferative phase of wound healing involves cellular proliferation, angiogenesis, new ECM deposition, and the formation of granulation tissue – processes that are largely mediated via the effects of the local microenvironment including pH and oxygen tension, and cytokines secreted by macrophages, T lymphocytes, and other cells within the wound site [91,98,99]. These cytokines include EGF, b-FGF, TGF- α , TGF- β , VEGF, and others depending on the nature of the injured tissue [86]. Importantly, and as will be discussed in the following examples, macrophage participation and polarization during this stage may have significant implications for the outcome of the following remodeling phase.

3.2.1.4. Remodeling phase: Following the deposition of significant amounts of ECM (predominantly collagens type I and III) during the proliferative phase, the remodeling phase of wound healing begins. This phase is characterized by MMP- and tissue inhibitor of metalloproteinase (TIMP)-mediated degradation and remodeling of the newly deposited collagen, generally resulting in scar tissue formation/maturation [86,100]. In some cases, as will be discussed in further detail, prolonged inflammation and remodeling leading to fibrosis or hypertrophic scar formation may occur due to dysregulation of the default healing process [32,86,101,102].

Although these wound healing events are described as part of the default response to tissue injury, many of these events also occur as part of constructive remodeling processes. That is, the selective activation of components of the inflammatory, proliferation, and remodeling phase can result in a constructive and functional outcome as opposed to scar tissue formation. In particular, the role of macrophages in promoting a constructive remodeling outcome following implantation of biomaterial scaffolds is discussed in further detail in the following section.

3.2.2. Skin—The skin represents one of the largest, most accessible and easily injured organs of the body. Therefore, it is not surprising that the default processes of tissue remodeling have been most extensively described in the context of injury and healing of the dermis. This process is known to include a number of immune cell populations including neutrophils, eosinophils, mast cells, and T-cells, depending on the stage of the tissue remodeling process [86]. Macrophages, however, have been recognized as the most long-lived and central cell population that plays a role in dermal wound healing largely through the production of the cytokines, growth factors, and MMPs described above [93,103,104]. In depth descriptions of the role of macrophages in wound healing have been reviewed at length elsewhere, and will not be reviewed herein [93,103,104]. However, despite the understanding that macrophages play an important and potentially determinant role in dermal wound healing, few studies to date have assessed the role of specific macrophage sub-populations in the remodeling process.

One recent study examined gene expression during cutaneous wound healing following skin biopsies of human patients [105]. Results showed upregulation of a wide variety of genes throughout the duration of wound healing. Of note, however, investigators identified two clusters of genes, associated with macrophage M1/ M2 polarization, which were upregulated

transiently during the wound healing process. The first was upregulated during the early, inflammatory stage of wound healing and contained a mixture of M1 and M2 associated genes (11 M1 genes and 7 M2 genes). The second was upregulated in the later tissue remodeling and angiogenesis stages of healing and contained predominantly M2 associated genes (1 M1 gene and 9 M2 genes). The results of this study suggest a role for M1/M2 polarization in the process of cutaneous wound healing.

The mechanisms of this switch from an M1 to an M2 phenotype during dermal remodeling are not well described, but are thought to include phenotypic changes caused by the phagocytosis of apoptotic cells and debris by M1 macrophages [94–96]. A recent study showed that depletion of macrophages during the proliferative phase of wound healing resulted in a defective transition to the remodeling phase and impaired healing [106,107]. It is interesting to note that diabetes and advanced age are also associated with delayed or impaired wound healing, and with a reduced ability to transition from an M1 to an M2 macrophage phenotype [63,108]. This phenotypic transition impairment suggests a potential, but as yet unexplored, link between defective macrophage polarization switching or disposition towards a pro-inflammatory phenotype and impaired tissue remodeling.

3.2.3. Muscle—While the mechanisms which underlie the role of macrophages in dermal wound healing have long been recognized, the role of macrophages as key mediators of tissue remodeling following muscle injury has been more recently described. However, more is known regarding the role of individual macrophage populations in skeletal muscle remodeling following injury than in skin remodeling [109].

In general, the regulatory mechanisms that affect skeletal muscle remodeling are similar to those that occur during muscle development. Briefly, the events that occur during skeletal muscle repair are thought to fall into three well defined stages including (1) proliferation, (2) early differentiation, and (3) terminal differentiation [110]. In development, these processes do not involve a significant immune cell population and similar processes are observed to occur without immune cell participation when muscle progenitor cells are grown in vitro. That is, when placed into culture, muscle progenitor cells proliferate, express early markers of differentiation along a muscle phenotype (MyoD, Myf5), and then fuse and form mature myotubes with a contractile phenotype in the absence of immune cells or their secreted products [110].

In vivo, however, the response following muscle tissue injury has been shown to include a significant number of infiltrating inflammatory cells which can exceed 100,000 cells/mm³ of tissue [111]. A number of studies have shown that reduction of the number of monocytes available to participate in the response following muscle injury via depletion of circulating cells results in slowed removal of cellular debris and delayed muscle tissue remodeling [112–114]. These cells have been shown to possess a phenotype that switches from M1 to M2 during the course of muscle remodeling [109]. Briefly, an initial M1 macrophage population is recruited to the site of tissue remodeling and participates in the proliferative stage of muscle remodeling. A switch to a more M2 phenotype is observed to occur concurrently with transition to the early differentiation and terminal differentiation stages of remodeling. The complex mechanisms by which the macrophage population within the site of remodeling affects the processes of skeletal muscle remodeling is the subject of a recent in depth review [109].

Briefly, a number of studies have shown that the chemokines and cytokines (TNF- α , IL-1 β , IL-6) produced by initially infiltrating M1 macrophages are chemoattractant and mitogenic for muscle progenitor cells, including Pax7+ satellite cells [115–119]. This endogenous cell recruitment process results in accumulation of satellite cells and mononuclear cells within

the site of remodeling. Elimination of these cytokines in vivo has been shown to diminish muscle repair [118]. However, it has also been shown that proinflammatory cytokines may inhibit muscle progenitor cell differentiation and that the reactive oxygen species that are produced by M1 cells (nitric oxide) can be cytotoxic if present in sufficient quantities, demonstrating the need for resolution of the proinflammatory response for muscle recovery to proceed beyond this initial stage [120–122].

Following the initial pro-inflammatory phase, a switch to an M2 macrophage phenotype and resolution of the inflammatory response has been shown to occur with the initial stages of differentiation of muscle satellite cells into differentiated skeletal muscle cells [123]. The transition to an M2 environment not only serves to attenuate the inflammatory response and potentially cytotoxic production of nitric oxide, but also to drive the differentiation and fusion of cells [114,124]. For example, IL-10 secreted by M2 macrophages is known both to drive a transition from an M1 to an M2 phenotype as well as to promote the fusion and maturation of myotubes [112,125]. A recent study examined the role of M2 macrophages in a mouse model of necrotic muscle injury repair [24]. In this study it was shown that the deletion of two of the binding sites in the CREB-C/EBP β pathway blocked the induction of M2 associated genes (Msr1, IL-10, IL-13ra, and Arg-1), while M1 gene (IL-1, IL-6, IL-12b, TNF α) expression was not affected. Necrotic injury was induced through the injection of a low dose of cardiotoxin (an injury which results in rapid recovery of the injured muscle tissue in unaltered wild type mice) and mice were then monitored for up to 10 days following injection. In those mice in which the CREB-C/EBP β pathway was blocked (i.e. lacked expression of genes associated with the M2 macrophage phenotype), clearance of cellular debris and recruitment of cells was observed at the site of tissue remodeling. However, the tissue that formed within the site of remodeling was highly fibrotic and associated with a decrease in myofiber size. This result was in direct contrast to wild-type mice that exhibited full recovery of the injured muscle. Additional studies have suggested that transition from specific M2a to M2c sub-populations may also play an important role in directing late stage differentiation and functional recovery [126].

Studies have also suggested that macrophage polarization may play an important role in the process of muscle wasting which occurs in models of muscular dystrophy [124,126,127]. Briefly, progenitor cells are recruited to sites of inflammation characterized by a mixed M1 and M2 cell population. Unlike the normal process of muscle tissue remodeling, this environment is not characterized by a switch from M1 to M2. Rather, the mixed polarization state persists, constantly recruiting progenitor cells which then fail to differentiate along normal pathways resulting in ineffective remodeling and depletion of progenitor cell reserves within the tissue.

3.2.4. Central nervous system—The tissue remodeling response that occurs following injury in the central nervous system, especially as it relates to the recruitment and involvement of macrophages, differs somewhat from the other examples discussed previously. The central nervous system is protected from the majority of the body's innate immune responders by the blood–brain barrier. Therefore, in the absence of a breach of the blood–brain barrier, tissue remodeling in the central nervous system largely involves the local tissue-resident innate immune cell population (microglia) at early time points following injury. These cells have been shown to possess a predominantly pro-inflammatory phenotype following injury, resulting in a microenvironment that is cytotoxic and propagates tissue damage and scar tissue deposition [128]. This response is in contrast to that seen in the periphery, which involves a circulating monocyte population, and in which spontaneous recovery has been observed depending on the degree of the injury. A small number of blood-derived monocytes have been observed to participate in the default tissue remodeling response in the CNS at late time points following injury, even in the absence of

a breach of the blood–brain barrier [129,130]. It has been suggested that these cells may be capable of adopting a more anti-inflammatory and regulatory phenotype which is beneficial for resolution of inflammation and promotion of functional recovery [131,132]. However, the level of recruitment of these cells to the CNS is likely insufficient to promote this type of response.

A recent study investigated the role of macrophages and macrophage polarization in the processes of tissue repair following injury of the spinal cord [128]. The results of the study showed that a predominantly M1 phenotype was induced immediately following injury and persisted within spinal cord lesions. A small number of M2 macrophages were also seen in the site of injury at early time points; however, these cells were not observed at later time points in the remodeling process, suggesting that the M1 to M2 phenotype shift does not occur in the same way in the spinal cord as it does in some other tissues following injury. This study also showed that M1 and M2 macrophages had distinct effects upon the survival of neurons and upon neurite outgrowth. Neurons which were exposed to media conditioned by M1 macrophages were shown to exhibit a decrease in viability as well as a decrease in neurite length, while those cells exposed to media conditioned by M2 macrophages showed improved survival and a greater degree of neurite extension. This bimodal outcome scenario suggests that the default response following CNS injury is a chronic pro-inflammatory, M1 type response which leads to decreased viability of the neurons within the site of injury, and that induction of a more M2 type response may be capable of promoting a more constructive tissue remodeling type environment.

A small number of studies have investigated the impact of injection of blood monocytes and polarized macrophages into spinal cord lesions upon scar tissue formation and recovery of motor function [133–135]. The findings of these studies have shown that injection of macrophages incubated with fragments of sciatic nerve, a nerve capable of regeneration, promoted functional recovery [135]. Additional experiments by the same group showed that skin derived macrophage populations also promoted functional recovery when injected into spinal cord lesions [133]. The exact mechanisms by which these cells promote recovery in the CNS are unknown. However, it has been suggested that these cells act through the secretion of MMPs which are crucial for scar tissue degradation, thereby allowing axonal growth to occur [131]. It was also shown that the optimal time for injection of these cells was one week post-injury, suggesting that early M1 polarization may play a beneficial role in CNS remodeling following injury, similar to that observed in skeletal muscle [136]. Finally, localization of blood-derived macrophages to the margin of the site of injury appears to be essential to functional recovery [137]. Contrary to many long-held views regarding neural inflammation and tissue remodeling, these results suggest that strategies that promote the recruitment of additional circulating monocytes and macrophages may promote functional recovery as opposed to tissue damage and lesion propagation.

3.2.5. Fibrosis—The above examples highlight the complex and tissue specific roles of M1 and M2 macrophage populations in tissue remodeling following injury in a number of settings. In each of these cases, a delicate balance and timely switch between M1 and M2 phenotypes with resolution of the inflammatory response is essential for tissue remodeling with functional recovery. Persistent or dysregulated inflammation can result in fibrosis following tissue injury or in the setting of infection [101,102]. Macrophages have been shown to be important regulators of the fibrotic response, and the mechanisms by which macrophages control the process of fibrosis have been reviewed elsewhere [101], but will be discussed briefly to emphasize the importance of phenotypic regulation of macrophages, a concept which is of critical importance in the context of the host tissue response to biomaterials as discussed in the following section.

Briefly, macrophages regulate the process of fibrosis through the production of cytokines which directly activate fibroblasts (TGF- β , PDGF, insulin like growth factor (IGF)-1), the production of MMPs and TIMPs which drive ECM remodeling, and M1 factors which drive a “feed-forward” state of inflammation and remodeling (IL-1b) [102]. Macrophages, however, can also regulate or reverse fibrosis through the secretion of matrix degrading MMPs and other M2 factors that have been shown to suppress fibrosis (IL-10, RELM α , Arg-1) [102,138–140]. The exact contributions of specific M1 and M2 populations in fibrosis are not well defined. However, a better understanding of the exact mechanisms by which macrophages are able to promote tissue specific remodeling responses and prevent fibrosis represents a major opportunity for biomaterials and regenerative medicine strategies that protect device function and/or promote disease resolution and appropriate functional recovery.

4. The role of macrophage polarization following biomaterial implantation

Each of the examples above demonstrates the potential positive and negative effects of macrophage participation in disease and tissue remodeling. Whether macrophage participation results in a positive or negative outcome depends largely upon context appropriate polarization towards an M1 or an M2 phenotype and the ability to shift and resolve polarized responses. It is likely, therefore, that similar paradigms and processes apply to the remodeling which occurs following the implantation of biomaterials.

Every material used in implantable medical devices, tissue engineered constructs, or scaffold-based approaches to regenerative medicine will evoke a host response which begins immediately upon implantation. The ability of the host innate immune system to resolve a polarized macrophage response resulting from implantation may also be of critical importance in determining the downstream functional success of implanted biomaterials, in much the same way described for tissue injury. Therefore, an effective biomaterial-based strategy for the replacement or repair of biologic structures requires not only an in-depth understanding of tissue specific mechanical and functional requirements, but also an in-depth understanding of the tissue specific interactions between the host innate immune system and the biomaterial of choice. A large number of studies have investigated the foreign body response and potential strategies for avoiding it. However, few studies have evaluated macrophage polarization specifically with respect to biomaterials implantation. Three examples are provided below which explore the effects of macrophage polarization following biomaterial implantation and demonstrate the potential benefits of strategies which are able to modulate the macrophage response in regenerative medicine.

4.1. The role of macrophage polarization following the implantation of long-term medical devices

Materials intended for use in long-term implantable medical devices include a wide spectrum of metallic and polymeric materials designed to be both mechanically robust and functional without evoking potentially pathogenic responses from the recipient. These materials are subject to a variety of host–material interfaces (i.e. soft tissue, bone, blood) depending on the nature of the device and its intended function. Biological processes at the host tissue interface often lead to complications or failure of the implanted device to perform as intended. While the specific mode of failure is highly context dependent, a major complication following the implantation of any material is the development of a foreign body reaction (FBR). The FBR consists of a set of well known and overlapping processes (reviewed in [141,142]) that result in the encapsulation of the material, thus isolating it from the surrounding tissues and, generally, negatively impacting the ability of the device to function as intended. One of the best-described examples of the adverse consequences of the FBR is the host response following the implantation of total joint replacements (TJR).

TRJ are continually subjected to wear of the bearing surfaces during daily activity. The byproducts of wear include particulate polymeric, ceramic and metallic debris (mostly in the nano, submicron, and micron size range) and in the case of metal-on-metal implants, metallic ions [143]. These byproducts are well known to cause pro-inflammatory responses which can persist for the life of the implant [144,145]. This type of chronic inflammatory and foreign body reaction to excessive wear debris has been studied for more than three decades. In the case of TJR, these processes result in periprosthetic bone resorption (osteolysis) which undermines the bony foundation of the implant and can lead to chronic synovitis, loosening and pathologic fracture [144]. Additionally, contamination with endotoxin and other bacterial byproducts may occur during the lifetime of the implant, leading to further pro-inflammatory activation and adverse outcomes [146]. With respect to these events, the macrophage plays a key role in determining the remodeling characteristics and functional outcome at the interface between the implant and the surrounding bone and soft tissues.

Tissue retrieval studies have begun to examine macrophage phenotype in periprosthetic tissues harvested from loose, surgically revised implants with osteolysis as compared to macrophage phenotype in the synovium harvested from osteoarthritic joints treated by primary TJR. In the former tissues, an M1 macrophage phenotype predominated, whereas M2 macrophages were more prevalent in the latter tissues, indicating that macrophage phenotype may play an important role in outcomes following TJR. These results also suggest that strategies which promote polarization to an M2 anti-inflammatory, pro-tissue healing phenotype may be a potential strategy to mitigate the adverse effects of particle-induced inflammation and osteolysis around TJR. However, exact strategies for eliciting this type of response and the ability of such strategies to prevent osteolysis have yet to be explored.

4.2. The role of macrophage polarization following the implantation of materials in regenerative medicine

In contrast to the materials intended for use in long-term medical implants, the materials most suitable for regenerative medicine applications are generally intended to serve as “scaffold” materials which facilitate the restoration of the normal structure and function of the tissue or organ of interest. These materials are typically manufactured from either synthetic or naturally occurring raw materials and commonly combined with bioactive molecules or living cells. The host response to these scaffold materials or multi-component constructs, therefore, is often distinctly different from that described for materials intended as permanent implants.

Invariably, however, the implantation of any material involves components of the host inflammatory response, comprising both innate and adaptive mechanisms, which begin immediately upon implantation and continue throughout the course of tissue remodeling. Without question, the manner in which the host responds to the intervention of choice will be a critical determinant of success or failure of any surgical implant or regenerative medicine strategy. To date, studies examining the specific effects of macrophage polarization following implantation of synthetic or naturally derived biomaterials in regenerative medicine have been few. Two examples of such studies are provided below to illustrate potential methods by which biomaterials may be able to control macrophage polarization with advantageous effects upon the tissue remodeling outcome.

4.2.1. Synthetic biomaterials—Synthetic non-degradable and slowly degradable biomaterials, following implantation in vascularized tissue, are generally encapsulated within an almost avascular, fibrous connective tissue within 2–4 weeks. Resident and

recruited macrophages cannot spread sufficiently to ingest these large objects (frustrated phagocytosis) and subsequently fuse into multinucleate giant cells, a hallmark of the classic FBR similar to that described above for long-term implantable biomaterials [142]. These multinucleate and mononuclear macrophages can be found at biomaterial surfaces even years following implantation.

Recent studies have demonstrated that porous biomaterials of the same composition as those which, in their non-porous form, elicit an FBR and encapsulation heal with less encapsulation and more vascularity when implanted into either the dermis or into cardiac tissues [147–149]. To assess the importance of porosity to healing, a series of materials were made in which pores were spherical, interconnected and of uniform size. It was noted that those materials possessing pores of 30–40 μm healed with minimal fibrosis and the highest vascularity [149]. These pores were also found to be heavily infiltrated by macrophages, but not foreign body giant cells. Further, when compared to the non-porous control, implants with 30–40 μm pores elicited a host response which was characterized by significantly higher ratios of M2/M1 macrophages [148]. Similarly, recent studies have also shown potential modulating effects of biomaterial surface topography upon macrophage polarization [150,151]. Thus, control of macrophage phenotype, with beneficial effects upon tissue remodeling, can be driven by biomaterial morphology. Although the exact mechanisms which underlie the observed responses still remain largely unknown, such biomaterials mediated control of macrophage phenotype has significant implications for improving outcomes in regenerative medicine.

4.2.2. Naturally derived biomaterials—Materials derived from mammalian tissue sources elicit a distinctly different host response than those composed of synthetic materials due to their unique surface topologies and ligand landscapes [152]. Further, naturally derived materials likely experience adsorption of a different repertoire of molecules than do synthetic materials and possess inherent surface functionality. A number of studies have shown that biologically derived scaffold materials, when prepared by tissue decellularization methods that largely preserve native structure and composition, are capable of promoting constructive tissue remodeling in a number of tissues and organs [153]. This constructive remodeling response has been directly linked to the ability of the material to modulate macrophage phenotype [154–157].

Briefly, scaffold materials composed of extracellular matrix (ECM) have been shown to promote a switch from a predominantly M1 cell population immediately following implantation to a population enriched in M2 cells by 7–14 days post implantation [154–156]. The phenotypic profile of the cells which respond to these scaffold materials at early time points has been shown to be a statistical predictor of the downstream outcome associated with their implantation [155]. The mechanisms by which ECM based scaffold materials promote the M1 to M2 transition remain unknown. However, modification of such scaffold materials with chemical crosslinking agents which delay or prevent macrophage mediated degradation inhibits the formation of the beneficial M2 response and results in downstream scar tissue formation [154]. These results suggest that interactions of host cells with ligands on the surface of the material, or their degradation products, may be responsible for the observed phenomena. While the exact surface ligands responsible have yet to be identified, a better understanding of macrophage-surface ligand interaction has significant potential to inform future biomaterial design and applications.

5. Conclusions

The boundaries between the classic inflammatory response, the processes of tissue remodeling and organ development, and the tissue adaptive response to implanted

biomaterials have become increasingly blurred. It is clear, however, that the macrophage plays a crucial and potentially determinant role in the outcome in each of these cases. The timely modulation of macrophage phenotype, in particular, appears to be a crucial event in the tissue remodeling process. Indeed, inappropriate polarization towards either an M1 or M2 extreme may have unintended and deleterious consequences. An increasing number of studies in the field of regenerative medicine have begun to apply these paradigms and concepts, and have shown that macrophage phenotype can be modulated by biomaterials with improved tissue remodeling and long-term functional outcomes as a result. This suggests that strategies which provide control of macrophage phenotype may meet with greater success in regenerative medicine applications. A better understanding of the context specific biological mechanisms which underlie the macrophage response and macrophage polarization switching is essential for the development of strategies which promote site-appropriate constructive and functional tissue remodeling responses as opposed to deleterious persistent inflammation and scar tissue formation.

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