Chapter 2
The Role of Macrophages in the Foreign Body Response to Implanted Biomaterials

Tony Yu, Valerie J. Tutwiler and Kara Spiller

2.1 Introduction

Biomaterials are part of the solution to many unmet clinical needs, from implantable sensors to drug delivery devices and engineered tissues. However, biomaterials face an inflammatory environment upon implantation, which represents a potential obstacle to their success [1]. In this chapter, we review the consequences of the foreign body response (FBR) for biomaterial function and strategies that have been used to inhibit the FBR. We focus on the role of the macrophage, the cell at the center of the inflammatory response, as the major regulator of the FBR, and discuss implications of changing macrophage behavior on biomaterial acceptance or rejection. Finally, we discuss recent discoveries in the role of macrophage phenotype, ranging from pro-inflammatory (M1) to anti-inflammatory (M2), and the role it plays in wound healing and biomaterial vascularization and integration. We conclude with a discussion of biomaterial design strategies that have been suggested to positively interact with and potentially control macrophages in order to improve interactions between biomaterials and the inflammatory response.

K. Spiller (✉) · T. Yu · V. J. Tutwiler
Biomaterials and Regenerative Medicine Laboratory, School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, PA, USA
e-mail: spiller@drexel.edu

T. Yu
e-mail: ty63@drexel.edu

V. J. Tutwiler
e-mail: Valerie.j.tutwiler@drexel.edu

L. Santambrogio (ed.), *Biomaterials in Regenerative Medicine and the Immune System,*
DOI 10.1007/978-3-319-18045-8_2

2.2 The Foreign Body Response and Consequences for Implanted Biomaterials

Upon implantation (Fig. 2.1a), the body mounts the FBR against the biomaterial, beginning with protein adsorption to the biomaterial surface (Fig. 2.1b) [2]. This creates a thrombogenic surface and results in the activation and aggregation of a platelet–fibrin meshwork (Fig. 2.1c) [3]. The resulting procoagulant surface results in the infiltration of inflammatory cells. Neutrophils are the first inflammatory cells to arrive at the biomaterial surface [4]. When these cells are unable to phagocytose the foreign body, cytokines are released, which result in the differentiation of macrophages from monocytes [5]. Of the recruited immune cells, macrophages are the main cell type that regulates the FBR. At this point in normal wound healing, the acute inflammation phase (Fig. 2.1d) would ebb, and proliferation of fibroblasts and eventually remodeling of the wound would occur [4]. However, in response to an implanted biomaterial, the macrophages continue to attempt to remove the foreign body via phagocytosis and secrete enzymes and reactive species that aggravate the inflammatory state [6]. As a result, the progression through normal wound healing is disturbed and a chronic inflammation phase ensues (Fig. 2.1e) [7]. If the biomaterial cannot be degraded, the macrophages fuse together to form multinucleated foreign body giant cells (FBGC) that surround the biomaterial [8]. FBGCs and recruited fibroblasts deposit collagen layers around the biomaterial to form granulation tissues [9]. Over time, the granulation tissue becomes a dense collagen capsule, the hallmark of the FBR (Fig. 2.1f) [9]. Isolation of the biomaterial within this

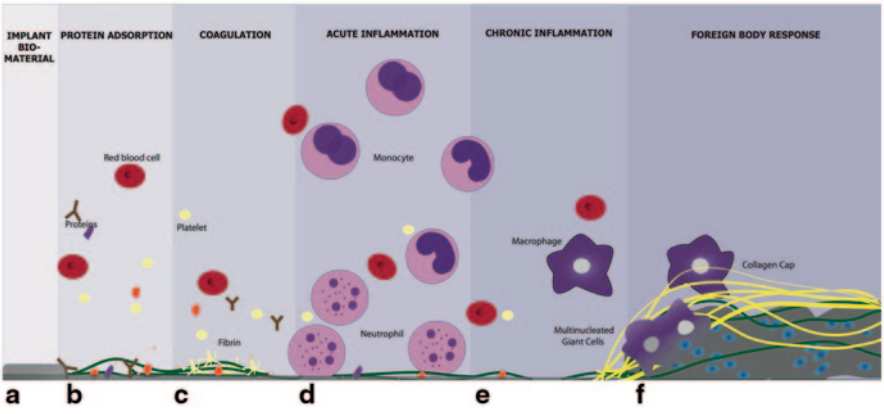

Fig. 2.1 Progression of the foreign body response. Upon implantation of the biomaterial (**a**), proteins from the blood and tissue nonspecifically adsorb to the biomaterial surface (**b**), and the coagulation cascade is initiated (**c**). Neutrophils and monocytes are recruited during the acute inflammatory phase (**d**). Monocytes differentiate into macrophages which attempt to degrade the biomaterial. If the macrophages cannot degrade the material, they fuse into foreign body giant cells, the hallmark of chronic inflammation (**e**). These multinucleated cells stimulate the formation of granulation tissue, which eventually becomes a dense fibrous collagen capsule that isolates the biomaterial from the rest of the body (**f**)

capsule as well as the secretion of damaging enzymes jeopardizes the functioning of the biomaterial. Some biomaterial applications that are particularly sensitive to the FBR include drug delivery devices, sensory devices, electrical devices, and tissue-engineered constructs.

2.2.1 Diffusion-Dependent Biomedical Devices

Biomaterials that depend on diffusion of molecules for their function include drug delivery systems, which are used to locally deliver drugs or growth factors to a particular area of the body, and sensors that measure the level of a molecule in the blood or tissue, including glucose sensors (Fig. 2.2a). Fibrous encapsulation can hinder diffusion, thus adversely affecting the function of the biomaterial [10]. For example, Anderson et al. investigated the drug release of gentamicin, an antibiotic, from silicone rubber rods [11]. Liquid-scintillation counting was performed to determine the release of radiolabeled gentamicin as well as the concentration in tissues adjacent to the implant. The rods were coated with a layer of silicon rubber to reduce the initial burst release and to prolong the drug release over time. The silicon rod drug release system with different gentamicin loading dosages (20, 35, and 40 wt.%) was implanted intramuscularly at the thigh muscles in dogs for 1 day and 1, 2, and 4 weeks. A fibrous capsule of about 5 and 11–15 μm thick was observed at 2 and 4 weeks post-implantation, respectively. Along with the observation of connective tissues forming around the implants, a reduction of the drug release rate was observed over the 4-week period. In addition, the difference in the gentamicin tissue level and serum levels over the first 3 weeks indicated that there was another factor with a different diffusion coefficient that may be responsible for the reduction in the drug release rate. As a result, it was suggested that the formation of the fibrous capsule inhibited the release of gentamicin [11].

One of the most common medical devices to monitor diabetes is the continuous glucose monitor (CGM), which monitors the blood glucose level via diffusion of blood glucose to the glucose sensor [12]. The FBR can hinder this function, as is demonstrated by a study in which macrophage depletion with diphtheria toxin driven by the CD11b promoter improved sensor performance [13]. While the creation of a diffusion barrier by the fibrous capsule likely inhibits sensor performance, it may also be a result of macrophage metabolic activity [14]. Klueh et al. injected macrophages at the implantation site of glucose sensors in mice [12]. The macrophages surrounded the sensors and sensor output quickly diminished, an effect that was not observed with injected lymphocytes. Interestingly, when serum glucose levels were artificially elevated, they were detected by the sensors, suggesting that their function was not permanently impaired by the presence of the macrophages. Moreover, companion in vitro studies showed that the presence of macrophages without the fibrous capsule or other biofouling effects also hindered sensor performance. The authors concluded that metabolism of glucose by macrophages also contribute to decreased sensor performance [12].

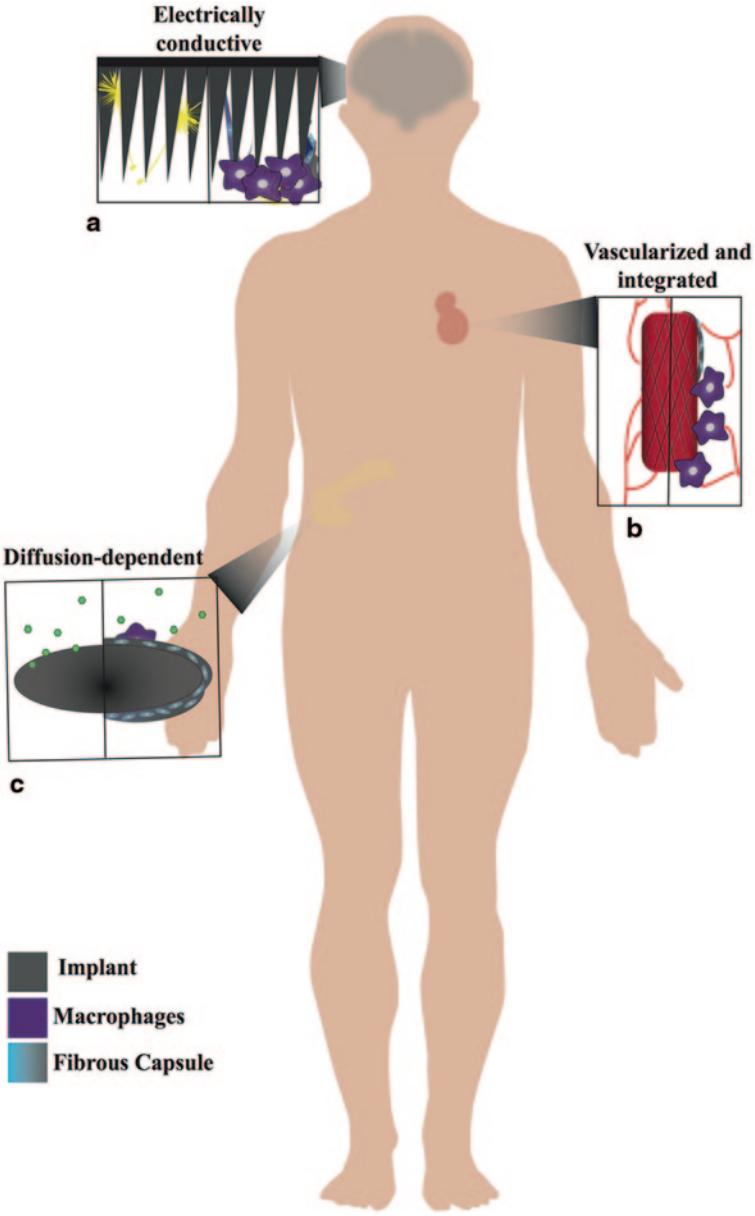

Fig. 2.2 Biomaterials particularly sensitive to the foreign body response. **a** Glial scarring around implanted microelectrodes can block the conductance of neuronal activity. **b** Vascularization, critical for the functionality of some implants, is blocked by fibrous encapsulation. **c** The fibrous capsule acts as a diffusion barrier, decreasing the performance of diffusion-dependent biomaterials such as drug delivery devices and glucose sensors

2.2.2 Transmission of Electrical Signals

Some medical devices require the conductance of electrical signals in order to monitor or pace the electrical activity of the brain, heart, or other muscles in the body, but the transmission of these signals can be inhibited by the presence of a fibrous capsule or a glial scar, as is the case in the central nervous system (Fig. 2.2b) [15]. A common electrical recording device is the silicon microelectrode array, a technology that measures the neuronal activity in the brain that is often used to monitor the activity of neurons and/or to investigate the correlation between the brain activity and behavior. However, one of the main limitations of this technology is inconsistency of performance in long-term applications. In a study by Biran et al. [16], the silicon microelectrode array was implanted into the brains of rats for 2 and 4 weeks to determine the mechanism of failures of the microelectrode arrays. Stab wounds were also created with the same microelectrodes as controls in order to distinguish whether it was the initial penetrating trauma or the FBR to the chronically implanted microelectrodes that caused device failure. After 2 and 4 weeks post-implantation, immunohistochemical analysis of the brain tissue indicated multilayered and dense regions of ED1-positive cells, a pan-macrophage marker in rats, along the implant–brain tissue interface in both the stab wounds and implanted microelectrodes. However, there were more ED1-positive cells surrounding the implanted microelectrodes compared to the stab wound. The intensity of glial fibrillary acidic proteins (GFAP) expression by reactive astrocytes was also significantly higher surrounding implanted microelectrodes compared to the control stab wounds. There was a significant amount of neuronal loss 2 weeks post-implantation in the nearby tissue of the implanted microelectrodes compared to the stab wound. Explants were rinsed with phosphate-buffered saline (PBS) and cultured in media for 24 h to assess cytokine secretion by adherent macrophages, which showed secretions of the inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and monocyte chemotactic protein (MCP-1). Thus, the presence of the foreign body increased inflammation, leading to neuronal loss and device failure [16].

2.2.3 Vascularization and Integration of Biomaterials

Tissue engineering holds tremendous potential to replace damaged tissues and organs. The success of most tissue-engineered constructs requires recruitment of endothelial cells and the formation of new blood vessels to provide nutrients and oxygen transport for implanted cells [17, 18]. However, the FBR and the fibrous capsule prevent direct contact between the biomaterial and the surrounding tissue, so that vascularization and integration are essentially blocked (Fig. 2.2c). Shin et al. showed that fibrous encapsulation of hydrogels based on oligo(poly(ethylene glyocol) fumarate) effectively prevented bone formation and vascularization in a rabbit bone defect model [19]. More recently, several studies have confirmed inverse correlation between fibrous capsule thickness and blood vessel ingrowth [5, 20].

2.3 Strategies to Inhibit the FBR

Clearly, the FBR and the formation of the fibrous capsule can drastically inhibit the function of biomaterials. Thus, researchers have turned to the development of strategies to inhibit the FBR. Because the FBR begins with protein adsorption and inflammatory cell interactions at the biomaterial surface, most strategies are based on modifications of the biomaterial surface [21, 22]. The main strategies include inhibition of protein adsorption, the use of bioactive coatings, and modifications to surface topography.

2.3.1 Inhibition of Protein Adsorption

Because the FBR begins with protein adsorption, inhibition of protein adsorption has been extensively researched as a tool to inhibit the FBR [23]. Polyethylene glycol (PEG)ylation, hydrogel coatings, plasma treatment, and other methods have shown substantially decreased protein adsorption in vitro with reduced fibrous capsule formation in vivo [24]. Ultimately, however, the sensors fail because blood proteins can still adsorb to a certain extent [14]. Recently, ultra-low fouling biomaterials have been prepared from zwitterionic materials [21]. Zwitterionic materials have both a positive and negative charge that are not dissociated in an aqueous environment. This property attracts water molecules via charge–dipole interactions resulting in extremely hydrophilic properties [25]. Thus, adsorption of relatively hydrophobic proteins is drastically reduced. The zwitterion carboxybetaine was shown to adsorb <0.3 ng/cm^2 proteins from 100 % blood serum, much lower than the 5 ng/cm^2 of absorbed fibrinogen that is required to initiate platelet adhesion [22]. When zwitterionic hydrogels based on poly(carboxybetaine methacrylate) (PCBMA) were implanted subcutaneously in mice for 3 months, the number of pro-inflammatory macrophages was reduced compared to control hydrogels prepared from poly(2-hydroxyethyl methacrylate) (PHEMA), and the presence of a fibrous capsule was not observed [21].

Thus, the inhibition of protein adsorption is an effective way to mitigate the FBR. However, without protein adhesion, cells from the body also cannot infiltrate the material, so these biomaterials may not be appropriate for applications that require integration with the body, such as in tissue engineering. Nonetheless, they may be extremely useful for applications in which the biomaterials are not intended to integrate with body, such as catheters.

2.3.2 Surface Modification with Bioactive Coatings

For biomaterials that are intended to integrate with the body, another strategy to mitigate the FBR and the formation of the fibrous capsule is to make the biomaterial

appear less foreign to immune cells, such as by coating with extracellular matrix (ECM)-derived molecules [26]. The ECM is mainly composed of collagen type I and glycosaminoglycans (GAG) such as hyaluronan (HA), chondroitin sulfate, and dermatan sulfate. Coating titanium rods with collagen chondroitin sulfate has been shown to inhibit fibrous encapsulation and to promote new bone formation in rat tibial defects [27]. Similarly, drug delivery strategies that actively increase integration with the body show decreased fibrous capsule formation. For example, controlled release of vascular endothelial-derived growth factor (VEGF) and nitric oxide (NO) from sensors has been shown to increase vascularization and decrease fibrous capsule formation [28, 29]. Controlled release of anti-inflammatory drugs such as dexamethasone has also been shown to reduce the FBR to sensors [30].

2.3.3 Surface Topography

Modifications to the surface topography of biomaterials have also been shown to affect the FBR [31]. The addition of porous poly(lactic acid) (PLA) coatings to glucose sensors decreased fibrous capsule thickness and increased vascularity following murine implantation [32]. Cao et al. investigated the orientation of the topography of electrospun nanofibrous poly(caprolactone) (PCL) scaffolds and reported the effect on the FBR [31]. The PCL was deposited in three distinct manners: aligned fibers, randomly oriented fibers, and a thin film; the scaffolds were then compared to an arginylglycylaspartic acid (RGD)-coated glass slide as a control. Interleukin (IL)-4 was added to human monocytes in vitro at days 3 and 7 in order to induce the formation of FBGC, mimicking the FBR in vivo. At day 10, the random fiber scaffolds resulted in the highest levels of cell attachment compared to the other scaffolds. In general, the cell density of all surfaces decreased over time as the macrophages fused into FBGC in the presence of IL-4. When the PCL scaffolds were implanted in Sprague-Dawley rats for 1, 2, and 4 weeks, the random fiber scaffold elicited a more severe FBR compared to the other scaffolds, while the aligned fiber scaffold resulted in the thinnest fibrous capsule [31]. Thus, biomaterial topography affects the FBR, and this behavior can be studied using in vitro models of macrophage–biomaterial interactions.

This relationship between in vitro and in vivo results was not supported in another study of the effects of biomaterial topography on the FBR. Expanded polytetrafluoroethylene (ePTFE) membranes with pore sizes of 0.2, 1, and 3 μm were seeded with primary human monocytes in vitro and compared to tissue culture polystyrene as a control [33]. Membranes with 3 μm pore size elicited a significant increase in the secretion of the inflammatory cytokine IL1-beta compared to the other pore sizes. The ePTFE biomaterials were also implanted subcutaneously in mice for 4 weeks to evaluate the formation of the fibrous capsule. Interestingly, despite showing more inflammatory activity in vitro, ePTFE membranes with 3 μm pores resulted in a significantly thinner fibrous capsule than the nonporous ePTFE [33]. Although these findings did show that biomaterial topography affects the FBR, they

also highlight the complexity of the relationship between inflammatory cell–biomaterial interactions and the FBR.

While the studies of the effects of biomaterial surface topography on the FBR have been largely empirical, they do suggest that modulation of topography may be a potential tool for mitigating the FBR. More systematic analyses are required to determine the mechanism of topographical effects on macrophage behavior and the FBR.

2.4 Macrophage Biology

It has been shown through many studies that macrophages play a crucial role in regulating the FBR [5]. A better understanding of macrophage dynamics may be the key to overcoming their ability to impair biomaterial performance. To understand the behavior of macrophages in response to biomaterials, it is helpful to consider biomaterial implantation as a chronic wound. Then, the behavior of macrophages can be assessed in comparison to normal wound healing in order to discover the mechanisms of impaired healing.

2.4.1 Macrophage Phenotypes in Normal Wound Healing

Normal wound healing in response to an injury generally consists of four distinct stages: hemostasis, the inflammatory stage, the proliferation stage, and the remodeling stage [4, 34, 35]. Macrophages can be polarized into a spectrum of phenotypes ranging from pro-inflammatory to anti-inflammatory and pro-healing depending on the environmental stimulus [36]. Pro-inflammatory macrophages are often referred to as “classically activated,” or M1, while the anti-inflammatory macrophages are referred to as “alternatively activated,” or M2 phenotype, following the T helper cell nomenclature of Th1 and Th2 [36]. At early stages of normal wound healing, M1 macrophages infiltrate the wound to promote inflammation and to stimulate the wound healing process (Fig. 2.3). M2 macrophages begin to accumulate around day 3 or 4 post-injury, while the level of M1 macrophages decreases [36]. M2 macrophages may accumulate via the direct transition of M1 to M2, the polarization of newly arriving macrophages to M2, and proliferation of other M2 macrophages [37]. The accumulated macrophages eventually emigrate to the draining lymph nodes returning back to the pre-injury state of resident macrophages after the wound is completely remodeled and healed [38].

Macrophages have been widely recognized as major regulators of wound healing and tissue regeneration over the past few decades. However, much of macrophage biology is still not well understood. Although the classification of the different macrophage phenotypes is widely accepted, a consensus has not yet been reached as to the overall effects and consequences of the diverse macrophages phenotypes on wound healing.

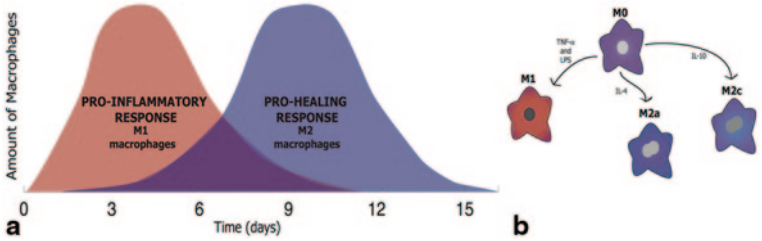

Fig. 2.3 Macrophages in normal wound healing. **a** In normal wound healing, macrophages initially express a pro-inflammatory response, but as time progress they transition to a pro-healing response. **b** Macrophages can be polarized into a spectrum of phenotypes that range from a pro-inflammatory to a pro-healing response. Those discussed here include M1 macrophages which are induced through by TNF-α and LPS, M2a macrophages which are stimulated by IL-4, and M2c macrophages which are activated by IL-10. *LPS* lipopolysaccharide

2.4.2 The Role of M1 Macrophages in Healing

Macrophages are polarized to the M1 phenotype by pro-inflammatory stimuli and cytokines such as bacterial lipopolysaccharide (LPS), TNF-α, and interferon-gamma (IFN-γ, Fig. 2.3b) [5]. M1 macrophages attempt to phagocytose any bacteria, cellular debris, and foreign invaders. However, controversies surround the role of M1 macrophages in wound healing. On one hand, chronic inflammation, characterized by persistent numbers of M1 macrophages is known to impair wound healing. For example, Kigerl et al. studied the effect of macrophage activation on central nervous system injury of C57BL/6 mice [39]. Moderate midthoracic spinal cord injury (SCI) was inflicted on the mice, while the sham mice receive a laminectomy without SCI. The tissue samples were collected at day 1, 3, 7, 14, and 28 post-SCI for immunohistochemical analysis. M1 macrophages were predominant at the sites of SCI as indicated by CD86 staining. The RNA from each of the wound sites at each time point was extracted for gene expression and showed that the genes associated with the M2 macrophages returned to pre-injury level at day 7 post-SCI, while genes associated with the M1 macrophages were maintained for 1 month post-SCI. These results suggest that the M1 macrophages were responsible for the defective wound healing over time. Furthermore, macrophage-conditioned media (MCM) was also collected from the supernatant of polarized macrophages to determine the effect of M1 and M2 MCM on cortical neurons in vitro. The M1—but not the M2—MCM was neurotoxic to cortical neurons [39]. Consequently, this study suggests that the M1 macrophages are detrimental to healing.

On the other hand, M1 macrophages have also been shown to be beneficial for wound healing [40]. M1 macrophages are highly angiogenic, stimulating endothelial cell sprout formation in vitro and in vivo in part by secretion of VEGF [5, 41]. When M1 macrophages were depleted in a mouse model of skeletal muscle injury via CD11b-diptheria toxin, muscle regeneration was completely prevented [42]. In contrast, when M2 macrophages were depleted, muscle regeneration was still possible, but was significantly impaired. However, persistent numbers of M1

macrophages mark chronic inflammation and impaired healing, highlighting the importance of the correct M1-to-M2 sequence in tissue repair.

2.4.3 The Role of M2 Macrophages in Wound Healing

M2 macrophages are generally associated with healing and tissue remodeling of the wound and are the dominant phenotype in the proliferation and remodeling stages of wound healing [43]. The M2 macrophages are usually responsible for the formation of connective tissue [44]. However, the granulation tissue may eventually lead to the formation of scar tissue or a fibrous capsule. For this reason, it is believed that the M2 macrophages contribute to the fibrous capsule formation and the FBR [4].

The M2 macrophage phenotype can be further classified into three subpopulations: M2a, M2b, and M2c [5, 45]. Macrophages are polarized to the M2a and M2c phenotype by environmental stimulation of IL-4 and IL-10, respectively (Fig. 2.3b) [5, 45]. The M2b phenotype is polarized by toll-like receptors (TLC) or other immune complexes [45]. Although the M2b is categorized within the M2 phenotype, the M2b macrophages are activated by an inflammatory environmental stimulus more similar to that of the M1 macrophages [46]. Their role in wound healing is not known. The traditional alternatively activated M2 macrophages are now referred to as the M2a phenotype, which promotes the production of ECM and collagen, a necessary part of healing [5, 46].

Preliminary studies have attempted to explain the functioning of M1, M2a, and M2c subpopulations in wound healing and vascularization [5]; however, there is still a great need for further research in this area. M1 macrophages secrete VEGF to initiate angiogenesis. M2a macrophages secrete platelet-derived growth factor (PDGF), a chemoattractant that stabilizes growing blood vessels and promotes anastomosis of new blood vessels into networks [5]. M2c macrophages secrete high levels of matrix metalloprotease 9 (MMP-9), a protease that is involved in the breakdown and remodeling of the ECM and vasculature [5]. M2c macrophages also express high levels of CD163, which has been shown to be associated with tissue repair and remodeling of the wound and promoting cell proliferation in mice [45]. Thus, it appears that M1, M2a, and M2c macrophages appear sequentially in normal wound healing, but more studies are required that distinguish between M2a and M2c macrophages in order to confirm this hypothesis.

2.4.4 Role of M1 and M2 Macrophages in the FBR to Biomaterials

Surprisingly, it is still not clear which macrophage phenotype is responsible for the formation of the fibrous capsule. M1 macrophages are widely believed to be the cause of the fibrous capsule as the inflammatory response upregulates the FBR, thereby increasing the thickness of the fibrous capsule [47]. However, IL-4, a

http://www.springer.com/978-3-319-18044-1

Biomaterials in Regenerative Medicine and the Immune System

Santambrogio, L. (Ed.)

2015, IX, 280 p. 82 illus., 59 illus. in color., Hardcover
ISBN: 978-3-319-18044-1