Chapter 13
Activation of Macrophages in Response to Biomaterials

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Abstract Macrophages are the initial biologic responders to biomaterials. These highly plastic immune sentinels control and modulate responses to materials, foreign or natural. The responses may vary from immune stimulatory to immune suppressive. Several parameters have been identified that influence macrophage response to biomaterials, specifically size, geometry, surface topography, hydrophobicity, surface chemistry, material mechanics, and protein adsorption. In this review, the influence of these parameters is supported with examples of both synthetic and naturally derived materials and illustrates that a combination of these parameters ultimately influences macrophage responses to the biomaterial. Having an understanding of these properties may lead to highly efficient design of biomaterials with desirable biologic response properties.

Abbreviations

Arg	Arginase
BMDM	Bone marrow-derived macrophage
CCR7	C-C chemokine receptor type 7
CD16	Cluster of differentiation 16
CD163	Cluster of differentiation 163
CD200	Cluster of differentiation 200
CD206	Cluster of differentiation 206 or mannose receptor

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CD47	Cluster of differentiation 47
CD80	Cluster of differentiation 80
CD86	Cluster of differentiation 86
CFA	Complete Freund's adjuvant
COX-2	Cyclooxygenase-2
CXCL10	C-X-C motif chemokine 10
FBGC	Foreign body giant cell
FGF	Fibroblast growth factor
HLA-DR	Human leukocyte antigen, antigen D related
IC	Immune complex
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-13	Interleukin 13
IL-17	Interleukin 17
IL-1β	Interleukin 1 beta
IL-4	Interleukin 4
IL-4RA	Interleukin 4 receptor antagonist
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-9	Interleukin 9
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
M(−)	Unstimulated, resting, or naïve macrophage
M(X)	Macrophages stimulated with molecule X
M1	Classically activated
M2	Alternatively activated
M2a	Wound healing macrophage
M2b	Type II macrophage
M2c	Deactivated macrophage
MARCO	Macrophage receptor with collagenous structure
MHCII	Major histocompatibility complex class II
NO	Nitric oxide
p(NIPAm-co-AAc)	Poly(N-isopropyl acrylamide-*co*-acrylic acid)
PBMC	Peripheral blood mononuclear cell
PCL	Poly(ε-caprolactone)
PDMS	Poly(dimethylsiloxane)
PDO	Polydioxanone
PE	Polyethylene
PEG	Poly(ethylene glycol)
PGE_2	Prostaglandin E2 or dinoprostone
PLA	Poly(lactic acid)
PLGA	Poly(lactide-*co*-glycolide)

PMMA	Poly(methyl methacrylate)
poly(HEMA)	Poly(2-hydroxyethyl methacrylate)
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PVA	Poly(vinyl alcohol)
PVC	Poly(vinyl chloride)
ROS	Reactive oxygen species
TGF-β	Transforming growth factor beta
Th1	Type 1 T helper
Th2	Type 2 T helper
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

13.1 Introduction

Macrophages are the workhorses of the immune system and have powerful and long-lasting impact on the integration or rejection of a biomaterial (Klopfleisch 2016; Garash et al. 2016; Ogle et al. 2016). Macrophages have an ability to influence both tissue integrative and destructive processes, stemming from their relatively plastic nature (Mantovani et al. 2004; Mosser 2003; Gordon 1986; Mosser and Edwards 2008; Gordon and Martinez 2010). In the simplest of conception, macrophages exist in one of two opposing forms: classically activated or alternatively activated phenotypes (Stein et al. 1992; Gordon 1986, 2007; Gordon and Martinez 2010; Gordon and Taylor 2005; Edwards et al. 2006; Mosser and Edwards 2008; Mosser 2003). In reality, macrophages often exist in the spectrum between these extremes (Fig. 13.1). We can understand and characterize macrophages by quantifying their activation-specific characteristics. This complex macrophage phenotype shapes the general reaction to a biomaterial (Mosser and Edwards 2008).

Each macrophage activation state elicits opposing inflammatory and anti-inflammatory reactions in response to the stimulus. One nomenclature for these activation states describes which T-helper cells the macrophages activate. Type 1 T-helper (Th1) cells are activated by M1 macrophages, while type 2 T-helper (Th2) cells are activated by M2 macrophages (Mantovani et al. 2004). Classically activated macrophages promote the inflammatory response and Th1 cells (Mosser and Edwards 2008; Mosser 2003; Gordon 1986). Interferon gamma (IFN-γ) activates macrophages to the classically activated (M1) state, and this phenotype serves in the innate immune response to protect against pathogens or other danger signals in the body, such as fragments of extracellular matrix components (Nathan 2008; Gordon 2007; Dale et al. 2008; Mackaness 1977; O'Shea and Murray 2008; Classen et al. 2009). For this reason, classically activated macrophages are programmed to phagocytize any foreign materials and release mediators that induce cellular death (Mackaness 1977; O'Shea and Murray 2008; Classen et al. 2009; Mosser and

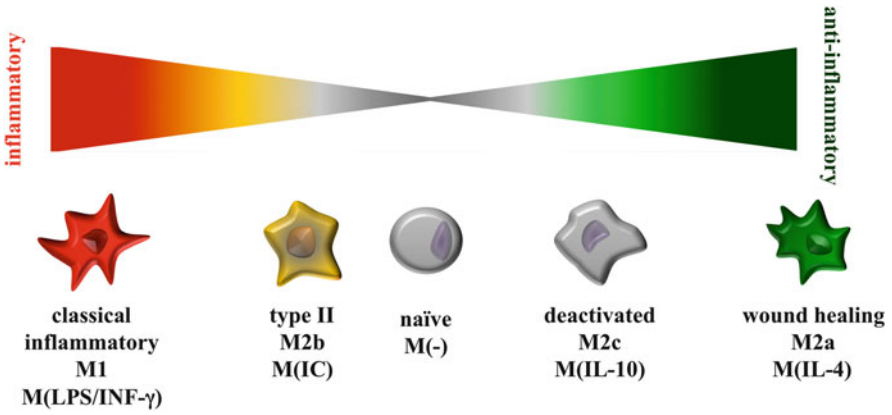
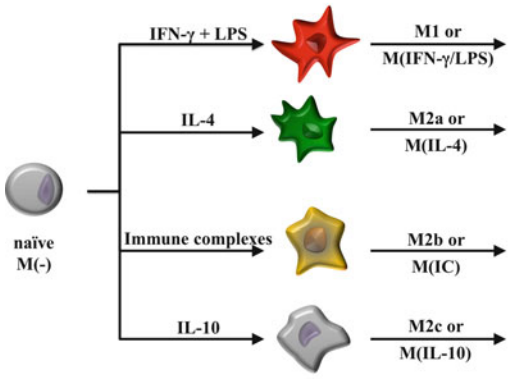


Fig. 13.1 Macrophage activation is a continuum. Macrophages exist in a wide variety of polarization states beyond those characterized in vitro. Classically activated, also known as inflammatory, macrophages and wound healing, also known as anti-inflammatory, macrophages are two extremes of this continuous spectrum. Many stimuli have the ability to polarize macrophages to points within this continuum. Stimuli that yield similar activation, such as LPS, IFN-γ, or the combination of LPS and IFN-γ, have also been identified with all three stimuli resulting in inflammatory macrophages with subtlety different characteristics. Naïve, also referred to as M(−), unstimulated or resting, macrophages lie at the center of the continuum. Type II and deactivated macrophages reside between the inflammatory and anti-inflammatory macrophages where activation characteristics are shared with both inflammatory and anti-inflammatory macrophages. This figure is adapted from several excellent reviews of macrophage activation (Murray et al. 2014; Mantovani et al. 2004; Gordon and Taylor 2005). Abbreviations: *ICs* immune complexes, *IFN-γ* interferon gamma, *IL-4* interleukin 4, *IL-10* interleukin 10, *LPS* lipopolysaccharide, *M1* inflammatory or classically activated macrophage, *M2a* wound healing macrophage, *M2b* type II macrophage, *M2c* deactivated macrophage, *M(−)* unstimulated or naïve macrophages, *M(X)* macrophage stimulated with molecule X

Edwards 2008; Mantovani et al. 2004; Mosser 2003; Gordon 1986; Murray and Wynn 2011). Classically activated macrophages also are potent producers of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 12 (IL-12), and nitric oxide (NO), each signals inflammation to the rest of the body and consequently induces tissue-destructive and foreign body destruction processes (Fig. 13.2).

On the other end of the spectrum is the alternatively activated macrophage, which stimulates Th2 cells (Loke et al. 2007; Edwards et al. 2006; Stein et al. 1992; Mosser and Edwards 2008; Mosser 2003) (Fig. 13.1). Traditionally, this classification has included all macrophage subsets that produce IL-10 and which do not fit the classically activated paradigm. This typically translates to one of three different "alternatively" activated macrophage subsets: wound healing, type II, and deactivated (Mosser and Edwards 2008; Mantovani et al. 2004; Derlindati et al. 2015). While there are a variety of alternatively activated macrophages (M2), literature on these subtypes may refer to any or all of the alternatively activated states as M2 without specifying the subtype. Due to the growing evidence of the



Cytokines	Receptors	Enzymes	Other
$IL\text{-}1\beta_{high}$, $IL\text{-}6_{high}$, $IL\text{-}12_{high}$, $TNF\text{-}\alpha_{high}$, $IL\text{-}10_{low}$	$CD86_{high}$, MHCII, $CD206_{low}$	$iNOS_{high}$, Arg_{low}	NO, ROS
$IL\text{-}10_{high}$, $IL\text{-}1\beta_{low}$, $IL\text{-}6_{low}$, $IL\text{-}12_{low}$, $TNF\text{-}\alpha_{low}$	$CD206_{high}$, MHCII, $CD86_{low}$	Arg_{high} $iNOS_{low}$	polyamines
$IL\text{-}10_{high}$, $IL\text{-}1\beta_{high}$, $TGF\text{-}\beta_{high}$, $IL\text{-}6_{high}$, $TNF\text{-}\alpha_{high}$, $IL\text{-}12_{low}$	$CD86_{high}$, MHCII		NO
$IL\text{-}10_{high}$, $TGF\text{-}\beta_{high}$, IL-4RA $IL\text{-}12_{low}$, $TNF\text{-}\alpha_{low}$, $IL\text{-}1\beta_{low}$, $IL\text{-}6_{low}$	$CD86_{high}$, $CD206_{high}$, $MARCO_{low}$		PGE_2

Fig. 13.2 Macrophage activation is classified by cytokine release, surface receptor expression, enzyme activity, and other reactive molecules. In the presence of IFN-γ and LPS, macrophages become classically activated (M1) and inflammation is stimulated. In the presence of IL-4, alternative activation of macrophages (M2a) occurs allowing the healing of wounds or injury. When stimulated with immune complexes, macrophages are stimulated toward type II activation (M2b) that functions in immunoregulation. If IL-10 is present, deactivated macrophages (M2c) act in immunoregulation and tissue remodeling. For each activation state, other stimulating molecules can be used to achieve a similar activation state. This figure is adapted from several excellent reviews of macrophage activation (Murray et al. 2014; Brancato and Albina 2011; Gordon and Taylor 2005; Biswas and Mantovani 2010; Mantovani et al. 2004). Abbreviations: *Arg* arginase, *CD86* cluster of differentiation 86, *CD206* cluster of differentiation 206 or mannose receptor, *ICs* immune complexes, *IFN-γ* interferon gamma, *IL-1β* interleukin 1β, *IL-4* interleukin 4, *IL-4RA* interleukin 4 receptor antagonist, *IL-6* interleukin 6, *IL-10* interleukin 10, *IL-12* interleukin 12, *iNOS* inducible nitric oxide synthase, *LPS* lipopolysaccharide, *M1* inflammatory or classically activated macrophage, *M2a* wound healing macrophage, *M2b* type II macrophage, *M2c* deactivated macrophage, *M(−)* unstimulated or naïve macrophages, *M(X)* macrophage stimulated with molecule X, *MARCO* macrophage receptor with collagenous structure, *MHCII* major histocompatibility complex class II, *NO* nitric oxide, PGE_2 prostaglandin E2 or dinoprostone, *ROS* reactive oxygen species, *TGF-β* transforming growth factor beta, *TNF-α* tumor necrosis factor alpha, where subscripts low and high indicate low and high expression

wide variety of macrophage polarization profiles, it has been suggested that the nomenclature be changed to classify the subtypes by polarizing stimulus (Murray et al. 2014). Since this review focuses on the response in vivo, typically inflammatory or anti-inflammatory, we will primarily refer to macrophage populations by their activity-based nomenclature: unstimulated (M(−)), inflammatory (M1), wound healing (M2a), type II (M2b), and deactivated (M2c).

Wound healing macrophages (M2a) are induced in vitro by treatment with interleukin 4 (IL-4) or interleukin 13 (IL-13) (Fig. 13.2) (Stein et al. 1992): the original "alternative" activation state. Wound healing macrophages express elevated levels of the non-opsonic mannose receptor (Stein et al. 1992) and fail to

produce nitric oxide through both elevated arginase activity and diminished nitric oxide synthase activity (Mantovani et al. 2004; Edwards et al. 2006; Mosser and Edwards 2008; Mosser 2003; Stein et al. 1992; Gordon 2007). Unlike classically activated macrophages, wound healing macrophages downregulate the production of inflammatory cytokines: TNF-α, IL-1β, IL-6, and IL-12. Additionally, wound healing macrophages upregulate immune-suppressive enzymes, including arginase, and tissue healing cytokines, specifically interleukin 10 (IL-10) (Ferrante and Leibovich 2012; Gordon and Martinez 2010). The wound healing macrophage subset is a potent mediator facilitating and organizing the wound healing process (Stein et al. 1992; Gordon 1986, 2007; Gordon and Martinez 2010; Gordon and Taylor 2005).

Other macrophage subgroups, which have been referred to as alternative macrophages, are type II macrophages (M2b) and deactivated macrophages (M2c) (Fig. 13.2) (Martinez et al. 2008; Mantovani et al. 2004). Both type II and deactivated macrophages share similarities with inflammatory macrophages, but do not directly oppose their actions by producing anti-inflammatory cytokines in the ways that wound healing macrophages do. Therefore, both type II and deactivated macrophages can be thought of as more within the spectrum of activation (Fig. 13.1), somewhere between classically activated and alternatively activated, rather than as two additional extremes of polarization.

Type II (M2b) macrophages are induced by immune complexes and share similarities with inflammatory macrophages (Fig. 13.2). Like wound healing macrophages, type II macrophages produce significant levels of nitric oxide, IL-6, TNF-α, and IL-1β. However, type II macrophages do not produce IL-12. Instead, like wound healing macrophages, they produce IL-10, which results in a Th2 response, as opposed to the Th1 response elicited by inflammatory macrophages (Gordon and Taylor 2005; Gordon 1986; Martinez et al. 2008; Martinez and Gordon 2014; Martinez 2011).

Deactivated macrophages are induced in vitro by IL-10 or glucocorticoids (Fig. 13.2). Like the wound healing macrophages, deactivated macrophages express significant levels of arginase and mannose receptor and do not produce inflammatory mediators. Deactivated macrophages are referred to as "deactivated" because they function in immune suppression and remodeling of the extracellular matrix by expression of factors, such as IL-10 and TGF-β (Martinez et al. 2008; Martinez and Gordon 2014; Gordon and Martinez 2010; Gordon and Taylor 2005).

Macrophages are highly plastic, meaning that macrophage activation is not static and that reprogramming may occur from one polarization state without dedifferentiation or deprogramming. Having biomaterials which promote the desired response, be it pro-inflammatory or anti-inflammatory, is highly desirable to tune overall biologic responses. A wide variety of biomaterial parameters have been shown to promote distinct macrophage responses as discussed below. By understanding these parameters and engineering materials with the appropriate properties, macrophages may be reprogramed to promote desirable responses to biomaterial implants.

13.2 Biomaterial Strategies for the Modulation of Macrophage Phenotype

Biomaterial success is highly dependent on macrophage polarization (Valentin et al. 2009; Greisler et al. 1989; Greisler 1988; Klopfleisch 2016; Garash et al. 2016; Ogle et al. 2016). Being able to predict or define these phenotypes prior to development of the biomaterial may help control therapeutic outcomes (Bryers et al. 2012). On the most simplistic level, one parameter may influence macrophage activation; however, it will be the combined properties that modulate responses to the material. The following sections discuss how selected parameters influence macrophage phenotype. Within each section, a table summarizing the activation of macrophages in response to this parameter is presented.

13.2.1 Size

Macrophage responses to foreign material are size dependent (Table 13.1). Macrophages are master phagocytic cells that scour the body for foreign materials ultimately attempting to phagocytize and destroy the structure. Macrophages can effectively phagocytize materials less than 10 μm in the longest dimension (Fig. 13.3). When material size increases (10–100 μm), single macrophages can no longer phagocytize the structure. Instead, a giant multinucleated cell, called a foreign body giant cell (FBGC), is formed following the fusion of several macrophages in an attempt to phagocytize the material (Anderson et al. 2008). When materials are even larger (bulk implants greater than 100 μm), macrophages undergo fusion and/or frustrated phagocytosis. At this level, it is understood that thickness may also modulate responses, where the thicker the material, the greater the foreign body and fibrotic responses expected (Nichols et al. 2013; Helton et al. 2011; Ward et al. 2002). Frustrated phagocytosis leads to highly inflammatory environment in which macrophages release a significantly elevated quantity of inflammatory cytokines, reactive oxygen and nitrogen species, and proteolytic enzymes in an attempt to degrade the material.

For example, biodegradable particles elicit a largely size-dependent influence on macrophage activation. For instance, poly(lactide-co-glycolide) (PLGA) copolymer microparticles were more inflammatory than their nanoparticle counterparts in J774 murine macrophages (Nicolete et al. 2011). Microparticles with a diameter of 6.5 μm induced the production of significantly more IL-1β and TNF-α from macrophages than nanoparticles with a diameter of 389 nm. PLGA nanoparticles (500 nm) reduced macrophage activation and led to T-cell tolerance and anergy via the scavenger receptor, MARCO, in a mouse model of experimental autoimmune encephalomyelitis, suggesting a role of these nanoparticles in inhibiting inflammatory response and modifying immune activation (Getts et al. 2012).

Table 13.1 Size-dependent influence on biomaterial-induced macrophage activation

Size (μm)	Material	Species	Model	In vitro/in vivo	Macrophage response	Geometry	References
6.5 ± 3.9	PLGA	Mouse	J774	In vitro	M1	Microparticle	Nicolete et al. (2011)
0.389		Mouse	J774	In vitro	M2	Nanoparticle	Nicolete et al. (2011)
0.500		Mouse	C57BL/6	In vivo	M2	Nanoparticle	Getts et al. (2012)
1500–1900	Alginate	Primate	Cynomolgus macaques	In vivo	M2	Implant	Veiseh et al. (2015)
300–1000		Primate	Cynomolgus macaques	In vivo	M1	Implant	Veiseh et al. (2015)

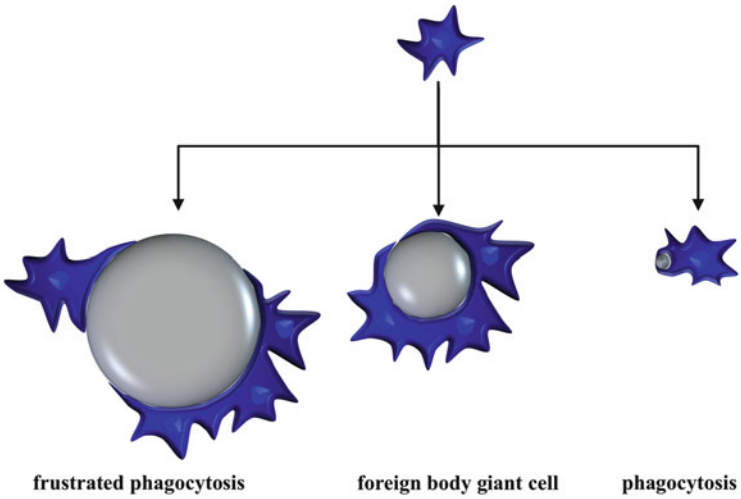


Fig. 13.3 The size of a biomaterial can influence macrophage response. Small particles (<10 μm) can be readily phagocytized, while large particles and materials can result in frustrated phagocytosis and/or foreign body giant cell, a multinuclear phagocyte, formation

Interestingly, the opposite has also been observed although the reasons for the difference can be readily explained. Large spherical implants (1.5–1.9 mm in diameter) were not easily recognized by macrophages, resulting in diminished inflammatory and fibrotic responses when implanted intra-dorsal in nonhuman primates compared to implants which were smaller (0.3–1 mm in diameter) (Veiseh et al. 2015). The diminished inflammation was confirmed to be due to sphere diameter and not total implanted surface area (Veiseh et al. 2015). This illustrated the importance of geometry of the biomaterial, as other geometries induced greater inflammatory response than did spherical implants, suggesting geometry as an additional parameter in understanding immune compatibility.

13.2.2 Geometry

Shape and geometry of the biomaterial have been argued to exhibit the greatest impact on macrophage responses (Table 13.2) (Champion et al. 2007; Champion and Mitragotri 2006). When different geometries, specifically circular, triangular, and pentagonal cross-sectional rods, were implanted in rat gluteal muscles, circular rods produced the smallest foreign body response (Matlaga et al. 1976). Intermediate response was observed for pentagonal rods. Triangular rods elicited the largest foreign body and inflammatory response from surrounding tissue macrophages (Matlaga et al. 1976). The angular aspect determined the severity of the foreign body response, suggesting that smooth, well-contoured implants have reduced inflammatory response than do those with acute angles (Salthouse 1984). More

Table 13.2 Geometry-dependent effects on biomaterial-induced macrophage activation

Shape	Material	Species	Model	In vitro/ in vivo	Macrophage response[a]	Architecture	References
Disks	PDO	Mouse	C57BL/6 BMDM	In vitro	M2	Electrospun fiber	Garg et al. (2009)
Disks		Human	PBMC	In vitro	M2	Electrospun fiber	Garg et al. (2013)
Circular rod	PVC, PE, PU, silicone, Teflon	Rat	Long-Evans	In vivo	M2	Implant	Matlaga et al. (1976)
Pentagonal rod		Rat	Long-Evans	In vivo	M1/M2	Implant	Matlaga et al. (1976)
Triangular rod		Rat	Long-Evans	In vivo	M1	Implant	Matlaga et al. (1976)
Ellipsoid	PS, PVA	Rat	NR8383 alveolar	In vitro	M2	Particles	Champion and Mitragotri (2006)
	PS	Mouse	RAW 264.7	In vitro	M2	Prolate	Sharma et al. (2010)
	PS	Mouse	RAW 264.7	In vitro	M1	Oblate	Sharma et al. (2010)
Spherical	PS, PVA	Rat	NR8383 alveolar	In vitro	M1	Particles	Champion and Mitragotri (2006)

[a]M1/M2 indicates mixed phenotype observed

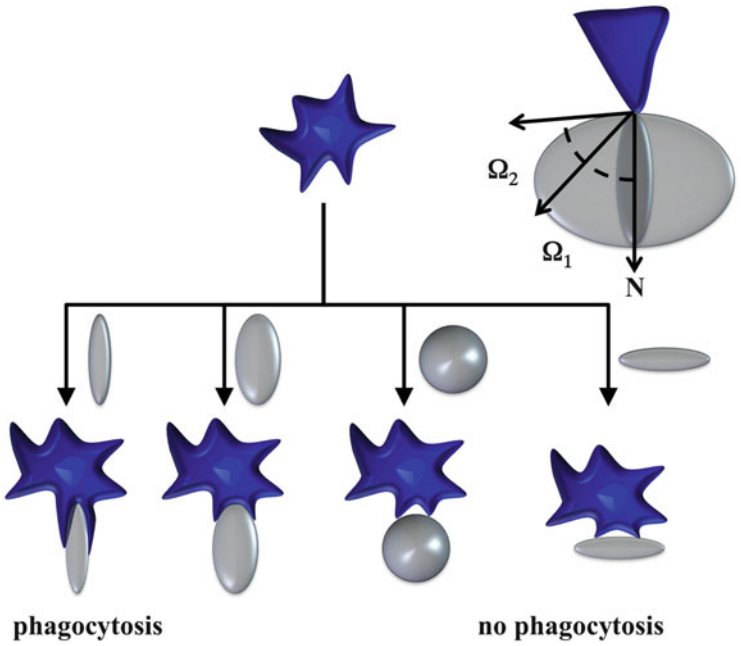


Fig. 13.4 Phagocytic response of macrophages to particles with varying curvature. When the curvature, or angle between the edge of the object at point of contact and a line normal (N) to the point of contact represented by Ω, is small (<45°), the macrophage will attempt to phagocytize the particle as represented by Ω_1. When the curvature is large as represented by Ω_2, macrophages are unable to phagocytize the particle. This figure is, in part, adapted from Moon et al. (2012)

recently, the angle of curvature at the point of macrophage attachment to the biomaterial was found to influence phagocytic activity and the rate at which it is undertaken (Fig. 13.4). Macrophages exposed to material surfaces with curvature greater than 45° had significantly less phagocytic activity (Champion and Mitragotri 2006).

Well-contoured shapes, such as disks, also resulted in alternatively activated and anti-inflammatory activities. Human macrophages, derived from peripheral blood mononuclear cells (PBMCs) and cultured on polydioxanone (PDO) disks, produced enhanced transforming growth factor beta (TGF-β), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) over a 28-day period compared to macrophages cultured on tissue culture polystyrene (Garg et al. 2009). Oblate ellipsoid disks made of polystyrene were taken up to a greater extent than prolate ellipsoids, suggesting that macrophages, which attached to prolate ellipsoids, were deactivated (Sharma et al. 2010). Anti-inflammatory and deactivated macrophage responses to biomaterials may be due to the aspect ratio of the material and the degree of actin remodeling required for the macrophage to phagocytize the material. In general, curved biomaterials with larger aspect ratios require more actin remodeling for uptake and are considered macrophage deactivating due to their

inability to induce phagocytosis (Gratton et al. 2008; Sharma et al. 2010; Champion et al. 2007; Champion and Mitragotri 2006). Despite these studies, consensus on a well-defined immune-compatible shape of an implant has not been defined and continues to be debated (Helton et al. 2011). Further complicating the general geometric recognition by macrophages is the larger architectural presentation of the material.

13.2.3 Architecture

In addition to the size of an implanted biomaterial, the architecture of the material may also influence macrophage response: the roughness of a surface; three-dimensional arrangement of two-dimensional structures, such as gratings or fibers; or porosity (Table 13.3). Early studies have shown that macrophages prefer to adhere to rougher surfaces (Rich and Harris 1981), but roughness also influences their activation status. When murine macrophages (P388D1) were cultured on poly (methyl methacrylate) (PMMA), surfaces with grooves 10 μm wide and 0.5 μm deep, macrophages were elongated and had enhanced migratory capacity (Wojciak-Stothard et al. 1996). Additionally, microgrooves on polystyrene beads increased the number of phagocytic macrophages, with groove depth being the predominant factor influencing migration and phagocytosis of the macrophages (Rich and Harris 1981). Larger grooves on surfaces enhanced classical activation of macrophages. Differences in surface topographies on titanium also affected the inflammatory cytokine profile of murine macrophages when introduced in the presence of lipo-polysaccharide (LPS) (Refai et al. 2004). Rougher surfaces synergistically induced elevated IL-1β, IL-6, and TNF-α mRNA transcription following LPS exposure compared to smoother surfaces, suggesting that these surfaces prompted classical activation in macrophages.

Other patterned architectures such as gratings have been used to regulate cellular interactions through a phenomenon called contact guidance (Dvir et al. 2011). On substrates made of poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), and poly (dimethylsiloxane) (PDMS), parallel gratings influenced macrophage activity. RAW 264.7 cells produced lower levels of TNF-α and VEGF as surface grating size increased, where macrophages exposed to 1 μm diameter gratings produced the lowest levels of both cytokines (Chen et al. 2010). In vivo, macrophage fusion into foreign body giant cells was reduced with grating size of 2 μm compared to planar controls or 500 μm gratings. The changes were topography induced and determined to be independent of material chemistries.

Fibrous architectures, such as electrospun polymer meshes, have patterns which most closely resemble native microenvironments and that macrophages should respond similarly to native matrix (Wang et al. 2013). For instance, bone marrow-derived macrophages grown within electrospun PDO fibers enhanced characteristics of alternatively activated macrophages, specifically increasing expression of arginase compared to inducible nitric oxide synthase (iNOS) along

Table 13.3 Architecturally mediated effects on biomaterial-induced macrophage activation

Architecture	Material	Species	Model	In vitro/ in vivo	Macrophage response[a]	Material type	References
Grooved	PMMA	Mouse	P388D1	In vitro	M2	Substrata	Wojciak-Stothard et al. (1996)
Grooved	PS	Mouse	Peritoneal macrophages from Swiss white mice	In vitro	M1	Beads	Rich and Harris (1981)
Rough/coarse	Titanium	Mouse	RAW 264.7	In vitro	M1	Disk	Rich and Harris (1981)
Small parallel grating	PCL, PLA, PDMS	Mouse, rat	RAW 264.7, Sprague-Dawley	In vitro/ in vivo	M1	Film	Chen et al. (2010)
Large parallel grating		Mouse, rat	RAW 264.7, Sprague-Dawley	In vitro/ in vivo	M2	Film	Chen et al. (2010)
Mesh	PDO	Mouse	BMDM	In vitro	M2	Electrospun	Garg et al. (2013)
Fibrous mesh	PLLA	Mouse	RAW 264.7	In vitro	M2	Electrospun	Saino et al. (2011)
Flat mesh	PLLA	Mouse	RAW 264.7	In vitro	M1	Electrospun	Saino et al. (2011)
Small fibers	PP, PE, PLA, PU	Rat	Sprague-Dawley	In vivo	Less inflammatory	Electrospun	Sanders et al. (2002); Sanders et al. (2000)
Large fibers	PP, PE, PLA, PU	Rat	Sprague-Dawley	In vivo	M1	Electrospun	Sanders et al. (2002); Sanders et al. (2000)
Nonporous	PU	Human	PBMC (CRL-9855)	In vitro	M1	2D films	McBane et al. (2011)
	Poly (HEMA)	Mouse	*BAT-gal*	In vivo	M1	Spherical implant	Sussman et al. (2014)
Porous	Poly (HEMA)	Human	*PBMC (CRL-9855)*	In vitro	M1/M2	3D porous scaffold	McBane et al. (2011)
		Rat	*Sprague-Dawley*	In vivo	M1/M2	Disks	Palmer et al. (2014)
		Human	–	In vivo	M2	Disks	Fukano et al. (2006)
		Mouse	*BAT-gal*	In vivo	M2	Spherical implant	Sussman et al. (2014)

[a]M1/M2 indicates mixed phenotype observed

with TGF-β, VEGF, and FGF (Garg et al. 2013). However, fiber diameter and alignment influenced macrophage activation. Aligned electrospun poly(L-lactic acid) (PLLA) fibers with micro- or nanoscale diameter enhanced macrophage adhesion when compared to randomly aligned or flat PLLA (Saino et al. 2011). In the presence of LPS, macrophages grown on fibrous PLLA secreted significantly less inflammatory cytokines than those cultured on flat PLLA films, suggesting that fibrous materials reduce classical activation in macrophages. In an in vivo multi-material study, fibers (1–5 μm diameter), regardless of material, led to smaller fibrous capsule formation than larger fibers (11–15 μm diameter) (Sanders et al. 2000, 2002).

Internal architecture of the material, such as porosity, may also interplay with other material properties to influence macrophage response. Highly porous materials, or those with defined size of pores or high total porosity, have been shown to have higher ratios of macrophage infiltration (Mitragotri and Lahann 2009). The presence of pores has also been shown to modulate macrophage responses and biointegration (Winter 1974; Sussman et al. 2014; Anderson and Miller 1984). For instance, human monocyte-derived macrophages enhanced TNF-α release when cultured on two-dimensional nonporous films made up of degradable, polar, hydrophobic, ionic polyurethane (PU), over a 28-day period, with the most significant TNF-α release at day 7. Although release of TNF-α waned over time, IL-10 release did not change significantly, suggesting that these macrophages remained in the classically activated state and did not transition to alternatively activated macrophages in response to the nonporous polymer films (McBane et al. 2011). Porous polyurethane scaffold, however, tended to induce enhanced migratory capacity of macrophages and reduced secretion of inflammatory cytokines compared to the films, suggesting an importance in porosity in modulating response to the material (McBane et al. 2011). In a human wound model, porous poly(2-hydroxyethyl methacrylate) (poly(HEMA)) disks showed enhanced healing and biointegration within the wound space (Fukano et al. 2006). When porous poly(HEMA) rods were implanted into the dorsal skin of C57BL/6 mice, rods showed signs of integration with the surrounding tissue (Isenhath et al. 2007). Both of these are examples of wound healing responses largely mediated by the wound healing macrophage (Gordon 2007; Sunderkotter et al. 1994; DiPietro 1995; Koh and DiPietro 2011; Mosser and Edwards 2008; Gordon and Martinez 2010), suggesting that porous scaffolds recruit or promote alternative macrophage polarization.

The size of pores is also important to modifying macrophage phenotype. Hydrogels composed of poly(HEMA) with 34 μm pores and 160 μm pores as well as nonporous hydrogels elicited differing macrophage activation responses (Sussman et al. 2014). When hydrogels were implanted, macrophages responded with significant fibrotic foreign body response in nonporous implants compared to porous ones, suggesting that macrophages took on a classically activated phenotype in response to the implant without pores. While the porous scaffolds had fibrotic responses as well, the implant with the smallest pore size (34 μm) elicited the least fibrotic response. Interestingly, histological analysis of macrophages directly surrounding the pores in the porous scaffolds showed that macrophages attached to and

directly surrounding the 34 or 160 μm pores actually enhanced the expression of classically activated markers, IL-1 receptor and iNOS, compared to those nonadherent to the pores, which showed enhanced mannose receptor expression (greatest in implants with 34 μm pores). While responses to a material may be based off of specific architectural cues, a fine interplay between internal and overall architectural cues determines totality of macrophage response.

Therefore, the response to porous scaffolds is not always easily predictable. For instance, polyurethane porous foams, 7 mm in diameter and 3 mm thick, were surrounded by tissue macrophages 6 weeks after implantation regardless of porosity. Polyurethane foams were positive for macrophages expressing CD80, CD68, mannose receptor, and iNOS (Table 13.3) (Palmer et al. 2014). Additionally, the foams were positive for foreign body giant cells expressing high levels of CD80 and absent levels of CD163, suggesting that these materials promoted a classically activated macrophage phenotype. Expression of mannose receptor, however, suggested a mixed phenotypic profile in response to the porous polyurethanes. An absence of CD80-positive and iNOS-positive cells was noted unless they were in direct contact with the material, suggesting that expression of these markers was dependent on direct activation by the underlying biomaterial. Once again, this suggests that it is the combination of the physicochemical properties of the biomaterial interplay to induce macrophage activation of either phenotype. There is a finely tuned response to architectural cues, and small changes may lead to significantly different macrophage responses.

13.2.4 Hydrophobicity

Hydrophobicity (Table 13.4) has long been regarded as a simple predictor of macrophage response. Under most healthy and normal biologic conditions, hydrophobic biologic surfaces are buried from the external environment and only become exposed during disease-related processes (Seong and Matzinger 2004; Matzinger 1994). Therefore, hydrophobicity is regarded as a danger-associated molecular pattern, and macrophages may respond to it as foreign and necessary to remove and destroy. As such, it is unsurprising that hydrophobicity of biomaterials has been shown to correlate with an increase in the number of inflammatory or classically activated macrophages (Seong and Matzinger 2004; Rostam et al. 2016; Bygd et al. 2015; Akilbekova et al. 2015). The increase in recruitment and local activation of classically activated macrophage subtypes in response to these materials can be explained as largely due to the nature of the hydrophobic surfaces and protein adsorption (Vroman 1962). For instance, nonpolar surfaces facilitated protein unfolding through decreased unfolding free energy barriers (Anand et al. 2010; Vroman 1962). The result of protein unfolding and refolding is a highly disorganized layer of proteins with newly exposed conformations, sometimes referred to as cryptic sites, on the surfaces. These cryptic sites generally lead to inflammatory

Table 13.4 Hydrophobicity of biomaterial and macrophage activation

Hydrophobicity	Material	Species	Model	In vitro/ in vivo	Macrophage response	Material type	References
Hydrophobic	Polystyrene	Human	PBMC	In vitro	M1	Polystyrene disk	Rostam et al. (2016)
	Ether and phosphonic acid-modified p (NIPAm-co-AAc)	Mouse	SKH1-E	In vivo	M1	Nanoparticles	Bygd et al. (2015)
	Latex	Mouse	RAW 264.7	In vitro	M1	Beads	Akilbekova et al. (2015)
	Silicone	Human	–	In vivo	M1	Implant	Barker et al. (1978)
	Silicone	Human	PBMC	In vitro	M1 to M1	Silicone foil	Vijaya Bhaskar et al. (2015)
	Silicone	Human	PBMC	In vitro	M2 to M1	Silicone foil	Vijaya Bhaskar et al. (2015)
	Silicone	Human	PBMC	In vitro	cytokine naive to M1	Silicone foil	Vijaya Bhaskar et al. (2015)
Hydrophilic	Poly(acrylic acid)	Rat	Sprague-Dawley	In vivo	M2	Implant	Brodbeck et al. (2002)
	Collagen/hyaluronan	Human	PBMC	In vitro	M2	Implant	Kajahn et al. (2012)
	Collagen/hyaluronan	Human	PBMC	In vitro	M1 to M2	Implant	Franz et al. (2013)
	Hyaluronan/gelatin	Human	PBMC	In vitro	M2	Carbylan-GSX	Hanson et al. (2011)
	Hyaluronan	Human	–	In vivo	M1	Cross-linked dermal filler	Edwards and Fantasia (2007)
	Hyaluronan	Human	–	In vivo	M1	Cross-linked dermal filler	Rongioletti et al. (2003)
	Hyaluronan	Human	–	In vivo	M1	Cross-linked dermal filler	Wolfram et al. (2006)
	Hyaluronan	Human	–	In vivo	M1	Cross-linked dermal filler	Vargas-Machuca et al. (2006)
	Hyaluronan	Human	–	In vivo	M1	Cross-linked dermal filler	Klein (2004)
	Hyaluronan	Human	–	In vivo	M1	Cross-linked dermal filler	Fernandez-Acenero et al. (2003)
	Dextran	Mouse	BALB/c	In vivo	M1	Sephadex (cross-linked dextran)	Blanckmeister and Sussdorf (1985)

responses from macrophages consistent with a classically activated phenotype (Seong and Matzinger 2004).

Natural responses to hydrophobic materials, such as silicone rubbers, have shown questionable histocompatibility with an increase in chronic inflammation with accumulation of monocytes and lymphocytes in the areas surrounding the implant (Iribarren et al. 2002; Barker et al. 1978). Human PBMC-derived macrophages show inflamed activation in response to silicone after being cultured on it for 24 h (Vijaya Bhaskar et al. 2015). Nonactivated human PBMC-derived macrophages showed higher levels of IL-8, C-X-C motif chemokine 10 (CXCL10), and IFN-γ when cultured on silicone compared to tissue culture plastic suggesting not only activation but reprogramming of the macrophages. Alternatively activated human PBMC-derived macrophages also secreted higher levels of TNF-α, interleukin 17 (IL-17), IFN-γ, IL-6, interleukin 9 (IL-9), and CXCL10 when cultured on silicone compared to tissue culture plastic. Finally, classically activated human PBMC-derived macrophages cultured on silicone exhibited higher levels of IL-8 and IFN-γ compared to those cultured on tissue culture plastic. Therefore, macrophages cultured on silicone, regardless of initial macrophage activation state, became more classically activated compared to those grown on tissue culture plastic, suggesting that silicone had the ability to classically activate human PBMC-derived macrophages.

Hydrophilic materials have generally shown the opposite effects. Hydrophilic anionic surfaces promoted anti-inflammatory IL-10 expression, reduced IL-8 expression, and inhibited macrophage fusion into foreign body giant cells, which indicated that these surfaces promoted an anti-inflammatory or alternatively activated macrophage response (Brodbeck et al. 2002). This may be due to the fact that on wettable surfaces, proteins are adsorbed in lower concentrations and in a more native state. In addition, protein reorganization on the surface provides binding sites for cell adhesion and not just cryptic site recognition (Anand et al. 2010). Depending upon the hydrophilic nature, however, proteins are bound with weaker forces which leads to protein detachment and poor or disabled cellular attachment. Hydrophilic surfaces also promote apoptosis of macrophages, through an unknown mechanism, thereby inhibiting their ability to form foreign body giant cells. The combination of which may further explain the lack of an inflammatory response to these types of materials (Brodbeck et al. 2001, 2002).

The macrophage response to hydrophobicity of a material is not always predictable as hydrophilic materials may also induce classical macrophage activation. For instance, cross-linked hydrophilic dextran induced classical macrophage activation (Blanckmeister and Sussdorf 1985). BALB/c peritoneal macrophages took on a polarization status not significantly different from that of macrophages treated with complete Freund's adjuvant (CFA). In other words, these macrophages were classically activated and had antitumor activity both in vitro and in vivo. Sometimes the same material may elicit widely different macrophage responses in different contexts. For instance, when hydrophilic hyaluronan was cross-linked with gelatin in an implant to support human mesenchymal stem cells, human macrophages responded with characteristics of alternatively activated macrophages, specifically

expressing low levels of cluster of differentiation (CD16), high levels of cluster of differentiation 206 (CD206), and low levels of HLA-DR expression after culture for 7 days (Hanson et al. 2011). However, cross-linked injectable hydrophilic hyaluronan implants have also been shown to elicit inflammatory or granulomatous reactions in humans over time (Edwards and Fantasia 2007; Rongioletti et al. 2003; Wolfram et al. 2006; Vargas-Machuca et al. 2006; Klein 2004; Fernandez-Acenero et al. 2003). Confounding these findings is the ability of hyaluronan to degrade into smaller fragments over time, making it unclear if the histological evidence of classical activation is due to the native material or the result of degradation of hyaluronan into smaller fragments.

In some instances, increasing hydrophobicity of a biomaterial, i.e., those with a water-air contact angle between 80° and 90°, leads to anti-inflammatory responses consistent with an alternative macrophage activation (Rostam et al. 2016). Possibly explaining these apparent contradictions, water contact angles can be subdivided into hydrophilic regimes, specifically contact angles between 0° and 45° representing "conditionally" nonadhesive regime, between 45° and 75° representing adhesive hydrophilic regime, and above 75° representing a nonadhesive regime. Materials in either of the nonadhesive regimes had the greatest potential for stimulating wound healing macrophage activation (Richards et al. 2012).

Another explanation for the differences in activation with hydrophilic materials resides in the charge of the surface. Hydrophilic neutral and anionic materials stimulated classically activated macrophages inducing production of IL-8, IL-1β, IL-6, and TNF-α, with low levels of IL-10 (Chang et al. 2008). On the other hand, hydrophilic cationic material surfaces promoted alternative macrophages activation (Chang et al. 2008). This suggests that while hydrophobicity of a material may have a role in mediating a certain response, it is not the sole material property that controls this.

13.2.5 Surface Chemistry

While the size, geometry, hydrophobicity, and surface topography of the surface have been shown to mediate macrophage responses, the surface chemistry (Table 13.5) of the material also dictates how much and what kind of proteins will adsorb onto the surface of the biomaterial and also plays a major role in determining macrophage responses to biomaterials. To examine the influence of varying surface chemistries, self-assembled monolayers were prepared on gold-yielding surfaces with similar physical properties (Barbosa et al. 2006). An increasing number of classically activated macrophages were recruited to and collected from the inflammatory exudates of implants functionalized with a methyl ($-CH_3$) compared to no functionalization. Surface functionalization with hydroxy ($-OH$) and carboxylic acid ($-COOH$) groups elicited intermediate numbers of macrophages. Surfaces coated with the methyl groups induced an in vivo capsule

formation, which was the largest out of all the functionalized groups, suggesting that the methyl-modified surfaces promoted the most significant inflammatory response from the macrophages. While hydroxy-modified surfaces recruited high levels of inflammatory macrophages, these surfaces did not induce the formation of a fibrous capsule to the extent of the methyl-coated surfaces, suggesting that the response elicited by this surface chemistry was not as polarizing as the methyl-modified surfaces. Unfortunately, the hydrophobicity could not be decoupled from the surface chemistry in this case.

Similarly, macrophage interaction with the differing surface chemistries was examined by formulating micron-sized particles made of polypropylene surface-modified with either hydroxy, amine, fluorocarbon, or carboxylic acid groups (Kamath et al. 2008). Using microparticles, macrophages had the opportunity to interact with the surface functionalities and potentially phagocytize the particles. Surfaces with hydroxy and amine surfaces induced thick fibrous capsule formation and cellular infiltration characteristic of inflammation in response to the implant. On the other hand, microparticles with surfaces of carboxylic acid or fluorocarbon modifications had the lowest inflammatory and fibrotic responses.

More recently, microparticles functionalized with acetal, ketone, and nitro groups on the surface induced macrophage infiltration into the implant site, with a lower ratio of classically activated to alternatively activated macrophages present in the site (Bygd et al. 2015). Macrophages exposed to these surfaces produced low levels of TNF-α, high levels of IL-10, and high levels of arginase compared to iNOS, suggesting that functionalization with those groups provoked a more alternatively activated state of the macrophage. On the other hand, sulfone, sulfonic acid, alkene, and epoxide groups on the surface did not stimulate significant macrophage infiltration into implant site, but these materials elicited a higher presence of classically activated macrophages. These macrophages tended to upregulate TNF-α production while decreasing IL-10 and arginase. Some functional groups analyzed yielded macrophage phenotypes which lay within the spectrum, not clearly classically activated or alternatively activated. For instance, particles functionalized with ester, ketal, acetal, sulfone, and oxime expressed intermediate levels of all cytokines or had mixed cytokine profiles, suggesting that either intermediate activation took place or that populations of macrophages polarized both alternatively and classically.

Modification of material with polymers also yields variable surface chemistry characteristics. For instance, mouse macrophages exposed to chitosan scaffolds exhibited properties dependent on the degree of acetylation of the chitosan. When implanted in mice, macrophages surrounding chitosan scaffolds with 5% acetylation were alternatively activated compared to those surrounding 15% acetylated chitosan, which were classically activated, as detected from immunofluorescent staining for CD206 and CCR7 (Vasconcelos et al. 2013). Additionally, there was a reduced IL-6 and TNF-α cytokine secretion of the 5% acetylated chitosan scaffolds compared to 15%, and the 5% acetylated scaffolds elicited enhanced secretion of anti-inflammatory IL-4 compared to 15% acetylation. Similarly, sulfonation of polyurethanes influences macrophage activation (Hunt et al. 1996). Both sulfated

Table 13.5 Influence of surface chemistry on macrophage activation

Surface chemistry	Material	Species	Model	In vitro/ in vivo	Macrophage response[a]	Material type	References
Methyl	Gold	Mouse	BALB/c	In vivo	M1	Monolayers over gold	Barbosa et al. (2006)
Hydroxy	Gold	Mouse	BALB/c	In vivo	M1/M2	Monolayers over gold	Barbosa et al. (2006)
	Polypropylene	Mouse	BALB/c	In vivo	M1	Microparticles	Kamath et al. (2008)
Carboxylic acid	Gold	Mouse	BALB/c	In vivo	M1/M2	Monolayers over gold	Barbosa et al. (2006)
	Polypropylene	Mouse	BALB/c	In vivo	M2	Microparticles	Kamath et al. (2008)
Amine	Polypropylene	Mouse	BALB/c	In vivo	M1	Microparticles	Kamath et al. (2008)
Fluorocarbon	Polypropylene	Mouse	BALB/c	In vivo	M2	Microparticles	Kamath et al. (2008)
Acetal	p(NIPAm-co-AAc)	Mouse	SKH1-E	In vivo	M2	Nanoparticle	Bygd et al. (2015)
	Chitosan	Mouse	BALB/c mice	In vivo	M1 or M2 dependent on acetylation	Implant	Vasconcelos et al. (2013)
Ketone	p(NIPAm-co-AAc)	Mouse	SKH1-E	In vivo	M2	Nanoparticle	Bygd et al. (2015)
Nitro	p(NIPAm-co-AAc)	Mouse	SKH1-E	In vivo	M2	Nanoparticle	Bygd et al. (2015)
Sulfone	p(NIPAm-co-AAc)	Mouse	SKH1-E	In vivo	M1	Nanoparticle	Bygd et al. (2015)
Sulfonic acid	p(NIPAm-co-AAc)	Mouse	SKH1-E	In vivo	M1	Nanoparticle	Bygd et al. (2015)
Alkene	p(NIPAm-co-AAc)	Mouse	SKH1-E	In vivo	M1	Nanoparticle	Bygd et al. (2015)
Sulfate	Polyurethane	Rat	Lister rat, Liverpool strain	In vivo	M1	Square sheet	Hunt et al. (1996)

[a]M1/M2 indicates mixed phenotype observed

and unsulfated PUs had similar amounts of TNF-α mRNA expression and release around the site of implantation in rats over a 14-day period. Although some specific surface chemistries and modifications have been found to elicit clear activation of macrophages, further study of the specific activation states and the reasons for these activations are needed.

13.2.6 Substrate Mechanics

Substrate stiffness and applied forces on substrates also influence macrophage activation (Table 13.6). In disease, tissue and extracellular matrix stiffness has been shown to change (Cox and Erler 2011; Bidan et al. 2015; Werfel et al. 2013); however, the relationship with macrophage phenotype in vivo has yet to be clearly elucidated. In vitro, clear relationships between substrate mechanics and macrophage activation are emerging.

In the absence of stimulation by LPS, murine macrophages (RAW 264.7) showed no differences in the cytokine expression on poly(ethylene glycol) hydrogels with stiffness ranging from 130 to 840 kPa (Blakney et al. 2012). However, in the presence of LPS, expression of TNF-α, IL-10, IL-1β, and IL-6 increased as implant rigidity increased. As such, stiffer implants tended to induce a more severe foreign body reaction than softer implants. These results were confirmed in vivo with increasing hydrogel stiffness correlating with a more severe foreign body response and activated macrophages surrounding the implant. PEG hydrogel implants in C57BL/6 mice have been shown to have similar foreign body response and cytokine activation in vivo as medical grade silicone (Lynn et al. 2010). When PEG hydrogels are formed into nanoparticles of differing elasticity, softer nanoparticles (10 kPa) had significantly reduced phagocytic uptake by murine macrophages (J774) than stiffer nanoparticles (3000 kPa), suggesting that regardless of size of PEG substrate, stiffer substrates induce greater inflammatory actions of macrophages compared to softer PEG substrates (Anselmo et al. 2015). However, this trend was both species and material specific. For instance, human macrophages (THP-1) showed enhanced TNF-α secretion on soft substrates (1.4 kPa) compared to stiffer ones (248 kPa) (Irwin et al. 2008). When compared side by side on polyacrylamide gels, RAW 264.7 murine macrophages and human U937 macrophages showed enhanced TNF-α secretion in response to softer gels in the presence of LPS (Patel et al. 2012). The variability in macrophage response to substrate stiffness may be due to the differences in other material properties and chemistries as well as the time at which response is analyzed (Brown et al. 2009; Brodbeck et al. 2002; Schutte et al. 2009).

Depending on where the biomaterial is being placed, macrophages surrounding or within the biomaterial may be exposed to a variety of mechanical factors that induce stretch. Stretch and shear stress elements are located in the promoter regions of pro-inflammatory genes, suggesting a role in modulating macrophage activation (Resnick and Gimbrone 1995; Shyy et al. 1995). For instance, primary peritoneal

Table 13.6 Mechanical factors influence macrophage activation

Mechanical factors	Material	Species	Model	In vitro/ in vivo	Macrophage response	Material type	References
Soft substrate	PEG	Mouse	C57BL/6	In vivo	M2	Hydrogel	Blakney et al. (2012)
	PEG	Mouse	C57BL/6	In vivo	M2	Hydrogel	Lynn et al. (2010)
	PEG	Mouse	J774	In vitro	M2	Nanoparticle	Anselmo et al. (2015)
	PEG	Human	THP-1	In vitro	M1	Interpenetrating polymer network	Irwin et al. (2008)
	Polyacrylamide	Mouse	RAW 264.7	In vitro	M1	Hydrogels	Patel et al. (2012)
	Polyacrylamide	Human	U937	In vitro	M1	Hydrogels	Patel et al. (2012)
Stiff substrate	PEG	Mouse	C57BL/6	In vivo	M1	Hydrogel	Blakney et al. (2012)
	PEG	Mouse	C57BL/6	In vivo	M1	Hydrogel	Lynn et al. (2010)
	PEG	Mouse	J774	In vitro	M1	Nanoparticle	Anselmo et al. (2015)
	PEG	Human	THP-1	In vitro	M2	Interpenetrating polymer network	Irwin et al. (2008)
	Polyacrylamide	Mouse	RAW 264.7	In vitro	M2	Hydrogels	Patel et al. (2012)
	Polyacrylamide	Human	U937	In vitro	M2	Hydrogels	Patel et al. (2012)
Static stretch	Collagen	Rat	Peritoneal macrophages	In vitro	M1	Coated wells	Wehner et al. (2010)
Cyclic stretch	Bioflex Silastic	Human	Alveolar derived	In vitro	M1	Coated wells	Pugin et al. (1998)
	Bioflex Silastic	Human	THP-1	In vitro	M1	Coated wells	Pugin et al. (1998)
	Poly-ε-caprolactone bisurea	Human	PBMC	In vitro	M1 to M2 with higher stretch	Strips	Ballotta et al. (2014)

macrophages from rats exposed to static stretch had elevated mRNA expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), IL-1β, and IL-6, suggesting that stretch induces pro-inflammatory or classical activation of the macrophage (Wehner et al. 2010). Unlike with substrate stiffness, the patterns with stretch are not material specific. For instance, human alveolar and THP-1 monocyte-derived macrophages exhibited high levels of IL-8 secretion in response to cyclic stretch compared to macrophages grown on materials under static conditions (Pugin et al. 1998). The stretch induced an even greater release of TNF-α and IL-6 when applied concurrently with LPS stimulation. The level of stretch within a material, however, is also important to macrophage polarization. For example, when compared to unstretched control, human PBMCs seeded onto poly-ε-caprolactone bisurea strips exposed to a 12% strain showed a higher shift of classically activated macrophages to the alternatively activated state over a 7-day period compared to those exposed to a 7% strain which showed higher alternative activation to classical activation polarization shift (Ballotta et al. 2014).

13.2.7 Protein Adsorption

Although many biomaterial properties influence macrophage response, the response to the property is generally best correlated with the proteins bound to a surface as well as the avidity with which the proteins bind to cells when on the surface. For this reason, protein adsorption onto the biomaterial is the most widely studied biomaterial parameter influencing macrophage activation (Table 13.7) (Anderson et al. 2008; Kastellorizios et al. 2015; Diekjiirgen et al. 2012; Walkey et al. 2012; Larson et al. 2012; Sanchez-Moreno et al. 2015; Yan et al. 2013; Jansch et al. 2013; Klopfleisch 2016). It is well understood that upon implantation, biomaterials quickly become coated with proteins from blood and surrounding fluid (Roach et al. 2005; Cross et al. 2016; Vogler 2012; Ihlenfeld and Cooper 1979). Adsorbed proteins differ based on biomaterial properties, but have the ability to modulate the cellular response to the material. The Vroman effect dictates what type of proteins become absorbed onto the surface and in what affinity they are adsorbed (Vroman and Lukosevicius 1964; Xu and Siedlecki 2007). Additionally, while on the surface, these proteins may undergo conformational changes, which may expose different bioactive sites of the protein for cells to interact with (Roach et al. 2005; Cross et al. 2016; Wilson et al. 2005; Gray 2004). While there remains a paucity of data on all protein interactions between biomaterial and macrophages, certain circulating proteins have made key impacts on modulating macrophage response, including fibronectin, von Willebrand factor, immunoglobulin G (IgG), albumin, vitronectin, and fibrinogen.

The blood-clotting protein, von Willebrand factor, for instance, inhibits the adhesion of monocytes and macrophages to biomaterials (Jenney and Anderson 2000b). With this inhibition, there is a decrease in macrophage binding to the biomaterial, and foreign body giant cell formation is inhibited (Anderson et al.

Table 13.7 Protein adsorption and macrophage activation

Protein	Material	Species	Model	In vitro/ in vivo	Macrophage response	Material type	References
von Willebrand factor	Glass	Human	PBMC	In vitro	M2	Plates	Jenney and Anderson (2000b)
Albumin	Polystyrene	Rat	Wistar	In vivo	M2	Nanospheres	Ogawara et al. (2004)
	Carbon	Mouse	RAW 264.7	In vitro	M2	Nanotubes	Dutta et al. (2007)
	Silicone	Mouse, Human	J774, THP-1	In vitro	M2	Nanoparticles	Parodi et al. (2013)
IgG	Plastek M	Human	PBMC	In vitro	M1	Plates	Jenney and Anderson (2000a)
	Polystyrene	Mouse	BMDM	In vitro	M1	Microbeads	Boyle et al. (2012)
	Polystyrene, Primaria	Human	PBMC	In vitro	M1	Plates	Shen et al. (2004)
Fibronectin	PEG	Mouse	C57BL/6 BMDM	In vitro	M1	Hydrogels	Lynn and Bryant (2011)
	mPEGmA	Rat	Sprague-Dawley	In vivo	M1	Implant	Kao and Lee (2001)
	Polystyrene	Mouse	C57BL/6	In vivo	M1	Microparticle	Zaveri et al. (2014)
Fibrinogen	PEG	Mouse	C57BL/6 BMDM	In vitro	M1	Hydrogels	Lynn and Bryant (2011)
	mPEGmA	Rat	Sprague-Dawley	In vivo	M1	Implant	Kao and Lee (2001)
	Polystyrene	Mouse	C57BL/6	In vivo	M1	Microparticle	Zaveri et al. (2014)
Vitronectin	PEG	Mouse	C57BL/6 BMDM	In vitro	M1	Hydrogels	Lynn and Bryant (2011)
	mPEGmA	Rat	Sprague-Dawley	In vivo	M1	Implant	Kao and Lee (2001)
	Polystyrene	Mouse	C57BL/6	In vivo	M1	Microparticle	Zaveri et al. (2014)

CD200	Polystyrene	Mouse	C57BL/6 BMDM	In vitro	M2	Microbeads	Kim et al. (2014)
IL-10	Nylon	Mouse	C57BL/6	In vivo	M2	Mesh	Higgins et al. (2009)
IL-4	PLGA	Rat	Lewis, BMDM	In vitro	M2	Microparticles	Minardi et al. (2016)
CD47	Polystyrene	Mouse	C57BL/6 BMDM	In vitro	None	Nanoparticle	Qie et al. (2016)

2008). Interestingly, macrophages have three von Willebrand factor receptors, which do not contribute to adhesion in the presence of the protein. This is in direct opposition to the adhesive properties of von Willebrand factor in other cells, such as endothelial cells (Carreno et al. 1993; Denis et al. 1993; Beacham et al. 1992).

Like von Willebrand factor, albumin has macrophage-repellant effects, which makes it useful in materials coatings for avoiding opsonization (Elzoghby et al. 2012; Orringer et al. 2009; Ogawara et al. 2004; Elsabahy and Wooley 2012). Synthetic nanoparticles coated with albumin have been shown to evade phagocytic activities of macrophages (Parodi et al. 2013). Additionally, albumin may actually inhibit inflammatory activities of macrophages when adsorbed onto the biomaterial. For instance, macrophages decreased COX-2 expression when exposed to single-walled carbon nanotubes with adsorbed albumin (Dutta et al. 2007).

On the other hand, surfaces coated with high levels of IgG tended to have enhanced adhesion of macrophages and high levels of pro-inflammatory cytokine release, including TNF-α (Jenney and Anderson 2000a; Boyle et al. 2012; Shen et al. 2004). IgG-coated surfaces also led to foreign body giant cell formation. This is thought to be due to complement receptors, which recognize IgG and may activate the opsonization process in macrophages (Shen et al. 2004; Brevig et al. 2005; Ademovic et al. 2006; Hu et al. 2001; Phillips and Kao 2005).

Other extracellular matrix proteins such as fibronectin, fibrinogen, and vitronectin facilitate macrophage adhesion and activation via integrin receptors on the macrophage. Materials coated with these proteins induce macrophage activation and foreign body giant cell formation. Using integrin-binding peptides, blocking integrin receptors on macrophages minimized inflammation and foreign body giant cell formation (Lynn and Bryant 2011; Kao and Lee 2001; Zaveri et al. 2014; Ratner and Bryant 2004).

Because of the importance of protein adsorption onto biomaterial and subsequent host response to the biomaterial modulated by this, some groups have taken advantage of this as a way to design implants and materials that evade the host immune response or promote positive outcomes for the implant. One example of this is macrophage activation inhibitory receptor, cluster of differentiation 200 (CD200), which has implications in modulating immune-mediated events including infection, allergy, autoimmunity, transplant response, and cancer progression (Nathan and Muller 2001; Liao et al. 2013). Coating materials with CD200 prior to subcutaneous implantation resulted in reduced macrophage activation and inflammatory response (Kim et al. 2014). Similarly, macrophage-deactivating cytokine (IL-4 or IL-10)-coated surfaces promoted tissue integrative and wound healing responses from macrophages in direct contact with the material (Higgins et al. 2009; Minardi et al. 2016). Coating with cluster of differentiation 47 (CD47), a member of the integrin-associated family, resulted in phagocytosis by macrophages due to integrin binding, but this did not influence macrophage polarization (Qie et al. 2016). The results of these studies and others illustrate that macrophages respond specifically to proteins coated on surfaces of biomaterials. It is potentially this interaction that is the final delineator of how a macrophage becomes activated in response to a biomaterial.

13.3 Conclusions

Recent advances in the literature have shown that macrophages have the ability to change polarization states and influence homeostatic and disease processes. Biomaterials differ in their innate properties that may induce a wide variety of responses in macrophages. Subsequently, these responses are key determinants of biocompatibility, controlling the foreign body reaction, and/or a tissue integrative and anti-inflammatory response. To elicit desired macrophage and immune responses to the biomaterial, several key components should be considered and tuned appropriately. Among these components are size, geometry, surface topography, architecture, hydrophobicity, surface chemistry, material mechanics, and protein adsorption. By manipulating biomaterial parameters, the material's innate properties may be used to control its overall destiny in the body. Ultimately, intelligent development and design of biomaterials will enhance desired biologic responses and activity.

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