Biomaterial based modulation of macrophage polarization: a review and suggested design principles

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Macrophages have long been known for their phagocytic capabilities and immune defence; however, their role in healing is being increasingly recognized in recent years due to their ability to polarize into pro-inflammatory and anti-inflammatory phenotypes. Historically, biomaterials were designed to be inert to minimize the host response. More recently, the emergence of tissue engineering and regenerative medicine has led to the design of biomaterials that interact with the host through tailored mechanical, chemical and temporal characteristics. Due to such advances in biomaterial functionality and an improved understanding of macrophage responses to implanted materials, it is now possible to identify biomaterial design characteristics that dictate the host response and contribute to successful tissue integration. Herein, we begin by briefly reviewing macrophage cell origin and the key cytokine/chemokine markers of macrophage polarization and then describe which responses are favorable for both replacement and regenerative biomaterials. The body of the review focuses on macrophage polarization in response to *inherent cues* directly provided by biomaterials and the *consequent cues* that result from events related to biomaterial implantation. To conclude, a section on potential design principles for both replacement and regenerative biomaterials is presented. An in depth understanding of biomaterial cues to selectively polarize macrophages may prove beneficial in the design of a new generation of '*immuno-informed*' biomaterials that can positively interact with the immune system to dictate a favorable macrophage response following implantation.

Introduction

Traditionally, macrophages (Greek: Macro – 'Large'; Phage – 'to eat') are thought of as providing the first line of defense to infectious microorganisms through their phagocytic activities; however, over the past two decades, their role in homeostasis, tissue repair and remodeling has become increasingly evident [1]. Together with dendritic cells, mast cells, granulocytes (neutrophils, basophils and easinophils) and natural killer cells, macrophages constitute the innate immune system and are responsible for recruiting other immune cells to the site of infection, removing foreign pathogens by phagocytosis and activating both the complement and adaptive immune systems [2]. While multiple cell types are involved in tissue healing after injury, macrophages play a pivotal role in mediating tissue remodeling by secreting chemokines and cytokines that directly impact tissue repair [3]. Therefore, understanding the exact role of macrophages in tissue healing processes, especially in events that follow biomaterial implantation, will aid in the design of '*immuno-informed*' materials that elicit a favorable immune response upon implantation.

'A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct,

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by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine' [4]. Biomaterials can be used to restore or augment the physiological function of diseased or damaged tissues via tissue replacement (e.g., permanent hip replacements) or regeneration (e.g., degradable tissue engineering constructs). Cells of the innate immune system are the first to respond to the implantation of a biomaterial in vascularized tissue. Following blood biomaterial contact, a layer of protein immediately adsorbs onto the biomaterial surface, resulting in the formation of a blood clot (provisional matrix) that is rich in growth factors, cytokines and chemoattractants capable of recruiting cells of the innate immune system to the injury site [5]. Subsequent to cell recruitment, the severity of the ensuing acute and chronic inflammation is dependent on the type of biomaterial implanted, the extent of provisional matrix formation and the time taken to resolve the inflammatory response. Without the provision of cues to direct otherwise (i.e., in cases involving biologically inert biomaterials), these events result in the formation of granuloma tissue, which

gives way to fibrous tissue formation and wound healing. These sequential steps represent the full extent of a foreign body response/reaction (FBR) following implantation of a biomaterial [6] (Fig. 1); however, as this review highlights, there are a multitude of ways in which these events can be altered to improve levels of tissue remodeling and reduce or eliminate fibrous tissue formation.

It is well established that microenvironmental cues presented by biomaterials play a crucial role in modulating the response of cells [7]. Physical properties such as substrate stiffness, topography, pore size and size of wear debris; chemical properties such as surface chemistry, ligand presentation and release of growth factors; and temporal properties, such as degradation rates, all influence the behavior of cells [8,9]. While much progress has been made in understanding these effects on both somatic cells [10] and stem cells [10,11], the effect of such biophysical and biochemical cues on immune cells, specifically macrophages, is less well known. This deficit in understanding macrophage responses is compounded by the complex interplay between inherent biomaterial
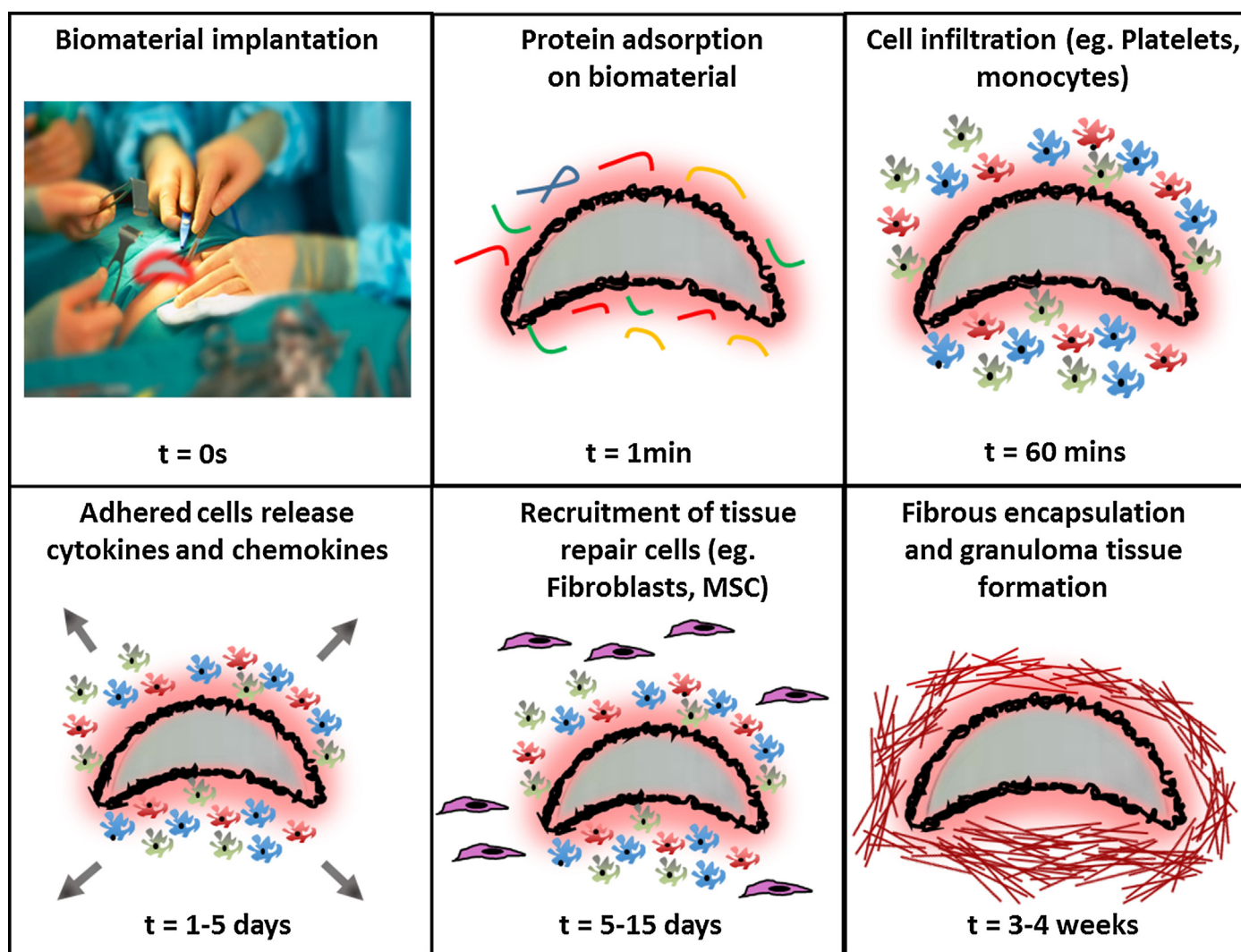

FIGURE 1

Natural innate immune response following biologically inert biomaterial implantation. Following the implantation procedure, a layer of proteins from the surrounding vasculature adsorbs onto the biomaterial surface. This leads to infiltration and adherence of cells such as platelets, monocytes and macrophages. These cells in turn release cytokines and chemokines that recruit tissue repair cells (e.g.: fibroblasts, Mesenchymal Stem Cells (MSC)) to the inflammation site. These cells deposit collagen matrix and encapsulate the biomaterial in a fibrous tissue layer.

properties and those that result from interactions with the local environment as a consequence of biomaterial interaction.

To fulfill their plethora of functions, macrophages exhibit a spectrum of transient polarization states that are influenced by varying microenvironmental cues, some of which may be biomaterial-based. In this review, we begin by briefly describing the origin of macrophages and their different polarization states. The main body of the review will focus on the response of macrophages to microenvironmental cues, primarily those inherently presented by biomaterials but also consequent cues that occur due to biomaterial implantation. To conclude, we will describe how these concepts can be integrated into biomaterial design to aid in the creation of immuno-informed biomaterials, a new generation of immunomodulatory biomaterials that incorporate specific design principles to actively modulate the immune response to implanted biomaterials.

Origin of macrophages

Macrophages can either reside in tissues or circulate in peripheral blood; accordingly, they originate from two distinct sources. Until recently, it was believed that macrophages were solely derived from circulating monocytes, which arise from precursors in the bone marrow as primary subsets of the mononuclear phagocyte system; however, recent evidence suggests that some tissue resident macrophages (e.g., brain, liver, heart) may be generated *in utero* during embryological development [12] (Fig. 2). These tissue resident macrophages sustain their local populations by rapid proliferation during injury events (Fig. 2b) [13]. In contrast, circulating monocytes circulate in peripheral blood for a few days after leaving the bone marrow environment, and differentiate into macrophages (monocyte derived macrophages (MDM)) by extravasation through the endothelium for steady state turnover (i.e., tissue homeostasis) or for mediating inflammatory events in response to chemoattractants (Fig. 2a). While different subsets of monocytes have been described, it is generally accepted that the CD14++CD16− subpopulation represents a significant proportion of circulating monocytes in humans [12].

Macrophage polarization and plasticity

Macrophages become activated following migration into inflamed tissue, where they exhibit a spectrum of polarization states related to their functional diversity. At one end of the spectrum there is the pro-inflammatory M1 and at the other end the anti-inflammatory M2 state (Table 1 for details and also the following excellent reviews [9,14–16]).

The 'classically activated' or M1 phenotype emerges as a result of macrophage interaction with pro-inflammatory signals such as Interferon-γ (IFN-γ) and microbial products such as lipopolysaccharide (LPS) [17]. M1 macrophages are capable of high antigen presentation, as well as promoting Th1 differentiation of lymphocytes that produce pro-inflammatory cytokines (such as IFN-γ and IL-2) in response to intracellular pathogens. These cells display a high level of iron retention and low iron export to restrict the availability of microenvironmental iron capable of aiding bacterial expansion, thereby preventing the growth of infections [18]. However, they also harm neighboring cells in the microenvironment by producing toxic reactive oxygen intermediates and by escalating the pro-inflammatory response [14]. In the context of biomaterial implantation, while the initial presence of M1 macrophages promotes a necessary inflammatory response, a prolonged M1 presence leads to a severe FBR, granuloma and fibrous encapsulation resulting in chronic inflammatory events and failure of biomaterial integration. This is especially detrimental for regenerative biomaterials where the goal is to replace lost tissue and avoid scar tissue formation [8].

The M2 phenotype of macrophages, which is referred to as 'alternatively activated', is the result of activation by signals (e.g., IL-4, IL-13) from basophils, mast cells and other granulocytes
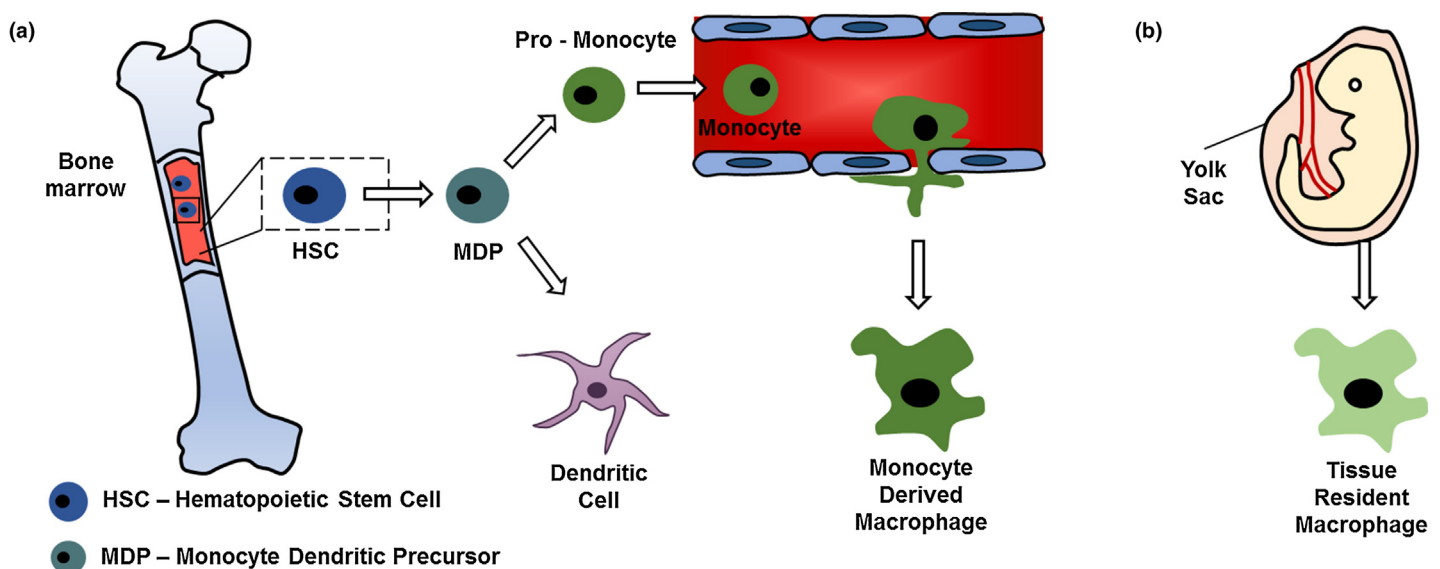

FIGURE 2

Origin of macrophages. (a) Circulating monocytes are primarily derived from committed progenitor cells in the bone marrow (derived from HSC), which migrate to peripheral blood. Monocytes extravasate through blood vessels when recruited as part of tissue homeostasis or injury events, where they subsequently differentiate into monocyte derived macrophages. (b) In contrast, tissue resident macrophages are derived *in utero* in the yolk sac and populate tissues such as the brain (microglia), liver (kuppfer cells) and the heart.

TABLE 1

Inducers and indicators of macrophage polarization.

	Induced by	Secretions (cytokines and chemokines)	Expressed markers	Functions	Refs.
M1	IFN-γ, LPS	IL - 1β, 6, 12, 15, 18, 23; TNF-α; CCL - 15, 20; CXCL - 9, 10, 11, 13	↑ **CD - 80, 86;** ↑ **MHC II;** ↓ **MRC1**	Pro-inflammatory, killing of intracellular pathogens by iron restriction, phagocytosis	[14,16,18]
M2a	IL-4, IL-13	IL - 10, 1ra; CCL - 13, 14, 17, 18, 22, 23, 24, 26; IGF-1; PDGF; TGF-β	CD - 163, 204, 206, 209; YM1, Fizz1; COX2, CLEC4A	Anti-inflammatory, parasitic immunity, allergic responses	[1,21,22]
M2b	LPS, ICs, IL-1β	IL - 10, 1β, 6; TNF-α; CCL - 1, 20; CXCL - 1, 2, 3	↑ **IL-10,** ↓ **IL-12;** MHC II; CD - 86, 163	Immuno-regulation, interaction with B cells, both pro-inflammatory and anti-inflammatory function	[1]
M2c	IL-10, TGF-β, GCs	CCL - 16, 18; CXCL13; TGF-β; matrix (versican, PTX3, α anti-trypsin)	↑ **CD - 163, 204, 206;** ↓ **IL-6, TNF-α;** TLR - 1, 8	Matrix deposition, tissue remodeling and pro-healing	[19,22,23]

IFN-γ – interferon-gamma; LPS – lipopolysaccharide; IL – interleukin; TGF-β – transforming growth factor-beta; IC – immune complexes; GC – gluco-corticoids; TNF-α – tumor necrosis factor-alpha; CCL – chemokine ligand; CXCL – α-chemokine ligands; IGF – insulin-like growth factor; PDGF – platelet derived growth factor; PTX3 – pentraxin 3; CD – cluster of Differentiation; MHC – major histocompatibility complex; MRC1 – mannose receptor, C type 1; COX2 – cyclooxygenase 2, CLEC4A – C type lectin domain family 4 member A; TLR – toll like receptor, SLAM – signaling lymphocyte activation molecule.

[2]. M2 macrophages consistently express scavenger and mannose receptors (CD206), release anti-inflammatory cytokines such as IL-10 [19], display a high level of iron export aiding in tissue remodeling [18] and encompass a range of different subsets (i.e., M2a, M2b, M2c) including 'wound healing' and 'regulatory macrophages' [20]. Within the M2 subsets, the M2a (induced by IL-4 and IL-13) and M2b (induced by immune complexes and toll like receptor (TLR) agonists) subsets perform immune regulatory functions by initiating Th2 lymphocyte anti-inflammatory responses (through the secretion of IL-10, IL-1ra and IL-6) [21,22]. Alternatively, the M2c subset is induced by IL-10 and plays a major role in tissue remodeling and suppression of inflammatory immune reactions by secreting transforming growth factor-β (TGF-β) and IL-10 [1,23]. The presence of such anti-inflammatory cytokines and the tissue remodeling response can aid in the vascularization of regenerative biomaterials by inhibiting fibrous tissue formation, which greatly improves the integration of the biomaterial and enables it to fulfill its intended function.

Unlike terminally differentiated cells, macrophages switch polarization states in response to their microenvironment. This is emphasized by the contrasting gene expression during early and late stages of the FBR [24], as well as their ability to adapt functionality in response to the temporal presentation of stimuli [25]. The transitory nature of macrophages is also related to their paracrine signaling mechanisms; for example, the induction of TNF-α and IL-12 production by the pro-inflammatory (M1 polarizing) cytokine, LPS, can be significantly dampened in the presence of IL-4; a M2 polarizing cytokine produced by nearby M2 macrophages, basophils and mast cells [26]. This suggests that the presence of macrophages in different states of polarization in the same microenvironment can be harnessed to induce constructive remodeling mechanisms that minimize the inflammatory reaction.

Harnessing microenvironmental cues to polarize macrophages

Cells receive a diverse range of signals from their surrounding environment through biochemical cues such as interactions with other cells [27] and interactions with extracellular matrix components [28], as well as biophysical cues such as externally applied

forces [29] and inherent material properties [11,30,31] (Fig. 3). The integral effect of these cues on the cell's current state directs its future behavior. Although it has been suggested that a high M2:M1 ratio in the vicinity of implanted biomaterials leads to better remodeling outcomes [32], prolonged presence of M2 macrophages can lead to the formation of detrimental foreign body giant cells (FBGCs) [5]. Understanding the control of this M2:M1 ratio through the modulation of biomaterial microenvironmental cues will therefore be a key step in the design of next generation immuno-informed biomaterials to enhance positive tissue remodeling, integration and regeneration. This section will first review a range of inherent cues presented by a biomaterial, followed by a section on consequent cues that are induced in the microenvironment as a consequence of biomaterial implantation.

Inherent cues
Biophysical cues
Cells use integrin–ligand interactions to probe, attach and respond to the properties of the underlying substrate, actively remodeling
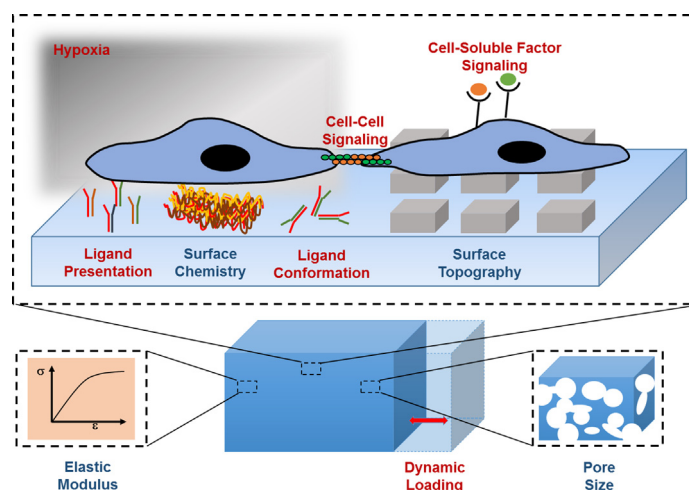

FIGURE 3

Inherent biomaterial cues (blue) and consequent cues (red) that affect macrophage polarization.

their cytoskeletal network and forming focal adhesions to affect cell shape, motility and function [33]. While anchorage dependent, low motile tissue cells rely heavily on stress fibers and reorganized F-actin structures to attach to substrates, highly motile cells derived from the myeloid lineage such as macrophages and neutrophils have been shown to not possess stress fibers, partly due to the fact that interactions with extracellular matrix components do not form part of their innate function [34]. Instead, macrophages rely on short lived focal complexes, point contacts and podosomes for migratory, phagocytic and mechanosensing roles [35]. Therefore, as detailed in the following sections, cytoskeletal mediated mechanisms form an integral part of macrophage response to biophysical cues.

Mechanical properties

One of the first studies exploring the effects of substrate stiffness on macrophage function used mouse bone marrow derived macrophages to show that stiff polyacrylamide particles were phagocytized preferentially over soft particles of identical chemistry through a Rac-1 mediated mechanosensory pathway [36]. This role of mechanosensitivity in macrophage function was further explored by Patel et al. [37], who reported that macrophage elasticity (elastic modulus), which is mediated by substrate stiffness, is actively dependent on actin polymerization and Rho-GTPase activity. RAW 264.7 cells (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice) consistently exhibited organized actin filaments and filopodial projections on stiff (150 kPa) polyacrylamide substrates; however, upon treatment with a Rho-GTPase inhibitor (C. Difficile toxin), cells appeared similar to those on softer (1.2 kPa) substrates, with an absence of organized actin fibers in projections [37] (Fig. 4h,i). Cell elasticity and phagocytic ability was also markedly higher for cells cultured on stiff substrates, suggesting that substrate elasticity modulates macrophage elasticity and phagocytosis through actin polymerization [37]. The authors also showed that addition of LPS/ IFN-γ to cells cultured on soft substrates increased cell spread area (Fig. 4j), suggesting that polarization signals impact cell shape, which has also been reported by several other groups [38,39].

Blakney et al. [40] showed that increasing the stiffness of 3D polyethylene glycol–RGD (PEG–RGD) hydrogels (130, 240 and 840 kPa) increased the FBR and the thickness of the fibrous capsule formed (~30 μm for 130 kPa and ~208 μm for 840 kPa). In vitro studies with the same gels revealed an increase in production of both pro-inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines with increasing stiffness of gels [40]. Based on these results, the authors concluded that softer gels are capable of a reduced inflammatory response; however, comprehensive quantitative studies on the in vivo host response would be more indicative of the mechanisms of hydrogel stiffness sensing by macrophages.

Micro and nano topography

From a biomaterial design perspective, modulating the surface topography is a simple method to modulate cellular response through control of cell shape and elasticity [41]. The modulation of macrophage function, phenotype and polarization to varying topography has been a subject of intense research for several decades [31,42–47]. It was recently found that topographical cues could override the effects of surface chemistry in certain materials,

especially in the first 6–48 hours after initial contact. RAW 264.7 cells seeded on substrates with three distinctive surface chemistries ((Poly (s-caprolactone) (PCL)), (Poly (Lactic acid)(PLA)), (Poly (Dimethyl Siloxane) (PDMS))) but different width parallel gratings (Fig. 4f,g) (with a range of 250 nm to 2 μm) showed increased elongation with decreasing topography irrespective of the underlying surface chemistry; however, cells were largely insensitive to topography changes smaller than 500 nm [46]. This study by Chen et al. [46] also showed that gratings that were 1 μm wide elicited the lowest inflammatory response in vitro (reduced levels of TNF-α and VEGF secretion) compared to nano gratings and planar controls. Interestingly, upon in vivo implantation, materials with 2 μm gratings exhibited the least number of FBGC (1 μm grating materials were not analyzed). This trend has been confirmed by Sanders et al. [48] who employed electrospun microfibers of different thickness and compositions; 1–5 μm diameter fibers exhibited the thinnest fibrous capsule formation compared to 6–10 μm and 11–15 μm for all surfaces tested. The above results suggest that topographical features in the size range 1–5 μm are well tolerated upon implantation and exhibit a minimal host response.

Recently, a study by McWhorter et al. [39] reaffirmed the influence of topography on macrophage polarization and identified a cell shape-mediated mechanism for this influence. Upon identifying that M1 polarized cells assume a rounded shape and M2 polarized cells assume an elongated shape, the authors used engineered cell culture substrates with 20 and 50 μm grooves to control cell shape and consequentially direct polarization (Fig. 4a–d). Moreover, the authors also reported that elongation of cells synergized with M2 inducing cytokines (IL-4, IL-13) to increase M2 polarization [39], suggesting that in addition to directing polarization, biophysical cues directly presented by biomaterials may be used to compliment the effects of factors already present in the native environment. Interestingly, while cytoskeletal inhibitors abrogated shape-induced polarization, their presence did not affect the cells' ability to respond to cytokine stimulation, suggesting that shape-induced and cytokine-induced polarization occur through distinct pathways [39].

3D geometric cues

While 2D substrates can be used as a means of easily answering important fundamental questions about the behavior of macrophages in response to individual stimuli, 3D models better represent the complex in vivo microenvironment. Indeed, pore size of 3D scaffolds has been implicated in modulating the macrophage phenotype. Sussman et al. [49] used Poly-hydroxy-ethyl-methacrylate (p-HEMA) scaffolds of small (34 μm) and large (160 μm) size pores to study the macrophage response in a subcutaneous mouse model three weeks after implantation. Interestingly, they observed increased vascular density, an increased presence of M1 phenotype and greater remodeling in the small pore size scaffold, suggesting that M1 cells are not always detrimental to the tissue remodeling process [49]. The authors were also the first to report specific locations of polarized cells; as the number of M1+ cells increased proximally to the implant while a smaller proportion of M2+ cells were found adherent on the implant. This study also indicated that rather than a fully polarized M1 or M2 phenotype, macrophages tend to assume a 'functionally active' phenotype, with up-regulation of both M1 and M2 markers simultaneously [49]. The
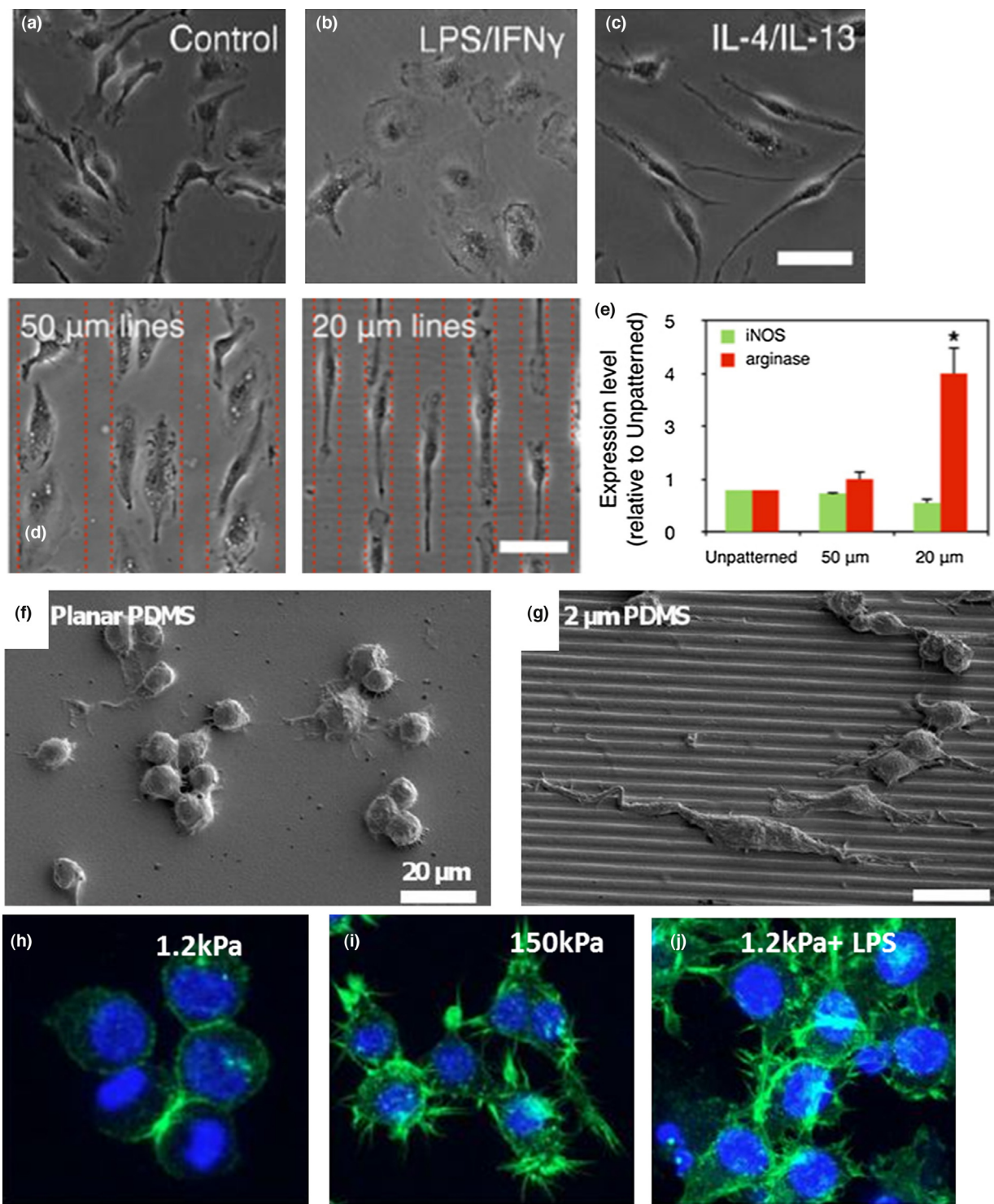
FIGURE 4

Morphological appearance of macrophages following cytokine stimulation and the effects of topography and stiffness on their polarization. (a–c) Macrophage polarization using cytokines results in an alteration in cell shape. Mouse bone marrow derived macrophages (a) assume a pancake shape when polarized with M1 cytokines (b) and an elongated phenotype with M2 cytokines (c) [adapted from [39]]; scale bar = 50 mm]. (d) By forcing these shape changes upon the cells by topological constraints (surface patterning; scale bar = 50 mm), the elongated cells take on a more M2 like phenotype in the absence of cytokines (decreased iNOS expression and increased arginase expression) (e) [adapted from [39]]. RAW 264.7 macrophages assume an elongated shape on PDMS substrates with 2 μm gratings compared to planar controls (f, g) [adapted from [46]]. When cultured on (h) soft (1.2 kPa) and (i) stiff (150 kPa) polyacrylamide gels, RAW 264.7 macrophages assumed pancake and elongated shapes respectively and more M2 like characteristics with increasing stiffness. Furthermore, cells on soft gels exhibited increased actin staining and cell spreading upon stimulation with LPS (j); cells were less responsive to LPS stimulation at higher stiffnesses [adapted from [37]].

influence of pore size on macrophage behavior was also observed by Almeida et al., who 3D printed chitosan and PLLA scaffolds of varying scaffold geometry and pore structure (orthogonal vs. diagonal pores). They determined that scaffolds with diagonal geometry had fewer FBGC and more elongated cells. This correlates well with the McWhorter study [39] that implicates an elongated cell shape in M2 responses. Interestingly, Almeida et al. [43] also observed that cells that had increased metabolic activity showed an increased inflammatory response. While this was an in vitro study carried out with MDMs, the results again suggest a 'functionally active' phenotype of macrophages, similar to that observed by Sussman et al.

Biochemical cues
Surface chemistry
In a previous study by Brodbeck et al. [50], it was reported that hydrophilic, anionic surfaces promoted the highest levels of apoptosis of biomaterial adherent FBGC. Since FBGC are detrimental to the biomaterial due to their ability to increase oxidative damage and recruit other inflammatory cells, the authors proposed that inducing apoptosis in the adhered FBGC would reduce the negative effects of adhered cells and improve tissue remodeling [50,51]. While the studies by Brodbeck et al. and others [52] suggest a dependence of the FBR on biomaterial surface chemistry, there is conflicting evidence in the literature regarding the extent of this dependence. For example, Schutte et al. and Castner et al. argued that there are no differences between inert, nondegradable materials with different surface chemistries one month post-implantation and hence any short-term effect observed is inconsequential [53,54]. This was also highlighted by Chen et al. [46] (see section 'Micro and nano topography') who demonstrated that subtle changes in topography override the effects of surface chemistry. Recent studies have progressed toward a possible explanation for the conflicting reports on the surface chemistry dependence of the FBR. McBane et al. [55] initially showed that a degradable hydrophobic, ionic polyurethane scaffold (D-PHI) was more successful than a tissue culture plastic surface (TCPS) in eliciting an anti-inflammatory phenotype from MDMs. This was characterized by significantly reduced levels of TNF-α in the D-PHI group after 24 hours and significantly increased levels of IL-10 at 72 hours when compared to the TCPS group. To understand a potential mechanism for this surface chemistry dependent immune response, a follow-up study by the same group demonstrated that the variation in protein adsorption, which was a function of surface chemistry, led to the varied cytokine release profile of adhered MDM on different surfaces [56]. This will be further discussed in the section 'Protein adsorption and ligand presentation'.

Consequent cues
As a consequence of biomaterial implantation, the local environment can be altered and subsequently affect macrophage behavior. These consequential cues include biophysical cues, such as dynamic loading; as well as biochemical factors, such as protein adsorption on biomaterial surfaces and local hypoxia resulting from the implantation procedure. Moreover, several tissue repair cells such as mesenchymal stem cells (MSC) selectively home to implant sites through paracrine signaling mechanisms [57]. The cytokines, chemokines and growth factors released by these infiltrating cells and cells already present in the microenvironment of the implant play a major role in determining the response of the local macrophage population to injury.

Dynamic loading
Upon implantation, a scaffold can undergo dynamic loading due to pulsing vessels, active joint loading, or proximity to contracting muscles resulting in dynamic or cyclical strain on cells. Indeed, an up-regulation of both M1 and M2 cytokine release was observed on applying a 7% cyclical load to PCL bisurea strips seeded with monocyte derived macrophages, with cells progressively polarizing toward an M2 phenotype with increasing time [58]. Another study quantified the effect of cyclical pressure on cytokine/chemokine production in cultured human macrophages, with an increase in pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) compared to controls for all levels of cyclical pressures tested (17–138 kPa) [59]. Moreover, this effect was further elevated with the presence of polyethylene particles (representing wear debris from implants). The authors proposed that this increase in release of pro-inflammatory cytokines in response to cyclical pressure and presence of wear debris contributes toward aseptic loosening of implants [59]. Hence, better implant design and fixation methods to modulate local levels of dynamic loading and minimizing wear debris are potential avenues for improving long term survival of implanted materials.

Protein adsorption and ligand presentation
Protein adsorption on the surface of biomaterials occurs post implantation and control over it is not inherently designed into the biomaterial, making it a consequent cue. Protein adsorption, through the subsequent presentation of ligands that differentially bind and activate integrins, can trigger the production of a wide range of inflammatory cytokines and chemokines through integrin–ligand interactions. The study by McBane et al. described in section 'Biochemical cues' observed that while surface chemistry per se may not modulate the FBR, the selective adsorption of proteins by different surfaces could account for observed differences in FBR. Moreover, earlier studies have indicated differences in cellular behavior on substrates with different ligand confirmations [60], suggesting that not only the type of protein adsorption, but also the orientation of ligands presented could affect the macrophage response.

Zaveri et al. demonstrated the effects of varying integrin binding on macrophage responses by subcutaneously implanting polyethylene terephthalate (PET) biomaterials and disrupting the function of integrin Mac1 (leukocyte integrin that binds to fibrinogen) and RGD (ligand present in fibronectin, fibrinogen, vitronectin and laminin [61]) binding integrins via a mouse knock out model and integrin blocking respectively [28]. Mac1 knockout mice displayed reduced cytokine secretion compared to the wild type controls and had reduced fibrous capsule thickness by 27%. Similarly, blocking RGD ligands by releasing a high affinity RGD peptide decreased the fibrous capsule thickness by 45% [28]. The authors conclude that this pronounced effect is likely due to a more widespread effect of RGD – which can bind to a large selection of integrins. This study demonstrates the potential for modulating macrophage behavior via inhibition of integrin

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interactions common to all cells (e.g., RGD). However, this could have detrimental effects on achieving sufficient biomaterial integration; hence, blocking targeted integrins may represent a preferred option for minimizing host response by minimizing integrin–ligand binding. From this section, it is clear that while protein adsorption (and ligand presentation resulting from protein adsorption) can considerably moderate the inflammatory response, protein adsorption itself can be modulated by changing inherent material properties such as surface chemistry. Moreover, emerging approaches such as direct ligand patterning on biomaterial surfaces to promote preferential protein attachment represent modifications to inherent cues presented by biomaterials to further affect consequent cues.

Hypoxia

Apart from changes in the surrounding biophysical and biochemical cues, implantation of a biomaterial can provide consequent cues via alterations to the local hypoxic environment caused by the destruction of blood vessels supplying the injured tissue [62]. Macrophages accumulate in hypoxic environments to release pro-healing and pro-angiogenic factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) [63] and enzymes such as Cyclooxygenase 2 (COX2) [64]. The response of macrophages to hypoxic environments is thought to be mediated by transcriptional factors hypoxia inducible factor (HIFs) 1 and 2 [65]. While the role of macrophages in a hypoxic wound environment is beneficial to promote vascularization of implanted scaffolds, it is noted that the hypoxic environment present in cancerous tissue actively recruits tumor associated macrophages (TAMs), which closely resemble the M2 phenotype. This has led to an intense research focus on TAM targeted therapies within the cancer field [66,67]. Undoubtedly, further research on hypoxia in the implant environment and its effect on macrophages will provide opportunities to take advantage of hypoxic conditions to aid in wound healing mechanisms.

Interaction with other cells in microenvironment

The capacity of biomaterials to act as a cell carrier and their impact upon host cells in situ can also provide a number of consequent cues to affect macrophage behavior and modulate the inflammatory response. In this section, we will review two main cell types known to play a major role in mediating macrophage response to biomaterials; lymphocytes and mesenchymal stem cells.

Lymphocyte/macrophage interactions

In addition to the well-established role of macrophages in response to implanted biomaterials, emerging research suggests that lymphocytes are key cellular determinants of biomaterial outcomes [68]. The bidirectional interaction between the two cell types is well established; indeed, the polarization paradigm of macrophages is derived from their interaction with lymphocytes [14]. TH1 lymphocytes release IFN-γ which polarizes cells toward an M1 phenotype, secreting several pro-inflammatory cytokines and chemokines, some of which (IL-12, CXCL-10) escalate the inflammatory response by recruiting more Th1 lymphocytes [68,69]. Likewise, Th2 lymphocyte derived signals such as IL-4 and IL-13 direct M2 polarization of macrophages that in turn produce chemokines such as CCL17, CCL22 and CCL24, which enhance the recruitment of Th2 lymphocytes [68]. Moreover, macrophages and lymphocytes regulate each other through the production of cytokines and chemokines through every step of the FBR; with TH2 derived cytokines (IL-4, IL-13) driving the formation of FBGCs [70]. Indeed, it has been shown in an in vitro lymphocyte/macrophage co-culture study that juxtacrine (cell-cell contact) and paracrine (soluble factors) signaling play a crucial role in determining inflammatory response; and that this response can be mediated by varying surface chemistry of materials [71]. This tightly controlled regulation and complex bidirectional interaction of macrophages is also observed with dendritic cells, neutrophils and other immune cells present in the environment (which are comprehensively reviewed in a number of publications [15,72,73]).

MSC/macrophage cross-talk

Mesenchymal stem cells or multipotent stromal cells (MSCs) are adult stem cells that are found in tissues such as the bone marrow and can differentiate toward a multitude of lineages that constitute tissues including fat, bone, cartilage, muscle and skin, making them popular in regenerative medicine strategies [74,75]. Due to their ability to secrete anti-inflammatory cytokines such as IL-10 and vascular endothelial growth factor (VEGF), they are additionally being explored for their immunomodulatory capabilities [76,77]. Their use in clinical trials for treating immune diseases such as graft versus host disease (GvHD) and Crohn's disease is ongoing [78]. Upon implantation of a biomaterial, there are two distinct sources of MSCs that can interact with macrophages in the implant environment: endogenously derived MSCs and those administered as part of the implant/therapy. Chen et al. [27] showed that factors secreted within bone marrow MSC (BM-MSC) conditioned medium (cultured under hypoxic conditions) actively recruit macrophages and endothelial cells to a wound thereby enhancing wound healing and that MSC conditioned medium increased in vitro migration of endothelial cells and keratinocytes. BM-MSC were shown to release chemo-attractants such as macrophage inflammatory protein (MIP) and monocyte chemo-attractant protein (MCP), which recruit more monocytes/ macrophages. Additionally, BM-MSC release cytokines such as VEGF, which promote vessel formation at site of implantation/ injury and allow for further recruitment of cells [27]. When injected in vivo in a mouse model, MSC conditioned medium led to greatest wound closure after 14 days compared to controls. It has also been shown that macrophages mediate MSC viability and proliferation in vitro [79], and that MSC are early regulators of inflammation [80] (Fig. 5).

Interestingly, it was recently observed by Seebach et al. that MSC-filled fibrin constructs promoted infiltration of M1 macrophages accompanied by early signs of vascularization, which was absent in fibrin constructs without MSC (Fig. 6c,d,g,h). Gene expression analysis at day 3 and 6 revealed no differences in expression of TNF-α, IL-1β or IL-10 between cell-free and MSC filled constructs [81]; however, later timepoints were not analyzed. Research from our group suggests that marked differences in M1 and M2 presence is observed 4 and 8 weeks after implantation of cell free and MSC filled porous collagen-based scaffolds (Fig. 6a,b,e,f)[82], hence analyzing later timepoints in the fibrin-MSC study might shed light on the beneficial effect of MSC in
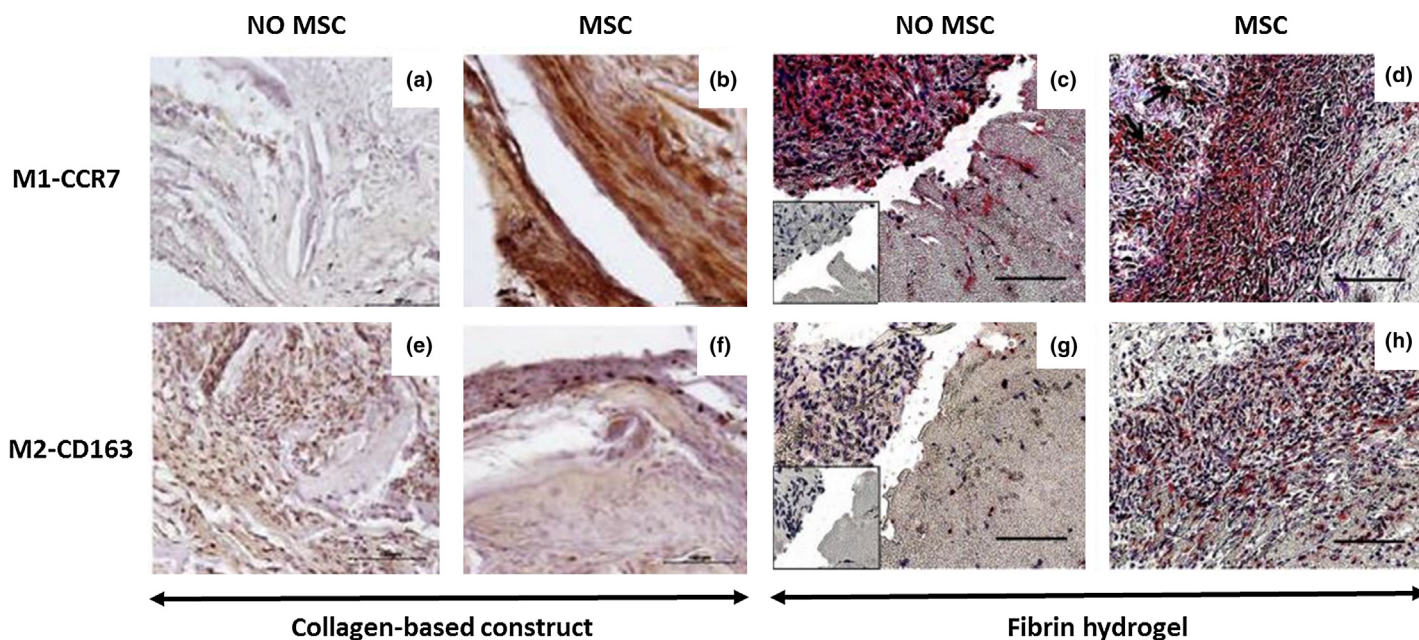
FIGURE 5

In vivo host response in bone defects to tissue engineered collagen based constructs (left) at 8 weeks and fibrin hydrogels (right) after 6 days. M1 macrophages were no longer present in the defect site after 8 weeks (cells were present at 4 weeks, data not shown) in cell free collagen-based scaffolds (a) but were present at the periphery and within the scaffold with MSC (b). M2 macrophages were observed in the periphery and inside the scaffold in cell free scaffolds (e) but only in the periphery in the scaffolds with MSC (f). For fibrin hydrogels, both M1 and M2 positive cells were scarcely present in the cell free constructs (c, g). In the MSC-filled fibrin hydrogel group, while M1 macrophages were found in cell dense areas (d), M2 macrophages had accumulated throughout the scaffold, especially in the migration front (h). Scale bars – 100 μm. [a, b, e, f [adapted from [82]] [c, d, g and h adapted from [81]].

promoting tissue integration. Apart from the materials used and the analysis time-points, one of the main differences between the two studies was the 4 week *in vitro* culture of the collagen-MSC scaffolds. Matrix deposition during this period of *in vitro* culture is likely to have hindered penetration by immune cells after implantation and may account for the limited tissue integration observed in these collagen-MSC scaffolds. However, results from both studies agree with conclusions by Brown *et al.* [83], who demonstrated that the presence of even an autologous cellular component attracted M1 cells, which was not observed in cell free constructs. This further suggests that the M1 response observed by Seebach *et al.* and Lyons *et al.* is a direct effect of the presence of a cellular component (and not necessarily due to the presence of MSC specifically). Nevertheless, it is clear that additional research needs to be undertaken with cell-free and MSC-seeded tissue engineering constructs to understand the effect of MSC presence in such scaffolds.

Peripheral blood monocytes co-cultured with MSC have been defined under a new paradigm by Kim *et al.*, and are referred to as MSC educated macrophages (MSC-Mo) [84]. These cells have a unique anti-inflammatory signature (IL-10 high, IL-12 low, IL-6 high, and TNF-α low) and are distinct from monocyte derived M2 macrophages, due to their increased production of IL-6 [85]. More research is requisite for understanding the *in vivo* functionality of this phenotype, and for shedding light on macrophage/MSC interactions especially in the presence of diverse biophysical and biochemical cues.

Designing immuno-informed biomaterials

With the many exciting advances in our understanding of macrophage-biomaterial biology, we can now begin to integrate this information into design choices for novel immuno-informed biomaterials. The design criteria of these biomaterials can be specified by broadly dividing them into two categories: (1) replacement biomaterials that integrate and remain permanently fixed upon implantation, with minimal inflammation and fibrous tissue formation; and (2) regenerative biomaterials that provide initial support and stimulate tissue formation while degrading at a controlled rate over time. Importantly, immuno-informed decisions have to be integrated into, and traded off against the other design goals of the device (such as tailored mechanical properties for bone or cartilage regeneration); however, our emerging understanding suggests that macrophage polarization can be affected in a plethora of ways, providing several potential avenues through which immuno-informed biomaterials can be designed (Fig. 6).

Replacement biomaterials

Replacement biomaterials include long term (15–20 years; after which even the best implants need replacement due to aseptic loosening and stress shielding) implantable devices made of polymeric/metallic materials that are mechanically stable and aim to exhibit minimal host response upon implantation [8]. Historically, such implants were preferred to be biologically inert, to minimize interactions between the implant and cells in the microenvironment [86]. This is achieved by careful selection of material components that: allow for native protein adsorption on the surface, which can contribute to provisional matrix formation and act as a buffer between the biomaterial and the host; and/or whose deterioration products (e.g., as a result of wear) are readily excreted through the kidneys. Furthermore, ensuring that motion between the implant and the host is minimized through appropriate surgical techniques will minimize scar tissue formation; this
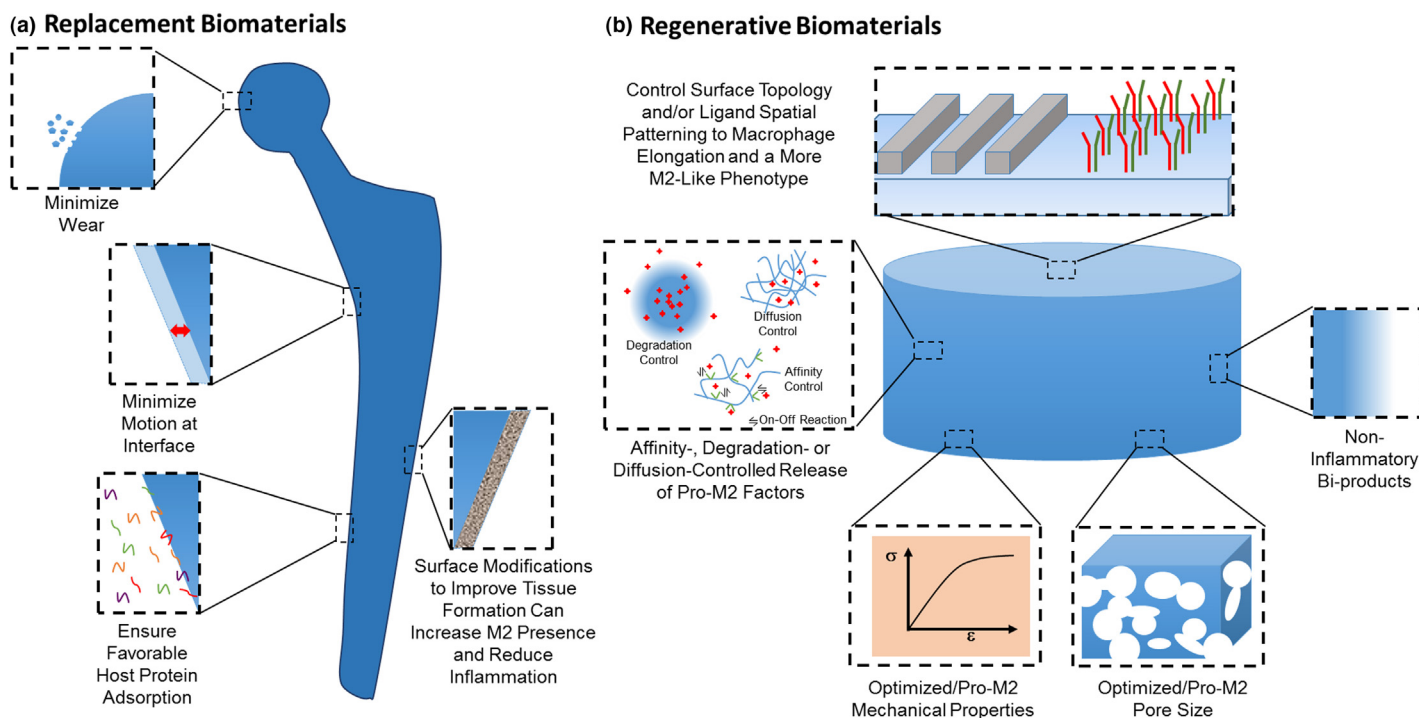
(a) Replacement Biomaterials

Minimize Wear

Minimize Motion at Interface

Ensure Favorable Host Protein Adsorption

Surface Modifications to Improve Tissue Formation Can Increase M2 Presence and Reduce Inflammation

(b) Regenerative Biomaterials

Control Surface Topology and/or Ligand Spatial Patterning to Macrophage Elongation and a More M2-Like Phenotype

Degradation Control

Diffusion Control

Affinity Control

⇌ On-Off Reaction

Affinity-, Degradation- or Diffusion-Controlled Release of Pro-M2 Factors

Non-Inflammatory Bi-products

σ

ε

Optimized/Pro-M2 Mechanical Properties

Optimized/Pro-M2 Pore Size

FIGURE 6

Current and evolving design principles for immuno-informed design of (a) replacement biomaterials and (b) regenerative biomaterials. The pros and cons of these effects on the immune response should be evaluated against their effects on all cell types involved in the host response.

is essential in implants placed in bone, for example, but may be of less importance for subcutaneous implants (e.g., implantable cardioverter defibrillator (ICD)) and irrelevant for implants in cavities (e.g., intrauterine devices).

Despite the desire to minimize the interaction between implant and host, recent evidence suggests that promoting specific interactions between cells and the implant can boost immune acceptance and integration. For example, titanium implants used for bone tissue replacement (e.g., hip/knee replacements, dental implants) show higher osseointegration when the surface is modified to permit attachment and migration of bone forming cells [87,88]. Such modifications to the biomaterial surface not only promotes greater tissue remodeling, but also has the potential to subsequently induce a pro-M2 response due to better tissue integration, thereby creating a favorable immune environment for remodeling. Current research in this area focuses on varying surface chemistries and roughness to modulate the macrophage response toward an M2 phenotype, which will in turn secrete pro-healing and anti-inflammatory factors to mitigate the formation of fibrous tissue [5,87,89]. To promote successful integration of the implant, the host tissue should ideally remodel and reform around the implant to restrict further inflammatory reaction. Furthermore, the boundary between replacement medical devices and regenerative medicine constructs is increasingly overlapping, as many coating technologies on replacement devices are now functionally similar to those used for regenerative medicine.

Regenerative biomaterials
The increasing exploration and use of regenerative biomaterials is due to their ability to restore lost structural and functional properties of injured and diseased tissue. Such constructs are typically designed to be biodegradable over a period of several days to months depending on the application, with regeneration of host tissue being structurally supported and promoted by matrices that eventuate in degradation products designed to be absorbed or excreted by the body [5,90]. Upon surgical implantation, it has been shown that the initial M1 response is responsible for recruiting inflammatory cells to the site of injury and for instigating the ensuing foreign body response [5,86,91], which are necessary events for wound healing. Following this initial response; however, the prolonged presence of M1 cells leads to the production of toxic reactive oxygen intermediates and results in excessive oxidative damage of the biomaterial [14,15]. Furthermore, fibrous capsule formation as a result of extended inflammation could impair the capacity of regenerative biomaterials to promote tissue formation or degrade in the intended manner. Therefore, a subsequent transition to the M2 phenotype – which promotes tissue remodeling and repair – is generally believed to be a favorable adaptation [8].

In designing an immuno-informed regenerative biomaterial, the most targeted method of controlling the immune response would be to release factors (e.g., IL-4, IL-10, steroids) that overwhelm native signaling and direct polarization [92,93]. This could be done by incorporating growth factors, gene delivery vectors or small molecule drugs (e.g., steroids) into controlled release systems [94], either alone or as part of a system that is designed for multi-factor release [93]. The advantages and effectiveness of this approach remains to be evaluated against the potential cost increase.

Beyond incorporation of stimulatory bioactive molecules, several biophysical and biochemical properties can be exploited

to affect macrophage polarization. Although the exact dependence of macrophage phenotype on stiffness is not fully conclusive, future investigations will no doubt add to the growing body of knowledge relating to the effect of biomaterial mechanical properties on macrophage behavior [37,38]. Investigations into the role of topography on polarization are strongly suggestive of the advantages of stimulating macrophage elongation for promoting M2 polarization [39]. This can be achieved by micro-patterning the surface – studies suggest around 1 μm thick strips or fibers are optimum [46] – to control attachment, or could be achieved by patterning macrophage ligands on the surface to promote elongation of cells. Pore size was also shown to affect macrophage response–as the incorporation of scaffolds with a pore size of 34 μm was shown to reduce fibrous encapsulation [49]; however, interestingly, more M1 cells were found on scaffolds with this pore size when compared with those with a larger pore size (160 μm), again suggestive of the necessity of the initial M1 response [49]. The consequential effects of adapting these biomaterial design considerations to directly modulate macrophage behavior need to be done with consideration for the effects on other cell types (e.g., the known dependence of MSC behavior on scaffold stiffness [95]).

While it is generally accepted that there are positive healing outcomes with the presence of M2 cells in the implant environment [8,96], it is still unclear whether positive healing outcomes are predominantly governed by the influence of macrophages and their orchestration of cellular events, or whether it is the overriding influence of other cells and microenvironmental cues, which are then also responsible for directing macrophage behavior. There is increasing evidence that MSCs stimulate polarization of macrophages towards the M2 state; whether this applies only to exogenously administered MSC or also endogenously homed MSC remains to be seen. Moreover, hypoxic effects and endogenous MSC could already be present in the injured tissue that requires biomaterial implantation; hence, efficient methods to harness these already existing M2 polarizing cues could warrant further investigation. Ultimately, it is evident that several routes exist for polarizing macrophages to assume a favorable M2 phenotype; however, a practical and efficient approach to the design of immunomodulatory biomaterials still warrants further research.

Conclusions

The recent surge in our understanding of macrophage polarization and its role in wound healing has seen an advance in knowledge from utilizing growth factors alone to affect polarization to an understanding of a diverse set of biophysical and biochemical cues that affect polarization. Ongoing research is unveiling more details of inherent biomaterial cues as well as consequent cues and their role in polarizing macrophages and immuno-modulation. With this in mind, current and future research should be aimed at gaining an increased understanding of such biomaterial-based factors involved in polarizing macrophages. This insight into macrophage-biomaterial biology and an improved understanding of other components of the immune system such as neutrophil and dendritic cell modulation will ultimately lead to a definitive set of design principles to aid in the design of a new generation of immuno-informed biomaterials that can actively direct the innate immune system.

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