

Immunomodulatory Biomaterials for Tissue Repair

Ricardo Whitaker,^{II} Beatriz Hernaez-Estrada,^{II} Rosa Maria Hernandez, Edorta Santos-Vizcaino, and Kara L. Spiller*



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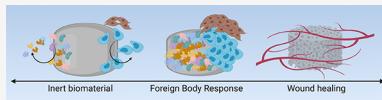
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ABSTRACT: All implanted biomaterials are targets of the host's immune system. While the host inflammatory response was once considered a detrimental force to be blunted or avoided, in recent years, it has become a powerful force to be leveraged to augment biomaterial–tissue integration and tissue repair. In this review, we will discuss the major immune cells that mediate the inflammatory response to biomaterials, with a focus on how biomaterials can be designed to modulate immune cell behavior to promote biomaterial–tissue integration. In particular, the intentional activation of monocytes and macrophages with controlled timing, and modulation of their interactions with other cell types involved in wound healing, have emerged as key strategies to improve biomaterial efficacy. To this end, careful design of biomaterial structure and controlled release of immunomodulators can be employed to manipulate macrophage phenotype for the maximization of the wound healing response with enhanced tissue integration and repair, as opposed to a typical foreign body response characterized by fibrous encapsulation and implant isolation. We discuss current challenges in the clinical translation of immunomodulatory biomaterials, such as limitations in the use of in vitro studies and animal models to model the human immune response. Finally, we describe future directions and opportunities for understanding and controlling the biomaterial–immune system interface, including the application of new imaging tools, new animal models, the discovery of new cellular targets, and novel techniques for in situ immune cell reprogramming.



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1. INTRODUCTION

Besides the need to ensure proper functionality of the device, biomaterials must address the immediate and aggressive response from the innate immune system upon implantation.

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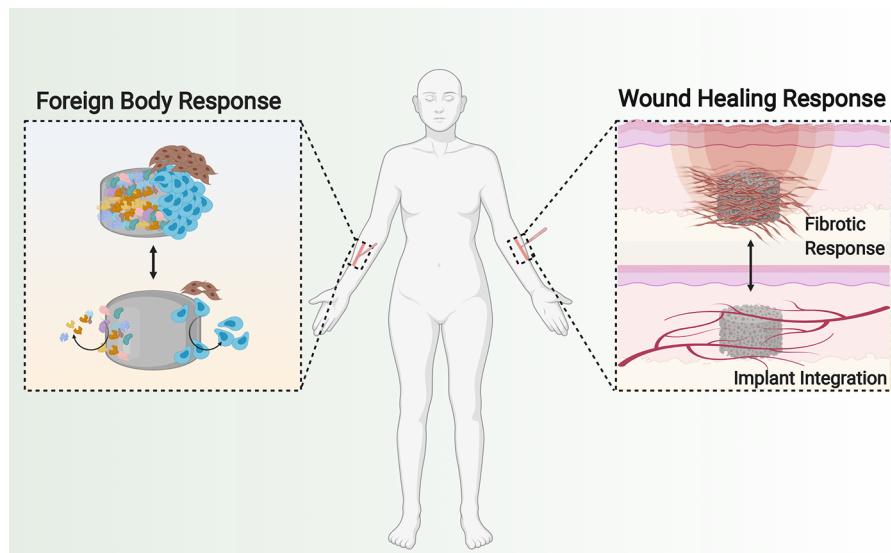


Figure 1. Overview. The two main types of inflammatory responses to implanted biomaterials include the foreign body response and biomaterial–tissue integration.

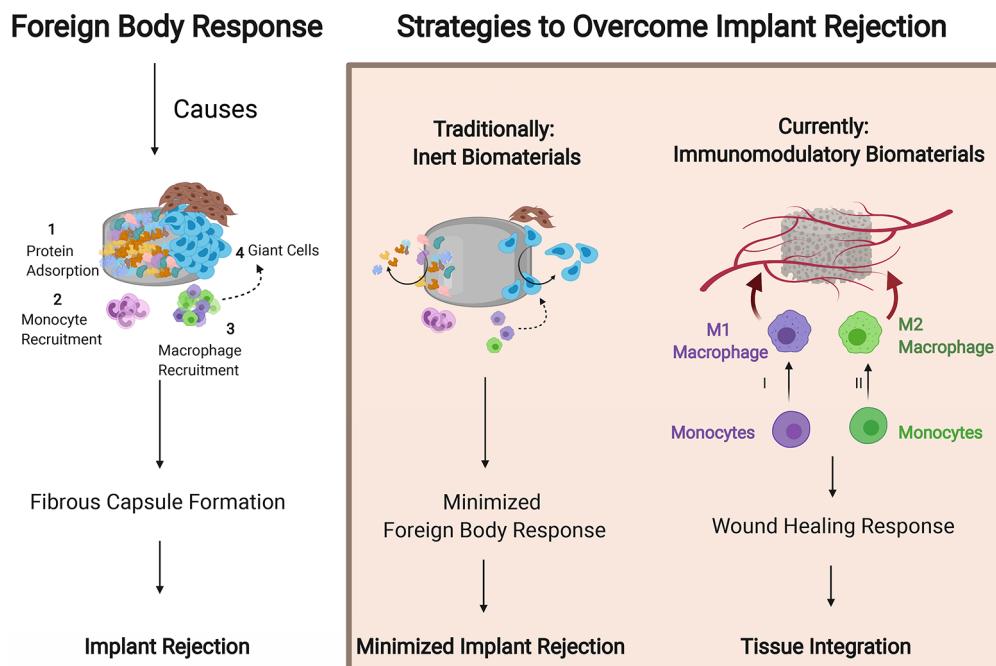


Figure 2. Foreign body response and strategies to overcome implant rejection.

An uncontrolled inflammatory response to implants results in fibrous encapsulation, which can lead to implant failure, in a process called the foreign body response (FBR). However, the inflammatory response is also essential for wound healing and tissue repair, making it an attractive target to leverage for biomaterial–tissue integration. While both responses involve similar immune cells and processes, including fibrosis, they are fundamentally different (Figure 1). Understanding these differences in the inflammatory response is key to designing successful biomaterials. Here we will briefly review these two main types of inflammatory responses to biomaterials, with a focus on design strategies to control these processes in order to augment biomaterial–tissue integration and tissue repair.

2. THE FOREIGN BODY RESPONSE

All implanted biomaterials trigger the inflammatory response, which is characterized by protein adsorption and the recruitment of innate immune cells like neutrophils, monocytes, and macrophages. When this process proceeds to fusion of macrophages into foreign body giant cells (FBGCs) and ultimately the formation of a fibrous capsule by FBGCs and/or fibroblasts that isolates the implant from the surrounding tissue, the process is called the foreign body response (FBR). This process is generally considered detrimental for most biomaterials, and therefore numerous strategies have been developed to interfere with one or more steps, with the level of success measured in terms of either reduction in fibrous capsule thickness or increased vascularization and integration, depending on the intended purpose of the implant (Figure 2).

Vascularization and integration are considered a wound healing-type response and will be covered in detail later in this review.

For some biomaterials designed to remain implanted in patients permanently, the FBR is a normal long-term reaction. For example, evidence of an FBR to cochlear implants, including macrophages that phagocytosed wear debris, was noted in patients post-mortem as long as 23 years after implantation.¹ Similarly, post-mortem analysis of the tissue surrounding cardiac pacemakers showed evidence of the FBR as late as eight years after implantation, with continued presence of macrophages and lymphocytes.^{2,3} In some patients, the long-term FBR may be characterized by dense fibrous tissue, with very few cells, while in others the FBR contains abundant cells, including multinucleated FBGCs and lymphocytes.^{1–3}

Inflammation is believed to be the driving factor behind the delayed failure of some long-term biomaterial implants, namely total joint replacements and bioprosthetic heart valves. Total joint replacements can last for 10–20 years, but then mechanical wear and tear on the biomaterial causes the generation of particulate wear debris, which are phagocytosed by macrophages to stimulate active inflammation, ultimately leading to implant loosening and the need for revision surgery.⁴ The mechanisms behind the inflammatory response to wear debris have been very well studied.⁵ The failure of bioprosthetic heart valves is poorly understood but may involve inflammation-mediated calcification of the implants, which are chemically cross-linked tissues of porcine or bovine origin.⁶ It is not known whether the failure of these materials, which occurs years after implantation, is triggered by a new inflammatory response to wear particles, or if it results from byproducts of low-grade, chronic inflammation that build up over time.

Breast implants are a prominent example of problems that can be caused by the FBR, as fibrous encapsulation can lead to pain and deformity. As a result, more than 29 000 revision surgeries were required in 2018.⁷ Prantl and colleagues analyzed the FBR to explanted smooth silicone gel implants from 24 females, ages 28–52, following an average implant duration of 34 ± 11 months.⁸ The goal of this study was to characterize the FBR to the implants and their correlation with clinical manifestations of implant failure, as measured by the Baker score, a commonly used metric of implant deformity and pain. The researchers observed a direct correlation between Baker score and both fibrous capsule thickness and inflammatory fibrosis, as measured by the Wilfingseder histological score. All patients presented fibrous capsules containing macrophages in the innermost layer. In addition, 62.5% of the patients also presented the formation of FBGCs within the fibrotic capsule.

Formation of fibrotic tissue around hernia meshes was also studied in humans. Here, Van den Hil and colleagues investigated the differences in the FBR comparing rats and human patients implanted intraperitoneally with surgical grade polyester hernia meshes.⁹ Eight out of ten implants in humans were recovered after 2–15 months of implantation, with high levels of fibrosis, which the authors attributed to the severity of the surgery but without substantial numbers of FBGCs or granulocytes. The FBR to the same materials in rats showed similar levels of general inflammation and adhesion formation but with less fibrosis and increased numbers of granulocytes and FBGCs compared to humans. This study highlighted the

clinical relevance of the FBR process while demonstrating potentially important differences across species.

This article will review the main cells and processes involved in the FBR, with a focus on biomaterial design strategies to modulate these processes for therapeutic purposes. For in-depth reviews of the fundamentals of the FBR, including the major molecular pathways involved, the reader is referred to refs 10 and 11.

2.1. Major Cells and Steps of the FBR

2.1.1. Protein Adsorption. The first step in the FBR is the adsorption of serum proteins to the surface of the biomaterial, which creates a chemoattractant gradient for neutrophils and monocytes and macrophages. This process is called opsonization. Among the numerous proteins capable of signaling foreign objects, the most potent is the complement protein family, especially C3 and C5, which fragment to coat the foreign material and to recruit circulating immune cells (for a thorough review, see ref 12). Additional opsonins include albumin, globulins, and fibronectin, among others.

In general, high levels of protein adsorption leads to increased cell adhesion and, therefore, increased fibrous encapsulation. As a result, a common strategy to reduce fibrous encapsulation of biomaterials is to coat them with nonfouling polymers. For example, zwitterionic polymers, which adsorb very low levels of proteins due to their highly hydrophilic nature and neutral charge, have shown promise for minimizing the FBR.^{13–18} Zhang et al. prepared zwitterionic hydrogels from poly(carboxybetaine methacrylate) (PCBMA) and compared them to poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogels implanted subcutaneously in mice for up to three months.¹⁸ The fibrous capsule surrounding the zwitterionic hydrogels was much thinner and had lower collagen density compared to the pHEMA hydrogels. Liu et al. extended this strategy to minimize the FBR to hydrogels containing transplanted islet cells for the treatment of type 1 diabetes.¹⁷ The group found that conjugation of zwitterionic moieties to alginate hydrogel particles resulted in less cell adhesion and improved survival of the transplanted islets in a diabetic mouse model and in larger animal models.

However, several studies have added complexity to this dogma. For example, fibronectin is a serum and extracellular matrix (ECM) protein that is critical for cell adhesion. Therefore, if increased cell adhesion always resulted in increased fibrous capsule thickness, then biomaterials implanted in the absence of fibronectin would be expected to result in thinner fibrous capsules. In contrast, Keselowski and colleagues found increased fibrous encapsulation of polyethylene terephthalate (PET) discs implanted subcutaneously in mice that were genetically deficient for fibronectin.¹⁹ These mice also showed a 3-fold increase in multinucleated cell formation around the implant compared to wild-type controls despite no differences in leukocyte recruitment. In another study, Swartzlander et al. showed that poly(ethylene glycol) (PEG)-based hydrogels conjugated with arginine–glycine–aspartic acid (RGD), the major cell-binding motif found in fibronectin, were encapsulated in thinner fibrous capsules compared to controls without RGD, even though they adsorbed similar levels of protein and supported higher levels of macrophage adhesion *in vitro* and *in vivo*.²⁰ However, it is possible that macrophages, an intrinsically adherent cell type, may upregulate receptors and proteins that allow adhesion in the absence of fibronectin. In summary, although reduced

protein adsorption is generally associated with lower levels of fibrous capsule formation, future studies are needed to elucidate the complexities of the system with respect to specific proteins.

2.1.2. Neutrophil Recruitment. Neutrophils are the most abundant cell type of the innate immune system in terms of concentration in the blood. Under healthy conditions, they develop mainly within the bone marrow, where they mature and are released into the bloodstream. Under inflammatory conditions, including an injury or implantation of a biomaterial, this process is potentiated, leading to greater extravasation to affected tissues. Neutrophils are phagocytic cells and are capable of degrading foreign objects up to a certain volume.²¹ While in a healthy wound, neutrophils are generally cleared within a few days, they have been shown to persist for several weeks around implanted biomaterials.²² The consequences of this persistence are poorly understood. To investigate the role of neutrophils in the FBR, Jhunjhunwala et al. implanted five different microparticles poly(lactic-co-glycolic acid) (PLGA), glass, polystyrene, poly(methyl methacrylate) (PMMA), and alginate of different sizes and shapes into the peritoneal cavity of mice.²² While under homeostatic conditions, the peritoneum is comprised mostly of resident macrophages, B cells, and T cells, the implantation of these materials provoked a 30–500-fold increase in the neutrophil population to around 8–35% of the total cell population, depending on the implanted material. Further evaluation of PMMA, polystyrene, and alginate microparticles demonstrated that neutrophils deposited extracellular material on these implants resembling neutrophil extracellular traps (NETs) or tangles of protein and chromatin that neutrophils use to kill invading pathogens. To investigate the implications of NET formation in the FBR, Fetz et al. examined the response of neutrophils to electrospun scaffolds of poly(dimethylsiloxane) (PDO) of two different fiber sizes, with or without collagen incorporation in vitro and in vivo.²³ In vitro studies with human neutrophils showed that the degree of NETosis, regardless of fiber size, was decreased in collagen-containing scaffolds. In addition, scaffolds with larger fibers (1–2 μm) stimulated less NETosis compared to those with smaller fibers (0.25–0.35 μm). When implanted subcutaneously in rats, the smaller fiber scaffolds resulted in fibrous encapsulation, while the larger diameter fiber scaffolds led to partial tissue integration, suggesting that NETosis propagates the FBR and hinders biomaterial–tissue integration.

Another study suggested that neutrophils are not required for fibrous capsule formation because the amount of fibrosis surrounding alginate microparticles was unaffected by depletion of neutrophils using Ly6G-neutralizing antibody.²⁴ However, the alginate microparticles did appear to clump together more, which the authors had previously found to be associated with fibrous encapsulation of other biomaterials,²⁵ suggesting that neutrophils may affect the rate of fibrotic tissue deposition. Collectively, these results suggest that neutrophils do play some role in the immune response to implants, although their precise functions are still poorly understood.

2.1.3. Macrophages and Foreign Body Giant Cells.

Concurrent with neutrophils, monocytes are also recruited to the site of injury, where they differentiate into macrophages and play indispensable and complex roles in the ensuing steps of the FBR. Monocytes, the precursors of macrophages, are formed and matured inside the bone marrow until they are released into the bloodstream in response to injury. Monocytes

are recruited to sites of injury via chemoattractive factors, and they differentiate into macrophages upon extravasation into the injured tissue. In addition to these blood-derived monocytes, long-lived tissue-resident macrophages are also recruited to sites of injury,²⁶ although their specific role in the FBR is not yet known. Monocytes and macrophages are highly plastic, existing as numerous distinct phenotypes. Some phenotypes may be more detrimental or beneficial than others, but the exact roles of each distinct phenotype in the FBR are still poorly understood (for review, see ref 27). While regulated macrophage activity is critical for biomaterial–tissue integration, which will be discussed in detail later in this review, macrophage fusion into foreign body giant cells (FBGCs) and their role in the propagation of fibrous capsule formation are hallmarks of the FBR.

In addition to many studies noting the prevalence of macrophages within the fibrous capsule surrounding biomaterials, several studies have shown that depletion of monocytes/macrophages via administration of clodronate liposomes inhibits fibrous encapsulation of biomaterials.^{24,28,29} Pharmacologic inhibition of the major receptor responsible for macrophage maturation, CSF1R, also decreased macrophage presence around implants and the ensuing FBR, although it did not affect general monocyte/macrophage recruitment to the implant area.²⁴ However, one study suggested that the mechanism of macrophage depletion may directly impact the results because macrophage depletion in macrophage fas-induced apoptosis (MaFIA) mice actually increased fibrous encapsulation surrounding subcutaneously implanted collagen scaffolds,³⁰ even though a previous study in the same mouse strain showed the opposite effects when alginate microparticles were implanted intraperitoneally.²⁵ Here, it is important to highlight that macrophage depletion using the MaFIA model can be highly toxic because of the excessive release of inflammatory cytokines,³¹ which can complicate the interpretation of any results derived from MaFIA mice. Furthermore, MaFIA mice also experience dendritic cell depletion, which may further skew results.³² Collectively, these studies show that manipulation of monocyte recruitment or macrophage maturation influences various aspects of the FBR, including FBGC formation and fibrous capsule deposition. Indeed, a few other studies have shown that directly interfering with macrophage signaling pathways, such as MyD88 (myeloid differentiation primary response 88), Nlpr3 (NOD-like receptor family pyrin domain containing 3), Asc (apoptosis associated speck-like protein containing a CARD), and caspase-1, affects the FBR.^{33,34}

Interestingly, one study showed that macrophage fusion into FBGC may not be required for fibrous encapsulation of biomaterials. Kyriakides and colleagues found that macrophage fusion surrounding subcutaneously implanted poly(vinyl alcohol) (PVA) sponges was reduced in mice genetically deficient for CCL2 (C–C motif chemokine ligand 2) (aka monocyte chemoattractant protein 1, MCP1), without affecting the total numbers of monocytes or macrophages.³⁵ Despite the reduction in macrophage fusion, collagen deposition and the thickness of the fibrous capsules surrounding the implants did not differ between groups, although scaffolds implanted in CCL2-null mice were 50% more intact compared to wild-type mice, indicating a role for FBGCs in biomaterial degradation. Together, these results suggest that while macrophages and their fusion into FBGCs do mediate fibrous capsule formation, there must be

redundancy in the system such that fibrous encapsulation can occur without these steps.

In addition to fusing into FBGCs to mediate biomaterial degradation, macrophages may also play a direct role in ECM deposition during the fibrous capsule formation process. Using MacGreen transgenic mice in which macrophages fluoresce green, Mooney et al. found that macrophages participating in the fibrous encapsulation of cubes of boiled egg whites implanted intraperitoneally accounted for 80% of the cells expressing α -smooth muscle actin (α SMA), a typical fibroblast marker, and further suggested that these cells should be classified as “fibroblastoid” macrophages.²⁹ Still, the current state of knowledge is that fibroblasts are the main producers of ECM, with macrophages playing a mostly regulatory role.³⁶ In summary, macrophages are indispensable for formation of the fibrous capsule, but their exact functions are still being elucidated.

2.1.4. Other Innate Immune Cells. Mast cells, another type of innate immune cell that is critical for wound healing, have been sparsely investigated for their effects on the FBR. Mast cells are distributed throughout connective tissues and in close proximity with blood vessels. Their activation results in release of their α -granules, stimulating recruitment of neutrophils and macrophages. Farrugia et al. investigated the infiltration of mast cells into chitosan or cellulose sponges subcutaneously implanted in rats.³⁷ Immunohistochemical analysis of mast cell markers confirmed their presence around blood vessels and within the fibrous capsule, depending on the material type. However, other studies have questioned the importance of mast cells in the FBR because the fibrous capsules surrounding PEG–PLGA, polyetherurethane (PEU), or polyethylene teraphthalate (PET) implants were unaffected in mast cell-deficient mice.^{38,39} Similarly, natural killer (NK) cells, another innate immune cell that is important in wound healing, appear to be dispensable for the FBR,³⁹ although this effect has not been widely studied.

It is also not clear to what extent dendritic cells (DCs) play a role in the FBR. DCs are considered the messengers between the innate and adaptive immune systems. One study found no evidence of DC presence in the fibrous capsules surrounding nylon meshes implanted subcutaneously in mice,⁴⁰ while another showed that DCs were present in the fibrous capsule surrounding ECM scaffolds implanted subcutaneously in mice.⁴¹ Another study retrieved DCs from PVA sponges implanted subcutaneously in rats, finding that these cells accounted for from 5% to 38% of total inflammatory cells within the sponge and that their capacity to promote T cell activation decreased over time of implantation.⁴² It may be that DCs do not typically reside in fibrous capsules but instead interact with biomaterials and then migrate to lymph nodes, where they activate T cells, which further influence the FBR (for review of DC–biomaterial interactions, see refs 43 and 44). This process is the main reason why DCs are considered detrimental for the survival of allogenic or xenogenic cell-based therapies.⁴⁵ Biomaterial-mediated modulation of DC behavior is an active area of investigation for immunotherapies targeting the adaptive immune system such as vaccines and cancer treatments.^{46,47} Collectively, these studies suggest that several innate immune cells that are clearly involved in wound healing may be less important in the fibrous encapsulation process of the FBR.

2.1.5. Adaptive Immune Cells. Lymphocytes, including T cells and B cells, are part of the adaptive immune system and

provide delayed and specific responses to invading pathogens. Their role in the FBR is a subject of debate. Some reports show that adaptive immune cells contribute to the formation of fibrous capsules, while others report minimal effects. For example, Rodriguez and colleagues investigated the FBR to elastane 80A (PEU), silicone rubber, and PET samples implanted subcutaneously in nude (T cell-deficient) Balb/c mice.⁴⁸ Leukocyte recruitment, FBGC formation, and cytokine levels were similar in immunocompetent and nude mice, although fibrous capsules were not measured. Doloff et al. used mouse models with more fine-tuned control of immune cell depletion and found that fibrosis around intraperitoneally implanted alginate microparticles was reduced in mice pharmacologically or genetically depleted of B cells, but this effect was eliminated in mice genetically depleted of both B cells and T cells, possibly because of a loss of regulatory T cells.²⁴ However, when macrophage function was further impacted in mice without B cells and T cells, as in the Rag2 $^{-/-}$ mice, fibrosis was greatly reduced again. These results implicate B and T cells in the FBR via their crosstalk with macrophages. Sadler and colleagues showed that the immune cell profile in response to ECM scaffolds differed in Rag1 $^{-/-}$ mice, which are also deficient for B and T cells, compared to wild-type mice.⁴¹ ECM scaffolds injected into Rag1 $^{-/-}$ mice recruited higher levels of macrophages (CD11b $^{+}$ F4/80 $^{+}$) within 1 week. Here it may be important to note that the ECM scaffolds were derived from porcine tissue, and as such may be more likely to induce adaptive immune cell activation than synthetic materials would be. Collectively, these studies suggest that the role of adaptive immune cells in the FBR is mediated through actions on macrophages, but further investigation in this area is needed and could provide new insights into how biomaterials can be tailored to control the FBR.

Studies evaluating explanted biomaterials from human clinical trials implicate an active role of the adaptive immune system in the FBR in humans. For instance, Chung et al. investigated the cell population surrounding breast implants removed from 12 female patients, ages ranging from 41 to 70 years.⁴⁹ The group identified a strong presence of CD3 $^{+}$ T cells and CD4 $^{+}$ T cells, both marked by high secretion of interleukin 17 (IL17). Gene analysis revealed a positive correlation between expression of the gene encoding IL17 and those that encode collagen type I alpha 1 chain (COL1A1), collagen type III alpha 1 chain (COL3A1), transforming growth factor-beta (TGFB1), and signal transducer and activators of transcription 3 (STAT3) expression. The COL1A1/COL3A1 ratio and TGFB β signaling are associated with a fibrotic environment, indicating a role for IL17-secreting T cells in the FBR around breast implants. In another study, Nadol and colleagues evaluated the cell population around cochlear implants of five patients, with average implant duration of around 12 years.⁵⁰ The group reported a noticeable presence of B cells and T cells, marked by CD20 and CD3 staining, respectively. In addition, the group also identified the presence of FBGCs in the interface between the implant and fibrous capsule. In summary, there is clearly a role for B and T cells in the FBR, especially in humans, but their precise roles remain poorly understood.

2.2. Effect of Biomaterial Properties on the FBR

Given the importance of the FBR for dictating the success or failure of implanted biomaterials, many studies have investigated how polymer chemistry, microstructure, surface

Table 1. Studies that Investigated the Effects of Structural or Surface Modifications of Biomaterials on Minimizing the FBR^a

type	animal model	polymer	modification	outcome	ref
molded substrate	Sprague-Dawley rats subcutaneous	silicone	surface modification	Ta–Si implant significantly reduced fibrous capsule thickness compared to bare silicone	66
films	BALB/c mice subcutaneous	PP	surface modification	–CF and –COOH reduced fibrous capsule thickness compared to –OH and –NH ₂	67
molded substrate	goats subcutaneous	PLA/silicone	topography	compared to smooth control, microgrooves promoted thicker capsules, while roughened surfaces reduced fibrous capsule thickness	68
accaffolds	Sprague-Dawley rats subcutaneous	PCL	topography	aligned fibers reduced fibrous capsule thickness compared to random fibers group and film control	69
molded substrate	Swiss Webster mice subcutaneous	PTFE	topography	substrates with greatest internodal distance reduced fibrous capsule thickness	70
hydrogel membrane	Sprague-Dawley rats intraperitoneally minipig intramuscular	PLL/PEG	surface coating	PEG-coated PLL particles reduced inflammatory response and fibrous capsule formation compared to uncoated PLL particles	71
medical grade silicone	Sprague-Dawley rats subcutaneous	cellulose	surface coating	cellulose-coated pacemakers reduced fibrous capsule thickness compared to uncoated controls	72
medical grade silicone	Sprague-Dawley rats subcutaneous	silk/silicone	surface coating	silk-coated implants reduced fibrous capsule thickness compared to uncoated controls	73
		PMPC/silicone	surface coating	PMPC-coated group reduced fibrous capsule thickness compared to uncoated controls	74

^aPP = polypropylene. PLA = poly(lactic acid). PCL = poly(ϵ -caprolactone). PTFE = poly(tetrafluoroethylene). PEG = poly(ethylene glycol). PLL = poly(L-lysine). PMPC = poly(2-methacryloyloxyethyl phosphorylcholine).

modifications, coatings, and topography of implants affect the FBR. In general, there are two main and very different goals behind decreasing fibrous encapsulation: (1) to minimize interactions with surrounding tissue, which would be desirable for removable biomaterial implants like sensors, and (2) to increase neovascularization and tissue integration, which is important for biomaterials intended to promote tissue repair (Figure 2).

2.2.1. Minimizing the FBR. The initial choice of polymer for the preparation of biomaterials has a profound impact on the ensuing FBR upon implantation. The effects of polymer chemistry were first described in detail by Anderson and colleagues in the 1980s.^{51–53} Recently, Rostam and colleagues designed a polymer library to investigate how polymer chemistry influences macrophage behavior in vitro and the FBR in vivo in a murine model.⁵⁴ The authors used machine learning to relate chemical descriptors of polymer structure to markers of macrophage inflammatory behavior. They found that alkoxy groups and methacrylamide molecular fragments were strongly linked to macrophage behavior, although no clear trends could be identified to identify how specific chemical functional groups influenced macrophage behaviors. Instead, the effects of the polymers on macrophages were linked to thickness of protein adsorption, with polymers that adsorbed thicker protein layers causing pro-inflammatory behavior and polymers that adsorbed thinner protein layers causing anti-inflammatory behavior. Interestingly, polymers that promoted strong pro-inflammatory or strong anti-inflammatory behavior of macrophages in vitro were equally encapsulated in thick fibrous capsules when implanted subcutaneously in a mouse model, while polymers that had more neutral effects on macrophages in vitro caused thinner fibrous capsules. This study illustrates that diverse polymer chemistries influence the FBR via effects on immune cell behavior, which may be partially predicted using in vitro studies of protein adsorption and macrophage response.

With respect to modification of biomaterials to inhibit the FBR, the general approach has been to decrease protein adsorption via coating with nonfouling polymers to minimize interactions with surrounding tissue, as previously described in section 2.1.1. On the other hand, coating biomaterials with

poly(ethylene glycol) (PEG), which generally decreases protein adsorption, has resulted in mixed effects, with some reporting decreased fibrous capsule thickness^{55,56} and others reporting increased fibrous capsules⁵⁷ or no effects.⁵⁸ It is relevant to note that PEG may also degrade in vivo, which would inhibit its ability to evade opsonization over time.⁵⁹ Moreover, PEGylated surfaces may trigger the formation of anti-PEG antibodies, which would further hinder any non-fouling capabilities,^{60–62} although this phenomenon is still poorly understood. For a thorough review on the use of PEG to modulate fibrous capsule formation, see ref 63.

Studies have shown that fibrous capsule formation can be reduced through physicochemical alterations of implants, including chemical and topographical modifications and polymer coatings, ultimately resulting in more inert biomaterials (Table 1). Other strategies to decrease fibrous encapsulation via promoting integration with surrounding tissue, which are generally applied to macroporous biomaterials, will be discussed in detail in section 2.2.2.

Some biomaterials have been discovered that result in such low levels of fibrous capsule formation that they appear nearly inert. Veiseh et al. showed that relatively simple design considerations such as implant shape and size have strong effects on the FBR.²⁵ They implanted alginate hydrogel spheres intraperitoneally in mice using eight groups with diameters ranging from 0.3 mm to 1.9 mm. Increasing sphere size resulted in reduced cell adhesion and formation of fibrous tissue, as measured by staining for α -SMA and F-actin and gene expression analysis of fibrosis-associated genes. Remarkably, this effect held true for a wide range of biomaterials, including hydrogels, ceramics, metals, and plastics, as well as across implantation sites, long time points (up to 6 months in mice), and animal models (including nonhuman primates).

Another approach sought to discover polymer modifications that reduced the FBR via a high throughput, unbiased screen. Vegas et al. created a library of 774 chemical modifications to alginate hydrogels that were 1.5 mm in diameter as described in the previous study, implanted them subcutaneously in mice and used a fluorescent probe for cathepsin activity to identify those that caused the least activity of this inflammatory marker.⁶⁴ The top 10 performers were then implanted

subcutaneously and intraperitoneally in mice for 14 days and analyzed for cell adhesion and fibrous capsule thickness, finally yielding three analogues that resulted in minimal cell adhesion and collagen deposition. The three candidates, all of which contained triazole groups, were further tested in primates for six months. All three modified alginates performed significantly better compared to unmodified alginate controls and had minimal fibrous capsule formation over six months, as demonstrated by insignificant levels of α -SMA and collagen deposition, common indicators of the FBR. In corroboration with this study, Liu et al. demonstrated that triazole-containing PHEMA hydrogels decreased collagen deposition when subcutaneously implanted in mice compared to controls.¹⁶

Other researchers have taken advantage of naturally occurring polymers to develop less reactive materials. For example, Yan and colleagues cross-linked the natural mucus biopolymer that covers the epithelium into hydrogels through the addition of tetrazine and norbornene.⁶⁵ The mucin hydrogels were surrounded by much lower collagen and cellular content compared to alginate hydrogels after 14 and 21 days of intraperitoneal implantation in mice.

2.2.2. Biomaterial–Tissue Integration. While the end result of inhibiting fibrous encapsulation may be the development of relatively more inert biomaterials, the goal of other strategies is to promote biomaterial–tissue integration. In general, porous biomaterials show lower levels of fibrous encapsulation and higher levels of implant integration compared to nonporous biomaterials,^{70,75,76} with larger pore sizes (~ 5 – $100\ \mu\text{m}$ and higher) allowing for higher levels of cell infiltration and expansion of neovascular networks.^{77–79} Adjustments to pore morphology, including size and shape, and microstructure also affect the balance between fibrous encapsulation and tissue integration. Madden et al. demonstrated the importance of pore size of microtemplated poly(2-hydroxyethyl methacrylate-*co*-methacrylic acid) (pHEMA-*co*-MMA) hydrogels when implanted in cardiac tissue.⁷⁶ Hydrogels with pores of 30 or $60\ \mu\text{m}$ in diameter allowed for more neovascularization after 4 weeks compared to nonporous hydrogels and those with $20\ \mu\text{m}$ pores. Interestingly, fibrous capsules were thicker around nonporous hydrogels and those with $60\ \mu\text{m}$ pores compared to hydrogels with 20 or $30\ \mu\text{m}$ pores, showing that neovascularization and fibrous encapsulation can occur concurrently. However, these results may depend on the specific application, the site of implantation, type of polymer, and animal model.^{80–82} Newly developed techniques have allowed for more precise and controlled mechanisms to control the architecture of porous structures. For example, Thorson and colleagues used a PEG-based hydrogel with a highly organized and interconnected porous structure created through bicontinuous interfacially jammed emulsion jet (bijels) to evaluate tissue integration compared to other hydrogels.⁸³ When implanted subcutaneously in athymic mice, these bijels PEG hydrogels were better vascularized and integrated than particle-templated and nontemplated PEG hydrogels.

Various surface modifications have also been shown to decrease fibrous capsule formation while increasing biomaterial–tissue integration. Among these modifications are the addition of functional groups to alter surface properties such as ionic charge and wettability,⁸⁴ as well as coating with other polymers⁵⁶ or bioactive moieties to mimic the natural tissue environment.⁸⁵ For example, the incorporation of RGD or other ECM- or growth factor-derived peptides decreases

fibrous encapsulation,^{20,86–88} probably through as-yet unknown effects on the inflammatory response.

The work mentioned within this session exemplifies that physicochemical modifications to biomaterials can not only hinder fibrous capsule formation, but also guide the cells involved in this process to promote integration with surrounding tissues. With increasing understanding of how inflammatory cells modulate tissue repair, it has become possible to direct their behavior for the enhancement of biomaterial-tissue integration, which will be discussed in more detail in section 3.

2.2.3. Strategies to Increase the FBR. A final consideration to discussion of the FBR, prior to moving on to strategies designed to actively promote biomaterial–tissue integration via modulation of the inflammatory response, is that some biomaterials are actually designed to augment the FBR for specific medical applications. For example, biomaterials and their ensuing fibrous capsules are used as embolic agents for the treatments of cardiovascular aneurysms, thinning of the blood vessel wall, which if ruptured would cause hemorrhage and stroke. Jung et al. investigated the usage of PVA particles as such an embolic agent in canine models with normal renal segmental arteries and aneurysms of the carotid wall.⁸⁹ PVA polymer coils were prepared by cross-linking them with tantalum particles. Complete occlusion of the segmental arteries occurred in all animals on day one and in 75% of animals by 4 weeks. Researchers noted increasing numbers of inflammatory cells and progressive thrombus formation over time.

A similar strategy is used for the treatment of uterine fibroids, or leiomyomata, which are benign uterine tumors that cause significant morbidity in the United States. A common treatment is the use of polymeric microparticles (less than 1 mm in diameter) of the uterine arteries in order to block the blood supply to the tumors, with the primary mechanism being the FBR.⁹⁰ These microparticles can be synthetic (PVA) or naturally derived (gelatin). PVA is not degradable, so this effect is considered to be permanent. No significant differences were noted in the safety or effectiveness of these different materials in a study of 100 patients over three months.⁹¹ In a five-year follow-up study of 200 patients, 73% of patients treated with PVA occlusion reported symptom control.⁹² Histologic studies of embolized uterine arteries removed for reasons other than complications due to embolization have noted long-term FBR, including the presence of macrophages and FBGCs, with no adverse effects on surrounding tissue.^{93,94} However, in a study of eight patients presenting with complications believed to result from the PVA embolization, evidence of necrosis of nearby endometrial tissue was found in five of the eight cases.⁹³

Others have even leveraged the FBR to enhance tissue regeneration. For example, the healing of nonunion bone fractures can be augmented by first implanting a PMMA block in the bone until a fibrous capsule forms and then replacing the block with a bone autograft, in a surgical procedure known as the Masquelet technique.⁹⁵ As another example, researchers have shown that the fibrous tissue that forms around a polymeric tube implanted in the abdomen can be surgically removed and used successfully as an autologous blood vessel graft in animal models.⁹⁶ Rothuizen et al. implanted poly(ethylene oxide terephthalate) (PEOT) and poly(butylene terephthalate) (PBT) rods, with dimensions similar to the carotid artery, subcutaneously in pigs for 4 weeks.⁹⁷ Rods,

together with surrounding fibrous capsules, were explanted and then implanted as vascular grafts in the carotid artery. Four weeks after vascular implantation, the grafts did not fail and underwent thorough alterations at the cellular and protein levels. Collagen, α SMA and desmin significantly increased and macrophage content significantly decreased post grafting, and a heavy capillary network was formed.

In summary, polymeric biomaterials typically elicit the FBR, which can be modulated to be minimal or robust as desired through chemical and structural modifications.

3. DESIGN OF BIOMATERIALS TO ENHANCE TISSUE INTEGRATION AND REPAIR

Many studies have shown that inhibiting the FBR can lead to more inert biomaterials with minimal interactions with surrounding tissue. This outcome is ideal for removable biomaterials like sensors, but it is not desirable for biomaterials that need to integrate with surrounding tissue, as is the case for all biomaterials intended to support tissue repair in injuries that would otherwise fail to heal on their own. For these biomaterials, success is measured in terms of cellular infiltration, neovascularization, and the development of functional tissue. Successful biomaterials tend to promote an inflammatory response that is reminiscent of the normal wound healing process, with beneficial effects like increased cellular infiltration and vascularization and reduced fibrosis compared to injuries treated without biomaterials. To understand how biomaterials can be designed to promote such a beneficial inflammatory response, it is helpful to first review how the inflammatory response proceeds in normal wound healing.

3.1. Inflammatory Response in Wound Healing

Any tissue injury initiates inflammation, which triggers the wound healing process. The recruitment of inflammatory cells is induced in response to damage associated molecular patterns (DAMPs, or alarmins) released from damaged cells and pathogen-associated molecular patterns (PAMPs) released from microbes.⁹⁸ The first step of inflammation is protein adsorption, followed by platelet adhesion, activation, and initiation of the blood-clotting cascade. A provisional fibrin matrix forms in the injury site, providing a scaffold for the infiltration of cells. Platelets release a multitude of growth factors and cytokines that activate and recruit inflammatory cells to the injury site.⁹⁹ Neutrophils are the first cells that are recruited, where they clear the wound of bacteria and foreign particles via enzymes and reactive oxygen species and by phagocytosis.¹⁰⁰ Neutrophil activation stimulates the release of monocyte-recruiting signals to the injury site. After 1–3 days, monocytes arrive at the site of injury and differentiate into macrophages, which are crucial for coordinating both early and late events in the wound healing cascade.¹⁰⁰ Neutrophils undergo apoptosis and are removed via phagocytosis by infiltrating macrophages, a process called efferocytosis that serves to regulate macrophage behavior.¹⁰¹

Macrophages are major regulators of all stages of tissue repair. During the normal healing process, distinct macrophage phenotypes emerge in at least two sequential phases. The initial phase is marked by the presence of mostly pro-inflammatory macrophages, whereas the second stage is characterized by macrophages with a distinct pro-regenerative phenotype.^{102,103} Injured tissues that fail to undergo normal repair are typically characterized by an impaired transition of

macrophages from pro-inflammatory to pro-regenerative, which has been demonstrated across numerous tissue types.^{104–106}

Pro-inflammatory macrophages are often referred to as classically activated or M1, while the second population of macrophages are referred to as alternatively activated or M2, although there is considerable debate over this nomenclature (for more information see Murray et al.¹⁰⁷ and Spiller and Koh¹⁰⁸). In addition, it is now known that the M2 population in particular is quite diverse, with numerous physiologically relevant stimuli leading to phenotypically distinct behaviors, including those induced by interleukin-4 (IL4), interleukin-10 (IL10), and efferocytosis of apoptotic neutrophils.^{109,110} Moreover, M2-type macrophages may derive from the infiltration of newly arriving monocytes at later stages of wound healing, or they may derive from M1 macrophage repolarization.^{108,111} Finally, while most macrophages within an injury site are monocyte-derived, prenatally derived tissue-resident macrophages may play important roles at the early stages of the response to injury by recruiting monocytes and initiating inflammation.¹¹²

Macrophages are critical regulators of angiogenesis. While depletion of macrophages from wounds hinders angiogenesis,¹¹³ exogenous addition of macrophages promotes angiogenesis.^{110,114} However, the angiogenic effects of the dynamic changes in macrophage phenotype that normally occur over time are poorly understood. Recently, Graney et al. used a tissue-engineered model of human blood vessel formation *in vitro* to interrogate macrophage–blood vessel interactions.¹¹⁰ First, they showed that coculture with M1 macrophages caused endothelial cells to upregulate genes associated with the early stages of angiogenesis, such as sprouting, while multiple M2-type phenotypes caused endothelial cells to upregulate genes associated with later stages of angiogenesis, such as regulation of pericyte differentiation. Then they demonstrated that M1 macrophages stimulated angiogenesis in a three-dimensional (3D) triculture model of human blood vessel network formation, but only if their presence was short-term (1 day). These results, in combination with numerous other studies, suggest that M1 and M2 type macrophages act sequentially to regulate angiogenesis (for review see, ref 115). With respect to other aspects of tissue repair, recent studies have suggested that M2-type macrophages, as well as hybrid M1/M2 macrophages, appear to promote tissue deposition by fibroblasts.¹¹⁶ While the detailed mechanisms regulating macrophage functions during tissue healing are still being elucidated, their critical roles in both the response to biomaterials and in tissue repair marks them as a primary target when designing biomaterials for regenerative medicine.

Because of the importance of macrophages for wound healing and angiogenesis, they are by far the most targeted immune cell for immunomodulatory biomaterials designed to enhance tissue repair. Thus, the remainder of this review will focus on the design of biomaterials to manipulate macrophage behavior, although it is acknowledged that biomaterials that target T cell behavior are an exciting area of future direction.¹¹⁷

3.2. Biomaterials that Manipulate Macrophage Behavior

Design strategies for promoting tissue regeneration via immunomodulation are progressing in conjunction with understanding of the crosstalk between the immune components, stem/progenitor cells, and other cells involved

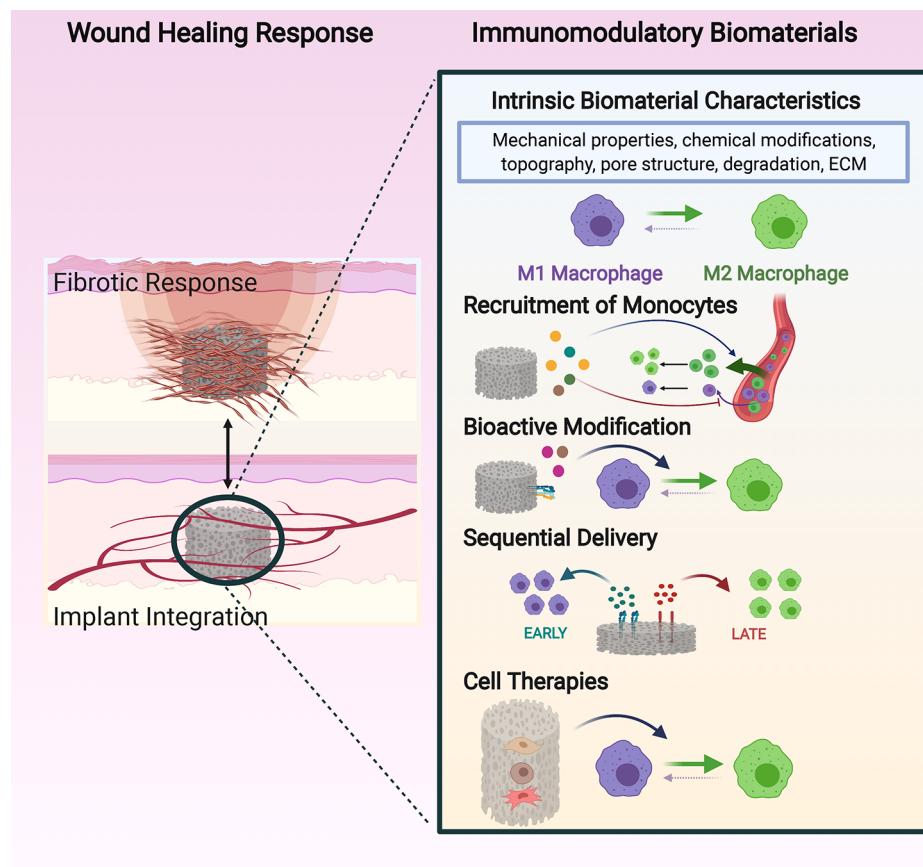


Figure 3. Design strategies for immunomodulatory biomaterials that achieve implant integration. Most strategies focus on manipulation of biomaterial-intrinsic properties, the addition of bioactive factors that mediate recruitment, and/or the M1-to-M2 transition of macrophages, or the inclusion of immunomodulatory cell types.

in the tissue healing process, such as endothelial cells and fibroblasts. Injuries characterized by defective healing, such as chronic wounds and large traumatic tissue defects, are distinguished by impairment in the M1-to-M2 transition (for review, see ref 118). Thus, many studies have been directed toward understanding how biomaterial properties affect macrophage phenotype, often with a focus on promoting M2 activation. Extending these findings, strategies to actively control immune cell behavior in order to promote biomaterial–tissue integration and tissue repair/regeneration generally focus on enhancing recruitment of M2-biased monocytes/macrophages, stimulating M2 phenotypes, and/or sequentially promoting M1 followed by M2. These effects have been achieved through manipulation of biomaterial properties, the addition of bioactive proteins or drugs, or the inclusion of immunomodulatory cell types (Figure 3).

3.2.1. Effects of Biomaterial Properties on Macrophage Polarization. Although it has long been known that macrophage behavior is critical for determining the success or failure of implanted biomaterials (for review, see ref 51), Badylak and colleagues were the first to introduce the importance of the M1/M2 paradigm to the biomaterials community.¹¹⁹ In a series of studies characterizing macrophages surrounding biomaterials with varying levels of success at being “constructively remodeled,” it was concluded that higher ratios of M2:M1 markers were predictive of a successful outcome.^{119–121} Together with the well-characterized importance of M2 macrophages in wound healing, these studies spurred a new subfield of biomaterials research focused on

promoting the M2 phenotype. However, it is important to remember that M2 macrophages can be detrimental for healing and that M1 macrophages are also critical for healing, as described above. Indeed, many studies have found higher M2:M1 ratios to be associated with thicker fibrous capsules surrounding biomaterials,^{122–124} which may be linked to pro-fibrotic processes by M2-like macrophages (for review, see ref 125). For these reasons, it is essential to understand the effect of biophysical clues such as biomaterial stiffness, pore structure, degradation, and incorporation of natural biomaterials on macrophage phenotype and how these factors influence tissue repair (Table 2).

3.2.1.1. Biomaterial Stiffness. Cells are able to sense physical properties of the surrounding biomaterial and respond accordingly, in a process called mechanotransduction. For example, macrophages are able to sense the deformation created by fibroblast contraction in fibrillar collagen matrices, migrating toward the source from a distance, in an $\alpha_2\beta_1$ integrin-dependent process.¹²⁶ Sridharan et al. showed that macrophage migration speed was dependent on substrate stiffness and was related to the migration mode adapted by macrophages and also to macrophage phenotype, with stiffer polyacrylamide gels (323 kPa) promoting slower migration and priming macrophages toward a pro-inflammatory (M1) phenotype compared to softer gels (11 and 88 kPa) that promoted faster migration and primed macrophages toward an M2-like phenotype (Figure 4A).¹²⁷ Similarly, other studies have also shown that stiffer substrates lead to more M1 activation^{127–130} and that M2 macrophages are more

Table 2. Representative Studies Illustrating How Biomaterial Properties Affect Macrophage Polarization^a

strategy	biomaterial	in vivo/in vitro	ref
stiffness	PA	in vitro: macrophages isolated from the femurs of C57BL/6 and TLR4-deficient mice in vitro: THP-1 cell line in vitro: THP-1 cell line in vivo: subcutaneous implantation in rats	128 127 129 134
stiffness	PA agarose	stiff gels increased M1 markers, while soft and medium stiffness gels increased M2 markers soft substrates increased M1 markers, while soft substrates increased M2 markers	136
stiffness	PUU-POSS	caffolds with dynamically decreasing stiffness increased vascularization and both M1 and M2 macrophage markers	153
enantiomer	PPFU/PLL/PDL	PPFU-PDL increased M2 markers compared to PPFU-PLL	154
sulfonation	PEEK	further sulfonation of PEEK increased M2 markers and decreased M1 markers	155
sulfonation	collagen/HAp	the matrix containing high-sulfated hyaluronan decreased M1 markers and increased M2 markers	156
biological coating	PE/PRP/MVF	PRP/MVF-coated and PRP-coated PE promoted M2 markers and accelerated vascularization	156
surface roughness	PDMS	micropatterned surfaces increased macrophage elongation, which enhanced M2 markers and inhibited M1 markers	140
surface roughness	PFPE	a microstructure of regular grooves and smaller posts with a shorter distance increased M1 marker; larger cylindrical posts increased M2 markers	144
surface roughness	PCL	nonmicrochamneled scaffolds increased M1 markers. Microchamneled scaffold increased M2 markers	156
pore size	PDO	increasing fiber/pore size increased M2 markers and decreased M1 markers	135
pore size	PCL Gelatin	scaffolds with smaller pores ($40\ \mu\text{m}$) and box shaped pores increased M2 macrophages markers small and soft pores increased M1 markers; larger and stiffer pores increased M2 markers	142 132
pore size	pHEMA-co-MAA	40 and $80\ \mu\text{m}$ pore size increased both M1 and M2 markers and vascularization	157
pore size	PCL	thicker fiber scaffolds increased M2 markers; thinner fiber scaffolds increased M1 markers	158
pore size	PLA/calcium phosphate glass/chitosan P(LLA-CL)	chitosan increased M1 markers; PLA increased M2 markers	159
fiber alignment	PLLA	aligned nanofibers increased M2 markers; random nanofibers increased M1 markers	160
fiber alignment	sP(EO-stat-PO)/PLGA	nanofibers scaffolds decreased the M1 response compared to films and microfibrous PLLA scaffolds	161
3D VS 2D	MAA-PEG	2D substrates enhanced M2 specific markers but also stimulated pro-inflammatory cytokine secretion (IL1 β , TNF α); 3D substrates promoted M1 specific markers, but higher release of pro-healing cytokines (IL8, CCL4)	162
different degradation rates	PGA	Degradation fast degrading group increased Arg1 (M2 marker), while slow group increasedFizz1 (another M2 marker), also decreasing M1 markers	163
structural differences		fast degrading increased M1 markers; slow degrading increased M2 markers	145
formulation concentration	dECM	ECM Properties dECM particles increased M1 markers, while gels increased M2 markers	164
tissue origin	dECM	5 mg/mL increased M2 markers compared to lower and higher concentrations	165
	scaffolds prepared from intestinal submucosa, UBM, brain ECM, esophageal ECM, and colonic ECM increased M2 markers; dermal ECM increased M1 markers; skeletal muscle did not change expression of M1 or M2 markers	149	

strategy	biomaterial	in vivo/in vitro	ECM Properties	outcome
synthetic vs ECM	PE/PEG/ECM	in vivo: volumetric muscle loss model of C57BL/6	ECM increased M2 markers compared to synthetic scaffolds	ref 148
synthetic vs ECM	PCL/UBM	in vivo: muscle injury in mice	UBM scaffolds upregulated M2 markers; pCL-induced fibrosis was driven by IL17	ref 152
dECM vs collagen I	collagen I/dECM	critical size calvarial defect model	dECM increased M2 markers	ref 166

^aHydroxyapatite (HAp), poly(urea-urethane) of terminated polyhedral oligomeric silsesquioxane (POSS), polyacrylamide (PA), poly(propylene fumarate) polyurethane (PPFU), poly-L-lysine (PLL), poly-D-lysine (PDL), polyethylene (PE), tissue-derived microvascular fragments (MVF), platelet-rich plasma (PRP), polydimethylsiloxane (PDMS), perfluoropolyether (PFPE), polydioxanone (PDO), polycaprolactone (PCL), poly(L-lactic acid-co-ε-caprolactone) (PLLA-CL), poly(L-lactic) (PLLA), polyetheretherketone (PEEK), poly(lactic acid (PLA), poly(l,L-lactide-co-glycolide) (PLGA), methacrylic acid (MAA), poly(ethylene glycol) (PEG), polyglycolide (PGA), extracellular matrix (ECM), decellularized extracellular matrix (dECM), urinary bladder matrix (UBM).

migratory than M1 macrophages.¹³¹ One study showed that increasing stiffness of PEG hydrogels modified with RGD from 130 to 840 kPa increased M1 related cytokine secretion when stimulated with LPS by a mouse macrophage RAW 264.7 cell line.¹³⁰ When implanted subcutaneously in mice for 4 weeks, stiffer gels were surrounded by thicker layers of macrophages. However, at least one study using porous gelatin hydrogel-based scaffolds reported decreasing M1 marker expression and increasing M2 marker expression with increasing stiffness both in vivo and in vitro,¹³² suggesting that the relationship between macrophage activation and substrate stiffness likely depends on the substrate.

Patel et al. linked substrate-dependent changes in macrophage phenotype to elasticity of the cells themselves.¹³³ First, they showed that pro-inflammatory (M1) activation of macrophages with LPS and IFNg caused their elastic moduli to increase, as measured by optical magnetic twisting cytometry. Then, using soft (1.2 kPa) and stiff (150 kPa) polyacrylamide gels, they found that macrophages cultured on stiffer substrates increased their own elastic moduli and their propensity to phagocytose latex beads but without clear effects on inflammatory cytokine secretion or gene expression. Wu et al. designed porous scaffolds with dynamic stiffness based on poly(urea-urethane) and polyhedral oligomeric silsesquioxane (PUU-POSS) using a 3D printing-guided thermally induced phase separation technique (3D-TIPS).¹³⁴ These scaffolds exhibited stiffness memory properties, with decreasing stiffness over time. When implanted subcutaneously in rats, these scaffolds were better vascularized than scaffolds with uniform stiffness, concomitant with increased numbers of macrophages expressing both M1 and M2 markers. Collectively, these results demonstrate the capacity of implant stiffness to influence macrophage behavior.

3.2.1.2. Pore Structure and Surface Topography. Pore size has also been demonstrated to exert an effect on macrophage behavior and the host immune response in vivo,^{132,135,136} with larger pores generally enhancing expression of M2 phenotype markers.^{137,138} However, one study highlighted the importance of context dependence, reporting that pro-inflammatory macrophage responses to chitosan scaffolds were exacerbated when pore size was increased.¹³⁹

In a recent study, Jiang et al. independently modulated pore size and stiffness of gelatin scaffolds to analyze the effects on macrophage and fibroblast behavior both in vivo and in vitro.¹³² Different concentrations of glutaraldehyde were used to adjust stiffness, while different concentrations of dimethyl sulfoxide (DMSO) were used to regulate pore size by ice crystal formation during cryogelation. They found that increasing pore size from 30 to 80 μm decreased M1 marker expression and increased M2 marker expression by murine macrophages in vitro, with more pronounced effects on softer substrates (20 kPa compared to 70 and 90 kPa). When implanted in cutaneous wounds in mice, softer scaffolds with smaller pores were more infiltrated by macrophages than stiffer scaffolds with larger pores, and the macrophages expressed higher levels of the antigen presentation-related marker MHC-II, although more commonly employed M1 and M2 markers were not assessed. Interestingly, softer scaffolds with larger pores achieved the most pronounced wound closure rate, suggesting that wound healing can be influenced by numerous cellular responses and different biomaterial properties (Figure 4B).¹³²

Table 2. continued

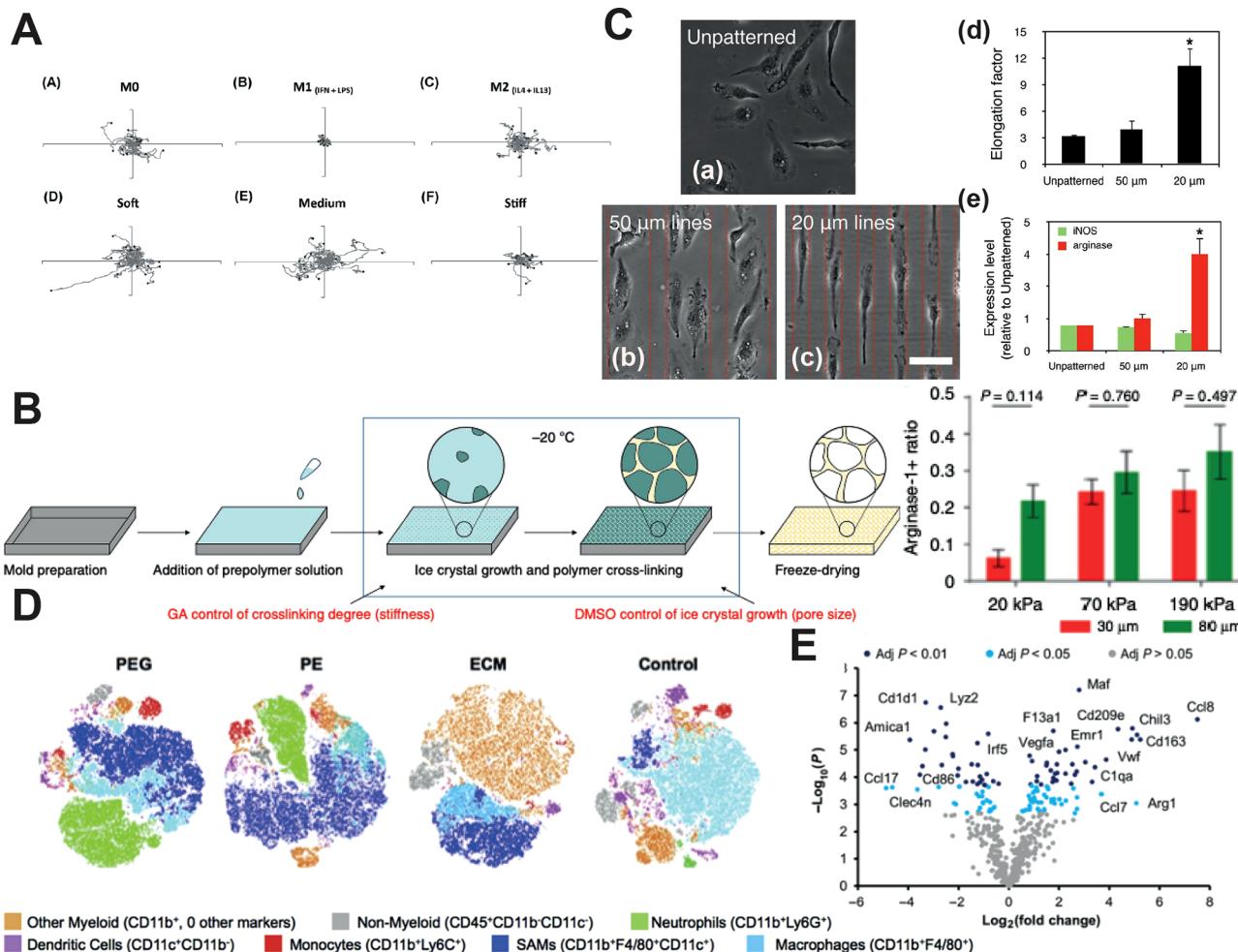


Figure 4. Intrinsic properties of biomaterials affect macrophage phenotype. (A) Macrophage motility is influenced by both phenotype and substrate stiffness. Reproduced with permission from ref 127. Copyright 2019 Elsevier. (B) Gelatin scaffolds were prepared with independent control over pore size and stiffness via the inclusion of cryoprotectant and cross-linkers, respectively. Quantification of macrophages expressing the M2 marker arginase 1. Reproduced with permission from ref 132. Licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0). (C) Micropatterning was used to increase elongation of murine macrophages, which led to upregulation of M2 marker arginase. Reproduced with permission from ref 140. Copyright 2013 U.S. National Academy of Sciences. (D) Multidimensional parameter reduction of a 13-color flow cytometry panel to illustrate the heterogeneity of the immune cells infiltrating ECM and synthetic scaffolds. Reproduced with permission from ref 148. Copyright 2019 Elsevier. (E) Volcano plot of up- and down-regulated genes by macrophages isolated from UBM-treated tumor microenvironment, highlighting their complex phenotype characterized by changes in both M1 and M2 markers compared to saline. Reproduced with permission from ref 147. Copyright 2019 The American Association for the Advancement of Science.

More recent studies have focused on the shape of the pores, following a landmark study that showed that M2 macrophages were more elongated than M1 macrophages and that micropatterning could be used to promote M2 polarization via cell elongation (Figure 4C).¹⁴⁰ Even though this link between morphology and phenotype is true only for murine and not human macrophages at least in 2D culture,¹⁴¹ a recent study showed that primary human macrophages upregulated M2 markers when cultured on pHEMA scaffolds with an elongated pore structure.¹⁴² In addition, another study screened drugs for those that promoted roundness of murine macrophages in order to find M1-promoting drugs for cancer treatment, and the selected drug also promoted M1 activation of human macrophages despite a lack of effect on their morphology.¹⁴³

As with 3D pore structure, macrophages also exhibit sensitivity toward surface topography. Bartneck et al. investigated the influence of different perfluoropolyether

(PFPE) microstructures (lines, large cylindrical post, widespread cylindrical post, small posts, and concentrated small cylindrical posts) on the inflammatory activation of human macrophages. They showed that smaller posts separated by a shorter distance increased expression of M1 markers compared to those with a longer distance, while larger cylindrical posts mostly upregulated M2 markers.¹⁴⁴

3.2.1.3. Degradation. Another biomaterial characteristic that affects the inflammatory response is the degradation rate. For example, Zhang et al. investigated how the degradation rate of polyglycolide (PGA) scaffolds influenced the host response in a mouse subcutaneous model for 6 weeks.¹⁴⁵ Degradation rate was controlled by changing the crystallinity of the polymers via varying the fabrication process of the scaffolds between gas foaming and fiber formation. Fast-degrading scaffolds (50% degradation in 1 week) increased the proportion of macrophages expressing the M1 marker CD86 compared to slow-degrading scaffolds (50% degradation in 2

Table 3. Bioactive Modification of Biomaterials to Promote M2 Macrophages^a

strategy	biomaterial	in vivo/in vitro	effects on macrophage	outcome
delivery of AT-RvDI	PLGA	murine dorsal skinfold window chamber oronasal fistula (ONF) C57BL/6 model	Monocyte Recruitment increased the accumulation Ly6C ^{low} monocytes and increased M2 macrophage markers	increased vascular remodeling; limited neutrophil infiltration 193
delivery of FTY720	PLCL/PLAGA	murine dorsal skinfold window chamber model	increased the accumulation of Ly6C ^{low} monocytes and M2 macrophage markers	increased of ONF healing 194
delivery of FTY720 entrapment of endogenous FKN into the scaffold	PLAGA PEGDA	murine dorsal skinfold window chamber model skin injury mouse model	increased the accumulation Ly6C ^{low} monocytes and increased M2 macrophage markers	increased vascular remodeling 170 195
delivery of FKN	agarose	model of nerve regeneration in rat murine partial thickness skin	increased the accumulation Ly6C ^{low} monocytes and increased M2 macrophage markers	increased in regeneration in the injury site 196
codelivery of FTY720 and SDF-1 α	aHep ^{-N} -PEG-DA	myocardial infarction model	increased the accumulation of Ly6C ^{low} monocytes and increased M2 macrophage markers	increased vascular remodeling 197
recombinant human collagen	rHCl and rHCIII matrices		rHCl reduced the accumulation of Ly6C ^{high} and Ly6C ^{low} monocytes; rHCl promoted cardioncytote survival and less pathological remodeling of the myocardium 198	
viral IL10 delivery	PLG HA-PEI	intraperitoneal fat mice mice intraperitoneal injection	increased M2 macrophage markers increased M2 macrophage markers	decreased inflammation 199
IL10 or IL4 plasmid DNA		subcutaneous implantation in mice rat subcutaneous model large cranial bone defect in rats	increased M2 macrophage markers increased M2 macrophage markers decreased M1 macrophage markers increased M2 macrophage markers	decreased inflammation 200
IL4 cytokine release	dermatan sulfate/chitosan/PP			improved tissue remodeling 201
IL4 cytokine release	collagen/PLGA-MSV DBM			improved wound healing 202
IL4 cytokine release	MHG-MS/heparin-modified gelatin	mandibular periodontal fenestration defect in a diabetic rat	decreased M1 macrophage markers increased M2 macrophage markers	promoted neovascularization and osteogenesis 203
IL4 cytokine release	T/DOP nanotubes and CG layer functionalized with RGD silk	in vitro: RAW 264.7 murine macrophage cell line	increased M2 macrophage markers	enhanced osteoblastic differentiation and bone regeneration 177
IL4 and dexamethasone release antagonir-133a miRNA-21		subcutaneous injections Swiss mice rat calvarian defeat rat model MI rat model of MI	increased M2 macrophage markers increased M2 macrophage markers increased M2 macrophage markers decreased M1 macrophage markers increased M2 macrophage markers	enhanced MSCs osteogenic differentiation 204
nimicking efferocytosis	PS			increased cell infiltration at earlier time points, and promoted M2 macrophage phenotype enhanced bone repair 205
mimicking efferocytosis	PLGA/PS	in vitro: bone marrow-derived mice macrophages	Monocyte Recruitment increased M2 macrophage markers increased M2 macrophage markers	promoted resolution of inflammation and improved cardiac healing 181
MCP-1 and IL4	multidomain peptides self-assembled into b-sheets	subcutaneous implantation in Wistar rats skin wound defect in diabetic mice	increased M2 macrophage markers increased M2 macrophage markers	promoted angiogenesis, preservation of small scars, prevented ventricular dilatation and remodeling 183
SDF-1 and SEV2871	gelatin			reduced NFkB activation 207
				allowed the formation of new blood vessels, as well as promoting an M2 macrophage phenotype enhanced MSCs recruitment and wound closure 167 176

Table 3. continued

strategy	biomaterial	in vivo/in vitro	effects on macrophage	ref
IFN γ (M1) and IL4 (M2)	decellularized bone	murine subcutaneous implantation model in vitro: THP-1 human monocyte line. old mice BM derived macrophages	M1 to M2 Promoting Agents promoted sequential M1 and M2 macrophage polarization promoted sequential M1 and M2 macrophage polarization	108
IFN γ (M1) and simvastatin (M2)	biomimetic calcium phosphate	subcutaneous implantation of C57Bl/6 macrophage line	promoted sequential M1 and M2 macrophage polarization	189
IFN γ (M1) and Si (M2)	CaSiO ₃ -TCP	in vitro: mouse RAW 264.7 macrophage line	promoted sequential M1 and M2 macrophage polarization	191
IFN γ (M1) and IL4 (M2)	CS/CMCS/TNT	in vitro: mouse RAW 264.7 macrophage line	promoted sequential M1 and M2 macrophage polarization	190
IFN γ (M1) and IL4 (M2)	CMCS/TNT	in vitro: mouse RAW 264.7 macrophage line	promoted sequential M1 and M2 macrophage polarization	192
IFN γ MCP-1 (M1) and IL4 (M2)	gelatin	in vitro: mouse RAW 264.7 macrophage line	promoted sequential M1 and M2 macrophage polarization	208
^a Aspirin-triggered resolin D1 (AT-RvD1), poly(D,L-lactide-co-glycolide) (PLGA), oronasal fistula (ONF), polycaprolactone (PCL), poly(lactic-co-glycolic-acid) (PLGA), poly(ethylene glycol) (PEG), fractalkine (FKN), poly(ethylene glycol) diacrylate (PEGDA), heparin desulfated at the -N position (aHep ^{-N}), poly(ethylene glycol) diacrylate (PEG-DA), recombinant human collagen type I (rHCl), recombinant human collagen type III (rHCIII), hyaluronic acid-poly(ethylenimine) (HA-PEI), polypropylene (PP), poly(lactic-co-glycolic acid)-multistage silicon particles (PLGA-MSV), decellularized bone matrix (DBM), nanofibrous heparin modified gelatin microsphere (NHG-MS), poly(dopamine) coated TiO ₂ (T/DOP), carboxymethyl chitosan hydrogel layer (CG), collagen-nanohydroxyapatite (coll-nHA), myocardial infarction (MI), collagen-hydroxyapatite nanoparticles (coll-nHA), phosphatidylserine (PS), monocyte chemoattractant protein-1 (MCP-1), sphingosine-1-phosphate agonist (SEW2871), stromal derived factors (SDF-1), mesenchymal stem cells (MSC), interferon- γ (IFNg), interleukin-4 (IL4), bone marrow (BM), calcium silicate/b-tricalcium phosphate (CaSiO ₃ -b-TCP), titania nanotube (TNT), chitosan (CS), carboxymethyl chitosan (CMCS),		193		

weeks), which were surrounded by macrophages expressing higher levels of the M2 marker CD163. These results corroborated previous findings that faster-degrading poly(lactic acid) (PLA)/PEG scaffolds provoked a more pronounced inflammatory response and thicker fibrous capsules when implanted subcutaneously in rats compared to more slowly degrading formulations.¹⁴⁶ The effects of biomaterial degradation rate on macrophage activation are likely a combination of the degradation byproducts as well as effects on the macrophages' ability to clear the foreign body.

3.2.1.4. ECM Scaffolds. ECM scaffolds, which are prepared by decellularization of human or animal tissue, have been widely investigated for their immunomodulatory properties because of their widespread success at promoting tissue repair and regeneration in animal models and clinically.^{117,147,148} The composition of the ECM scaffolds varies depending on its tissue origin.^{149–151} Dziki et al. cultured murine macrophages on ECM scaffolds derived from eight different tissue sources.¹⁴⁹ They found that macrophages exposed to small intestinal submucosa, urinary bladder matrix (UBM), brain, esophageal, and colonic ECM expressed higher levels of M2 markers and lower levels of M1 markers, while macrophages exposed to dermal ECM expressed higher levels of M1 marker and lower levels of M2 markers, and macrophages exposed to liver ECM and skeletal muscle ECM did not significantly change the expression of these markers.¹⁴⁹ Sadtler and colleagues performed detailed analyses of the immune cell response to ECM scaffolds derived from UBM, bone, or cardiac tissue and found that macrophages upregulated multiple M2 markers compared to macrophages infiltrating collagen or synthetic scaffolds prepared from PEG or polycaprolactone (PCL) (Figure 4D).^{41,117,148} Here it is also important to note than these studies evaluated ECM scaffolds derived from different species (i.e., bovine, and porcine), which could also influence the results. Interestingly, while M2 macrophages are typically believed to promote tumor progression, Wolf et al. showed that UBM scaffolds actually inhibited tumor formation despite promoting increased expression of M2 macrophages markers (Figure 4E).¹⁴⁷ Gene expression analysis of the macrophages showed that macrophages isolated from the UBM-treated tumor microenvironment were phenotypically distinct from traditional M2 cells and from classical tumor-associated macrophages. A recent study used single-cell ribonucleic acid (RNA) sequencing to further analyze macrophages sorted from UBM-treated muscle injuries compared to PCL, which led to a more pro-fibrotic response.¹⁵² The authors found that macrophages infiltrating the ECM scaffolds did upregulate many M2 markers, but also highlighted the limitations of conventional M1/M2 markers for accurately separating pro-regenerative and pro-fibrotic behavior. They found that the PCL-induced fibrosis was driven by IL17 and identified CD9 and CD301b as new cell surface markers to distinguish pro-regenerative from pro-fibrotic macrophages. These studies demonstrate that macrophage phenotypes are complex and caution against drawing broad conclusions about the role of M2 macrophages in tissue regeneration.

3.2.2. Incorporation of Bioactive Factors to Modulate Immune Cell Behavior. Beyond empirical investigations of how changes in biomaterial properties affect macrophage behavior and biomaterial-mediated tissue repair, the incorporation of immunomodulatory cytokines and drugs has contributed a great deal to our understanding of how immune

cell behavior can be harnessed to promote tissue repair. Such immunomodulatory design strategies typically involve enhancing monocyte recruitment, promoting M2 polarization, or sequentially stimulating M1 and M2 activation (Table 3).

3.2.2.1. Biomaterials That Recruit Monocytes to the Site of Injury. Because monocytes are naturally recruited to sites of injury where they differentiate into macrophages, biomaterials that augment this process have been developed. Several studies have shown beneficial effects of biomaterials that enhance monocyte recruitment to a site of injury, especially if the monocytes quickly take on an M2 phenotype. For example, Kumar et al. designed multidomain peptide hydrogels that sequentially delivered MCP-1, to recruit monocytes, followed by IL4, to promote M2 activation of the macrophages derived from those monocytes.¹⁶⁷ The hydrogels released 80% of loaded MCP-1 in the first 2 days, while a much slower release of IL4 occurred over the next 16 days, with these divergent release profiles resulting from differences in diffusivities of MCP-1 and IL4 as well as possible matrix–protein interactions. Subcutaneous implantation in rats confirmed that the sequential cytokine delivery increased the number of infiltrating monocytes and shifted the macrophage population toward M2.

Other studies have taken advantage of the fact that monocytes themselves exist as two different populations in the blood (three in humans), which appear to be biased toward M1 or M2 macrophages as they extravasate into tissues. The so-called classical or inflammatory monocytes are distinguished by the Ly6C^{hi}CX3CR1^{mid}CCR2⁺CD62L⁺CD43^{low}) surface receptor profile (in mice). Nonclassical or alternative monocytes appear to be biased to become M2 macrophages and are distinguished by Ly6C^{low}CX3CR1^{hi}CCR2⁻(CD62L⁻CD43^{high})¹⁶⁸ (for review, see ref 169). However, it is important to remember that classical monocytes can also be converted to nonclassical monocytes, so the terms "M1-biased" and "M2-biased" may not always be accurate.¹⁶⁸ Awojodu et al. showed that M2-biased monocytes can be selectively recruited via the delivery of FTY720, a sphingosine 1-phosphate receptor (SP1) agonist, from PLGA thin films.¹⁷⁰ Implantation into a dorsal skinfold window chamber model in mice showed that the release of FTY720 increased the recruitment of alternative monocytes, resulting in higher numbers of M2 macrophages, lower inflammatory cytokine secretion, and increased microvascular density. This strategy was also shown to be effective at enhancing repair of skeletal muscle¹⁷¹ and bone defects.^{172–174} Similar effects on angiogenesis were achieved by preferentially recruiting alternative monocytes via controlled release of stromal cell-derived factor-1 (SDF-1 α).¹⁷⁵ Kim et al. modified the release profiles of proteins from gelatin hydrogels by changing the isoelectric points of the hydrogels.¹⁷⁶ They incorporated SDF-1 α and SEW2871, an SP1 agonist, which have both been shown in other studies to recruit alternative monocytes as well as other cells. The rapid release of SDF-1 α combined with the sustained release of SEW2871 resulted in higher numbers of macrophages expressing M2 markers and faster wound closure when compared to sustained release of SDF-1 α and rapid release of SEW2871.¹⁷⁶

3.2.2.2. Bioactive Modification with M2-promoting Stimuli. A variety of techniques have been used to actively promote M2 polarization of macrophages responding to biomaterials. Biomaterials that release IL4 and IL10 in particular have been extensively investigated because of the

potent effects of these cytokines on M2 polarization. For example, Hu et al. designed injectable heparin-modified gelatin microspheres to deliver IL4 to macrophages within bone defects under diabetes mellitus (DM) conditions.¹⁷⁷ Because IL4 has heparin-binding domains, the release of IL4 was sustained for 3 weeks *in vitro*.¹⁷⁸ The system was able to increase the presence of macrophages expressing M2 markers and enhanced osteoblastic differentiation and bone regeneration in diabetic rats.

Another strategy to promote M2 polarization is using microRNAs (miRNAs). miRNAs are small (~22 nucleotides) noncoding RNAs that regulate translation of coding RNAs. Some miRNAs have been shown to regulate macrophage polarization and subsequently affect inflammation (for review see refs 179 and 180). A number of studies have determined miRNA expression profiles in M1- and M2-polarized human and murine macrophages using microarray and RT-qPCR arrays techniques, identifying potential targets for therapeutic manipulation. For example, Bejerano et al. presented a new therapeutic strategy to manipulate macrophage phenotype using hyaluronan-sulfate (HS) nanoparticles loaded with miRNA-21, which promotes an M2 phenotype.¹⁸¹ The nanoparticles were delivered intravenously to a murine model of myocardial infarction. The nanoparticles increased M2 polarization of macrophages in the infarcted zone, increased angiogenesis, and improved cardiac outcomes.¹⁸¹

3.2.2.3. Mimicking Efferocytosis. Considering another mechanism by which the M2 phenotype is generated *in vivo* is by efferocytosis, or the uptake of apoptotic neutrophils, some studies have sought to mimic this process using biomaterials. Apoptotic cells display phosphatidylserine (PS), which is a phospholipid typically hidden inside the cell membrane in nonapoptotic cells. The PS acts as an “eat me” signal for the macrophages, and the binding to this receptor triggers an anti-inflammatory phenotype in macrophages characterized by the secretion of IL10, TGF β , and prostaglandins (for review, see ref 101). Huynh et al. were the first to propose the use of PS-containing liposomes to mimic apoptotic cells as a tool to promote the resolution of inflammation.¹⁸² Harel-Adar et al. showed that PS-presenting liposomes promoted M2 polarization of cardiac macrophages after myocardial infarction in rats.¹⁸³ After PS-liposome uptake by macrophages *in vitro* and *in vivo*, macrophages secreted high levels of anti-inflammatory cytokines such as TGF β and IL10 and upregulated the M2 cell surface marker CD206 along with downregulation of proinflammatory markers such as TNF α and the M1 cell surface marker CD86. Finally, PS-liposomes have been shown to decrease inflammatory cytokine production and improve symptoms in a carrageenan-induced model of mouse hindpaw inflammation.¹⁸⁴

3.2.2.4. Biomaterials that Promote Sequential M1 then M2. Even though the precise roles of each population of macrophages in tissue repair is not fully understood, dysfunctional regulation of macrophage phenotype can impede proper healing. The early presence of M1 macrophages is key for the initiation of tissue regeneration, but chronic M1 activity could impede or prevent proper healing.^{104,185} On the other hand, uncontrolled M2 activation could lead to fibrosis.^{186–188} Moreover, M1 and M2 macrophages appear to act sequentially in their regulation of normal angiogenesis and wound healing, and improper activation in either phase is detrimental for healing (for review see ref 115). For these reasons, numerous studies have recently reported the design of biomaterials that

can promote sequential M1 and M2 activation of macrophages.^{108,115,189–192}

One popular strategy to achieve sequential activation of M1 and M2 macrophages is the sequential release of a proinflammatory agent, such as interferon- γ (IFN γ), which induces the M1 phenotype, followed by an M2-promoting cytokine or drug such as IL4 or simvastatin.^{190–192} This idea was first introduced by Spiller et al. using decellularized bone as a scaffold, with M1-promoting IFN γ released by desorption, with subsequent M2-promoting IL4 released via biotin–avidin interactions.¹⁰⁸ This sequential release promoted the M1-to-M2 phenotypical change of primary human macrophages *in vitro* but failed to significantly enhance vascularization of bone scaffolds in a murine subcutaneous implantation model, an effect that was attributed to the overlapping of M1 and M2 phases at early time points. Following up on this study, Li et al. loaded IFN γ into calcium silicate/ β -tricalcium phosphate scaffolds, so that IFN γ release would be followed by release of silicon (Si) ions, which promote M2 polarization.¹⁸⁹ When the scaffolds were implanted subcutaneously in mice, the sequential delivery of IFN γ and Si ions upregulated M1 marker expression on day 3 and M2 marker expression on day 7 and increased the number of infiltrating blood vessels. Along similar lines, Alhamdi et al. designed a calcium phosphate (β CaP) drug delivery system to release IFN γ followed by the M2-promoting drug simvastatin, with release tied to the activity of macrophages, for a potentially self-regulating patient-specific biomaterial designed for bone repair.¹⁹⁰ Sequential M1-to-M2 activation was achieved in both a human monocyte cell line and in bone marrow-derived macrophages obtained from both young and old mice, although the expression of M1 and M2 gene markers was blunted in aged macrophages. Nonetheless, the system showed potential applicability even for elderly patients, whose immune systems are compromised.¹⁹⁰ Externally controlled dual release of macrophage-modulating cytokines was achieved by Tolouei et al., who designed a two-compartment biomaterial system composed of an outer gelatin scaffold and an inner biphasic ferrogel.¹⁹² The outer porous gelatin layer was loaded with the IFN γ and the chemoattractant MCP-1, for rapid yet passive release via diffusion. In contrast, the inner compartment was composed of a biphasic ferrogel with an Fe₃O₄-laden region in the top half and a Fe₃O₄-free, porous, and deformable region on the bottom. In the presence of a gradient magnetic field, the ferrogel physically deformed, releasing loaded IL-4 in a magnetically triggered fashion.¹⁹² Although the bioactivity of this system was not assessed, it could be a promising approach to precisely control the release of macrophage-modulating cytokines, which would be useful for investigations of macrophage timing *in vitro* and *in vivo*.

4. BIOMATERIAL-ENABLED CELL-BASED THERAPIES FOR IMMUNOMODULATION

While the inclusion of macrophage-modulating cytokines has shown considerable promise, others have explored the delivery of cells with immunomodulatory properties because they have the potential to respond to the changes in the local environment. While numerous attempts have been made to deliver macrophages themselves to sites of injury (for review, see¹¹⁸), only recently have biomaterials been employed to support this strategy. For example, Hu et al. used pullulan-collagen hydrogels to deliver supraphysiologic numbers of inactivated macrophages to a murine cutaneous wound model

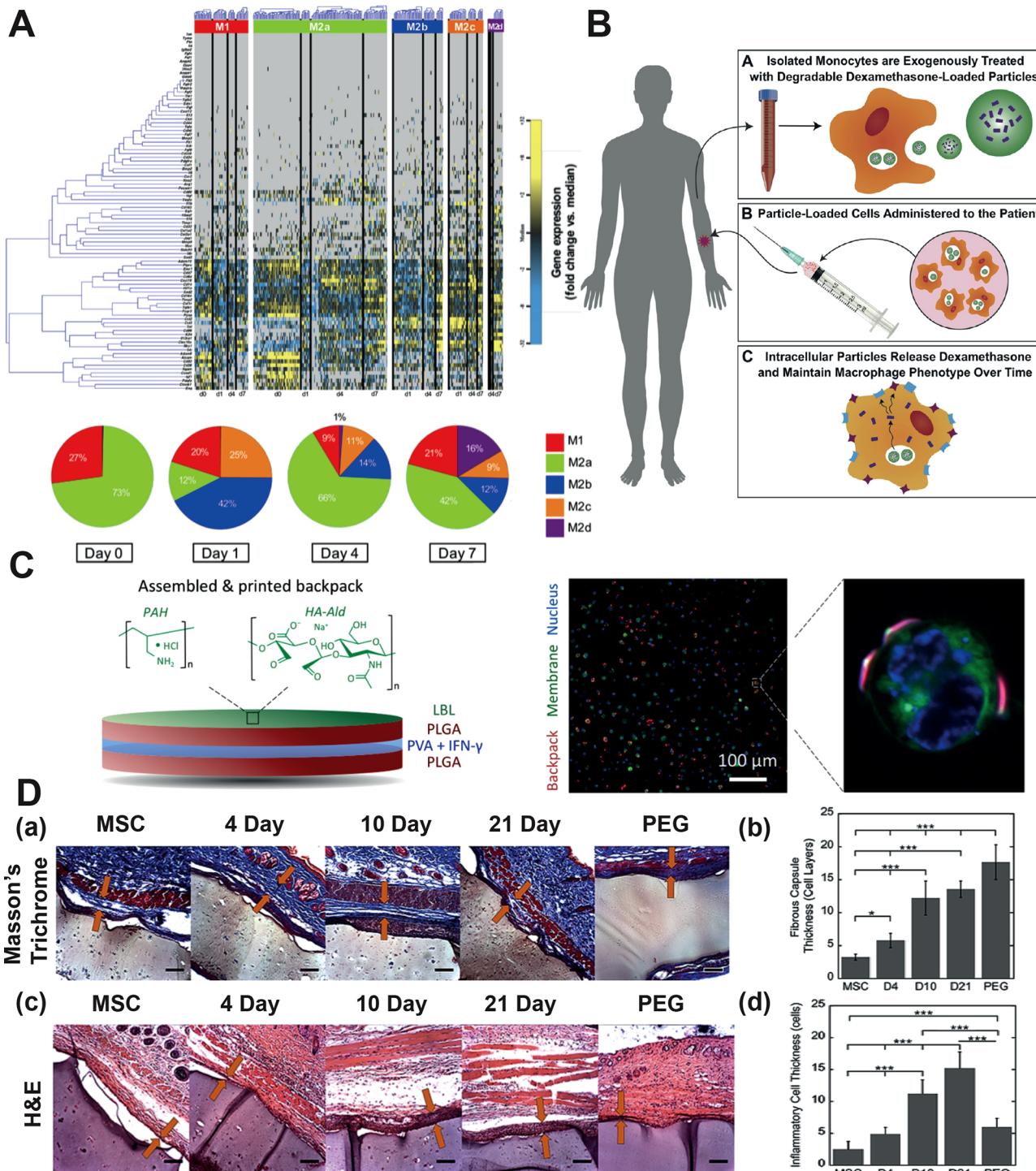


Figure 5. Cell therapies to promote wound healing response. (A) Macrophages delivered within hydrogels to cutaneous wounds took on complex phenotypic profiles. Reproduced and adapted with permission from ref 209. Licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0). (B) Strategy to control the phenotype of monocyte-derived macrophages by loading monocytes with drug-loaded microparticles, which release drug over time as the monocytes differentiate into macrophages. Reproduced with permission from ref 212. Copyright 2020 Elsevier. (C) Cytokine-loaded “backpacks” that adhere to the surface of macrophages and release cytokines to control their phenotype. Reproduced and adapted with permission from ref 213. Licensed under a Creative Commons Attribution-Non Commercial 4.0 International (CC BY-NC 4.0). (D) Ability of MSCs embedded within hydrogels to decrease fibrous capsule formation decreases with stage of differentiation. Reproduced and adapted with permission from ref 223. Copyright 2015 Elsevier.

(Figure 5A).²⁰⁹ The transplanted cells survived for at least 7 days *in vivo*, migrated into the middle and lower dermis, and acquired mixed M1/M2 phenotypes. This treatment accelerated wound healing and angiogenesis. Interestingly, the

authors showed that macrophages from diabetic mice also accelerated diabetic wound healing, and even transplantation of human diabetic monocytes accelerated wound healing in immunocompromised mice, although survival of the trans-

planted cells or their incorporation into the mouse tissue were not assessed.

Despite these encouraging results, the efficacy of monocyte/macrophage-based therapies has been limited due to inability to control the phenotype of exogenously administered macrophages because their high plasticity causes them to take on the phenotype induced by microenvironmental stimuli at the injury site.^{210,211} To address this challenge, Wofford et al. designed a strategy to promote and preserve an anti-inflammatory macrophage phenotype through the use of intracellular microparticles.²¹² In this approach, monocytes would be isolated from patients, incubated with drug-loaded microparticles to allow their uptake by phagocytosis, and then readministered back into the patient, so that the intracellular release of drug can maintain macrophages derived from those monocytes in an anti-inflammatory phenotype over time even in inflammatory environments (Figure 5B).²¹² The authors showed that dexamethasone-loaded PLGA microparticles were retained within monocyte-derived macrophages for several weeks *in vitro*, inhibited expression and secretion of pro-inflammatory cytokines even in the presence of pro-inflammatory stimuli, and did not interfere with subsequent phagocytosis of tissue debris and bacteria.²¹² Recently, Shields et al. described a strategy to lock macrophages in an M1 phenotype for cancer therapy (Figure 5C).²¹³ They designed discoidal shaped particles referred to as “backpacks”, whose anisotropic shape prevented phagocytosis by macrophages and instead promoted adhesion to their surfaces. Macrophages carrying IFN γ -releasing backpacks maintained a pro-inflammatory phenotype in the immunosuppressive environment of a murine breast cancer model, where they slowed tumor growth and reduced metastasis compared to control macrophages carrying blank backpacks. These studies demonstrate promising strategies to modify and maintain phenotypes of transplanted macrophages.

Mesenchymal stromal cells (MSCs) are a widely investigated cell source for diverse applications in regenerative medicine and have been used clinically for decades.²¹⁴ While originally touted for their ability to differentiate into multiple cell types in the mesenchymal lineage (e.g., bone and cartilage), they are now even more widely used for their immunomodulatory properties (for review, see ref 215). In particular, they exhibit low immunogenicity even when transplanted allogenically, and they inhibit T cell proliferation. In addition, crosstalk of monocytes/macrophages with MSCs or MSC-derived conditioned media induces a unique macrophage phenotype sometimes referred to as MSC-educated macrophages.^{216–218} As a result, MSC therapies have the potential to modulate macrophage phenotype for enhanced tissue repair. However, MSC transplantation suffers from poor survival *in vivo* and low cellular retention.^{219–221} Therefore, biomaterials have been developed to protect MSCs in numerous applications (for review, see ref 222). The inclusion of MSCs within hydrogels has been shown to reduce their fibrous encapsulation,^{223,224} with the magnitude of this effect decreasing with more differentiated cells (Figure 5D).

Recently, Clark et al. investigated how MSC functionality can be tuned by delivering them within hydrogels modified with different integrins.²²⁵ PEG hydrogels were modified to present peptides capable of binding to different integrins expressed by MSCs. They compared GFOGER, derived from type I collagen, which has binding specificity for $\alpha_5\beta_1$ integrin, with RGD, which can be found in different ECM proteins,

such as fibronectin, and which has binding specificity toward $\alpha_1\beta_3$, $\alpha_1\beta_1$, and $\alpha_5\beta_1$ integrins. GFOGER-presenting hydrogels prolonged MSC survival and caused them to upregulate numerous genes and cytokines associated with inflammation and enhanced their abilities to stimulate bone repair in a segmental defect model in mice.

In a wound healing environment, inflammatory cytokines prime MSCs to increase their immunomodulatory properties.²²⁶ Recently, Gonzalez et al. took advantage of this finding to design immunomodulatory hydrogels for the delivery of MSCs.²²⁷ The hydrogels consisted of an interpenetrating network of functionalized alginate and fibrillar collagen embedded with IFN γ -loaded heparin-coated beads. The inclusion of IFN γ -loaded beads prolonged the expression of immunomodulatory genes by bone marrow-derived primary human MSCs compared to 2D tissue culture.

Genetic modification of MSCs to amplify the expression of immunomodulatory factors is another promising strategy.^{228,229} Ueno et al. developed lentivirus-transduced IL4 overexpressing MSCs (IL4-MSCs) to promote M2 polarization of macrophages.²³⁰ IL4-MSCs were delivered in a microporous gelatin-based microribbon scaffold to critical-size long bone defects in mice. IL4-expressing MSCs increased M2 marker expression by surrounding macrophages without inhibiting M1 marker expression in the early stage and augmented macrophage migration into the scaffold, ultimately resulting in enhanced bone healing. Collectively, these studies show that biomaterials are useful delivery vehicles for immunomodulatory MSCs.

5. CHALLENGES AND FUTURE DIRECTIONS

Designing new and more effective strategies to prevent the FBR and/or promote tissue repair and regeneration will undoubtedly require a greater understanding of the complex mechanisms that govern these processes. To that end, researchers are developing better *in vitro* and animal models while exploring innovative avenues to study and manipulate the inflammatory response to biomaterials. At the same time, this is providing invaluable insight to identify hitherto unknown action points and new cellular targets.

5.1. Considerations for Interpretation of *in Vitro* Studies and Animal Models

The mechanisms underlying the inflammatory response to biomaterials and the advancement of novel immunomodulatory therapies were supported by *in vitro* and animal studies, and numerous studies have shown corroborating results between *in vitro* studies and *in vivo* outcomes.^{52,54,231,232} However, challenges in extrapolating results from such models to human patients are well-known to be major factors limiting clinical translation of novel therapies. These challenges may be particularly problematic for strategies that target the immune system, considering the highly responsive nature of immune cells to their microenvironment, the highly evolved state of the human immune system, and the dependence of immune cell behavior on a patient's particular medical history.

For example, there are several notable differences between murine and human inflammatory responses²³³ and even *in vitro* macrophage behavior,²³⁴ such as the lack of human macrophage expression of nitric oxide and arginase, which are popular murine markers of M1/M2 polarization.^{235,236} As another example, murine macrophages become elongated upon M2 polarization with IL4 *in vitro*,²³⁷ but human macrophages

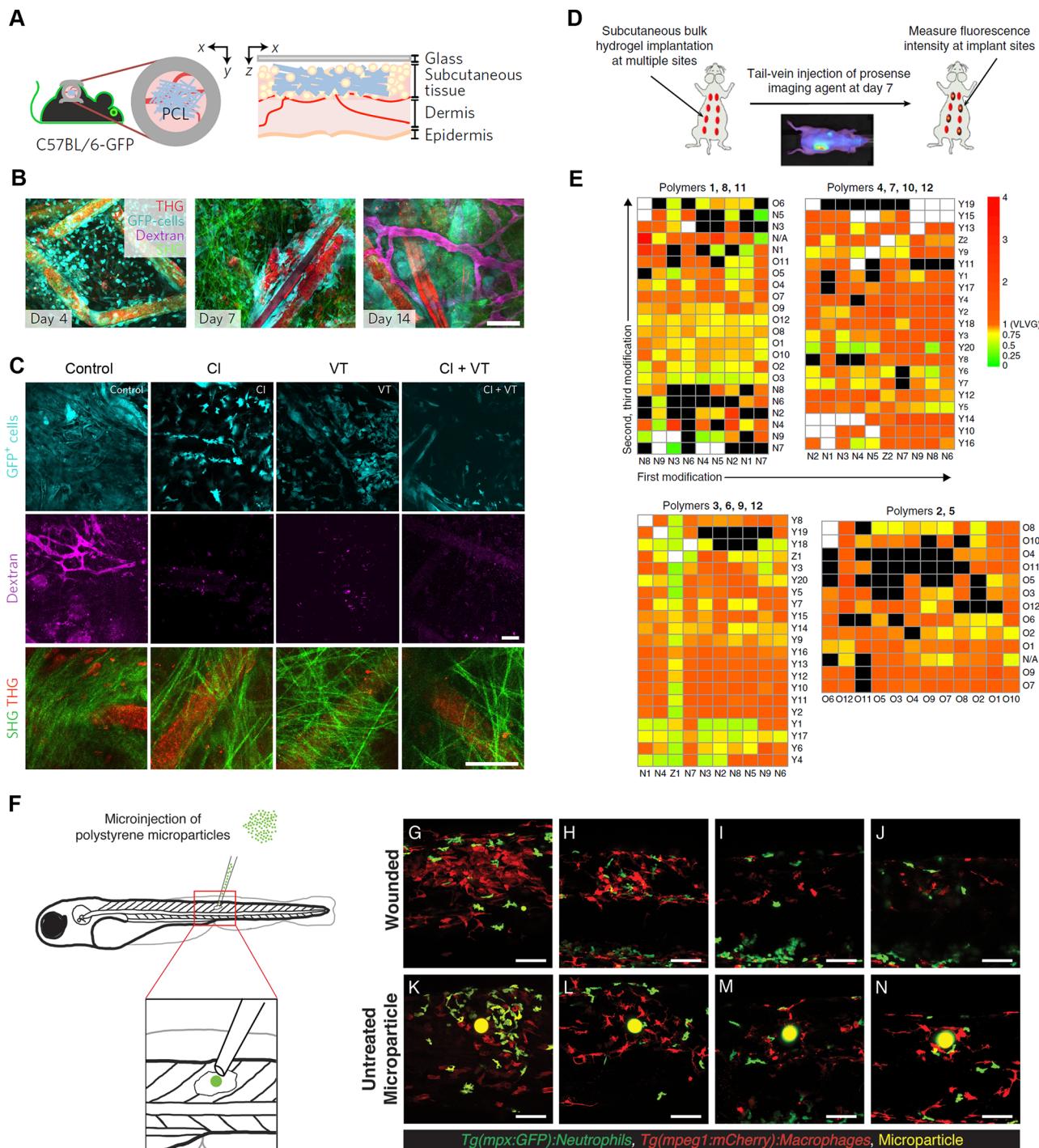


Figure 6. Representative examples of emerging tools for studying the inflammatory response to biomaterials. (A) Implantation of biomaterials in dorsal skinfold window chambers in transgenic mice to study the inflammatory response using intravital imaging. Reproduced with permission from ref 28. Copyright 2016 Springer Nature. (B,C) Longitudinal intravital imaging of green fluorescent protein (GFP)⁺ cells in combination with perfusion agent (dextran) and SHG and THG signals showing collagen fibril orientation. Reproduced with permission from ref 28. Copyright 2016 Springer Nature. (D,E) High throughput evaluation of fluorescent inflammatory activity in response to a library of modified alginates injected subcutaneously into mice. (E) Heat map summarizing gelation and cathepsin activity of a library of 774 modifications to alginate. Reproduced with permission from ref 64. Copyright 2016 Springer Nature. (F) Injection of fluorescent polystyrene microparticles into transgenic larval zebrafish with fluorescently labeled neutrophils and macrophages. Reproduced and adapted with permission from ref 260. Copyright 2018 American Chemical Society.

do not.²³⁸ Murine wounds heal primarily via contraction, while human wounds do not.²³⁹ These and other major mouse-human differences in inflammation and immunity have been reviewed elsewhere.²⁴⁰

In every field, it can be challenging to translate findings from in vitro studies to animal models and then to humans, but in vitro studies of macrophage behavior are particularly controversial. Studies that carefully characterized the phenotypes of macrophages derived from animal models have

pointed out that while macrophages *in vivo* do exist on a spectrum of diverse phenotypes, they do not clearly map to phenotypes that can be prepared *in vitro* with defined chemical stimuli like LPS and IFN γ (for M1) or IL4 and IL13 (for M2).¹⁵² On the other hand, some studies have shown that gene expression profiles from *in vitro* polarized macrophages can be a useful framework for describing the complex phenotypes observed *in vivo*, even if they are not perfect models.^{241,242} Another problem that complicates utility of *in vitro* studies of macrophage behavior is that macrophages are activated by various stimuli, including tissue culture polystyrene and probably other aspects of the *in vitro* environment that do not exist *in vivo*. One study reported relatively little consensus in gene expression signatures between just three publicly available data sets derived from whole transcriptome gene expression analysis of M1 and M2 macrophages prepared with the same protocols *in vitro* but by different research groups.²⁴³ Another study showed that the response of macrophages to IL4 may be affected by the presence or absence of apoptotic cells in cell culture media,²⁴⁴ which could vary widely across different cell culture conditions. The presence of integrin-binding motifs within biomaterials has also been shown to influence the macrophage response to IL4,²⁴⁵ but this type of interaction between biophysical and biochemical cues that may be present in the wound environment is rarely assessed in studies of the macrophage response to biomaterials. Nonetheless, *in vitro* studies were critical for advancing key concepts in macrophage regulation of tissue repair, such as the role of M1 macrophages in initiating angiogenesis,^{110,246} the role of M2 macrophages for regulating ECM assembly,¹¹⁶ and the sexual dimorphism of the macrophage response to oxidized lipids in the context of atherosclerosis.²⁴⁷ Thus, *in vitro* models of macrophage behavior are indispensable for dissecting mechanisms and evaluating novel biomaterials, but care must be taken upon extrapolation to *in vivo* studies.

Finally, preclinical evaluation of novel biomaterials must take into account that the inflammatory response is affected by patient-specific factors that may not be adequately represented by animal models. For example, at least 40% of the U.S. population is obese or diabetic,²⁴⁸ with major effects on immune cell behavior,^{249–252} but the efficacy of biomaterials is not typically evaluated in obese or diabetic animals. As examples of how heterogeneity in the human immune response can influence the failure of biomaterials, 28% of breast implants require revision,²⁵³ and successful biomaterials in diabetic wound healing are successful in only around 55% of cases.^{254,255} Similarly, total joint replacements fail after a wide range of implant duration, ranging from 15 to 25 years on average.²⁵⁶ It remains poorly understood why some patients respond better to certain biomaterial treatments than others and why some biomaterials fail more rapidly in some patients compared to others.

5.2. Emerging Tools and Models for Studying the Inflammatory Response to Biomaterials

New techniques that allow detailed characterization of the inflammatory response to biomaterials in real time using nondestructive means can help elucidate some of the differences that lead to patient-specific responses to biomaterials. Typically, the FBR has been assessed by means of histopathological analysis of retrieved implants after staining with hematoxylin and eosin (H&E), Masson's trichrome, and/

or Sirius Red. The fibrous capsule can be analyzed via semiquantitative analysis for thickness, degree of fibrosis, and cell overgrowth. Supplementary methods include the use of gene expression analysis, cytokine arrays, and multidimensional flow cytometry. However, the need for high-throughput, real-time, comprehensive, and biologically relevant data is leading researchers to the development of innovative ways to explore the FBR.

Multiphoton intravital microscopy can provide valuable information to unravel some of the still unknown complex and dynamic biological interactions driving the inflammatory response. This technique allows imaging living biological responses to materials implanted deep into the skin with excellent signal-to-background noise ratio and minimal photodamage (Figure 6A).²⁵⁷ Besides fluorescence detection, it is possible to exploit nonlinear processes such as second harmonic generation (SHG) and third harmonic generation (THG).²⁵⁸ In this vein, 3D porous electrospun PCL scaffolds implanted in the subcutaneous space of mice have been monitored by means of the generated THG signal, while following the deposition of SHG-positive collagen fibers and sprouting of red fluorophore-tagged neovessels (Figure 6B,C).²⁸ Intravital microscopy is usually performed in transgenic mice, whose cells express fluorescent protein markers, to analyze kinetics of cell infiltrates.²⁴

In vivo molecular imaging techniques are also valuable tools, especially when combined with innovative strategies to conduct high throughput investigations. For example, by injecting eight different polymer modifications subcutaneously into one mouse and using an injected imaging probe that fluoresces in proportion to the level of inflammation, researchers performed a rapid assessment of 774 variants of chemically modified alginates aimed at mitigating the FBR (Figure 6D,E).⁶⁴ Likewise, Yang et al. employed mice with a luciferase reporter gene to enable real-time, noninvasive monitoring of expression of the pro-inflammatory cytokine interleukin-1 β (IL1 β) by bioluminescence imaging in response to subcutaneously implanted functionalized PLLA scaffolds.²⁵⁹ Strategies that can combine these techniques with multiple reported outputs will be key to rapid advances in understanding the inflammatory response.

Along these lines, Witherel et al. examined the FBR to polypropylene sutures and polystyrene microparticles in zebrafish, which are optically translucent and genetically tractable model organisms that have been used extensively to study human disease.²⁶⁰ By implanting these biomaterials in zebrafish with fluorescent macrophages and neutrophils, the dynamics of these immune cells interacting with model biomaterials could be visualized noninvasively and in real-time (Figure 6F). Zebrafish are amenable to numerous genetic and chemical modifications, which enables researchers to fluorescently label key cellular and molecular mediators, including immune cells, pro-inflammatory signals, and blood vessels, to evaluate the complex and multifaceted interplay between biomaterials and surrounding tissues.^{260–262}

New animal models that will facilitate increased understanding of the human immune response to biomaterials include humanized mouse models, which are engrafted with human hematopoietic stem cells or peripheral blood mononuclear cells. For example, one study showed that humanized mice display distinct responses to human- or porcine-derived ECM hydrogels, unlike wild-type mice without human immune cells.²⁶³ Beyond animal testing, however, there

are currently no reliable and accepted methods to assess the inflammatory response to biomaterials in a way that accurately represents the human response. To address this problem and reduce the burden of experimental animals, some researchers direct their efforts toward the development of more clinically relevant *in vitro* models. For example, Jannasch et al. attempted to recapitulate the wound environment by exposing biomaterials (PTFE and titanium) to macrophages integrated in fibrin clots and coculturing them with fibroblasts embedded within soft tissue-resembling 3D matrices (made of either collagen or fibrin).²⁶⁴ Their results generally agreed with preclinical and clinical studies described in the literature. With the development of more sophisticated “organ-on-a-chip” model systems, it may become possible to comprehensively evaluate the human response to biomaterials *in vitro*.²⁶⁵ For example, the use of a FBR-on-a-chip platform combined with peripheral blood mononuclear cells (PBMCs) directly extracted from the patient has recently been proposed.²⁶⁶ Such *in vitro* model systems may also help to address another major challenge in understanding the inflammatory response to biomaterials in humans, which is that patients may have very different responses based on their own clinical factors and medical history. It is generally not known how variations in the immunological profile of individuals related to age, sex, or comorbidities affect the FBR or the ability of biomaterials to support tissue repair, although some studies are beginning to tackle these questions.^{267–269} The effects of comorbidities in particular are especially difficult but important to consider because biomaterials are typically used to repair tissue in diseased sites. For example, the inflammatory response to dendrimer/dextran hydrogels was shown to vary greatly in healthy vs pathologic models of colonic tissue.²⁷⁰ These findings suggest that a personalized evaluation of the inflammatory response to biomaterials may be required.

5.3. Identification of New Cellular Targets

By harnessing spontaneous and knockout mutations or producing chemically induced immune cell depletions in mice, it is possible to delete specific immune targets to study their role in the FBR and the inflammatory response to biomaterials.^{271,272} Using these models, independent studies targeting different immune mediators have arrived to similar conclusions: neutrophils,^{24,41} mast cells,^{38,39} natural killer cells (NK cells),³⁹ and T lymphocytes⁴⁸ seem to have little, if any, effect on fibrous capsule development. However, these cells do have major effects in wound healing, and T cells in particular have been demonstrated to be key regulators of biomaterial-mediated tissue repair.¹¹⁷ Therefore, the similarities and differences between cellular regulation of the FBR and the wound healing response to biomaterials are still poorly understood.

A recent study by Chung et al. described so far overlooked potential therapeutic targets to reduce the FBR.⁴⁹ They analyzed the cellular components of fibrous capsules surrounding silicone breast implants in humans and identified IL17-producing $\gamma\delta^+$ T cells and T helper 17 (Th17) cells as well as senescent stromal cells. Senescent cells, which accumulate in aging and in chronic disease, are nonproliferative cells that secrete numerous inflammatory cytokines as part of the senescence associated secretory phenotype (SASP). Clearance of these cells has been shown to ameliorate numerous diseases.²⁷³ Using animal models, senescent cells were identified to be key mediators of the fibrotic response to

PCL particles, inducing differentiation of Th17 cells and giving rise to IL17-secreting antigen-dependent adaptive response.⁴⁹ This study opens the door to alternative therapeutic interventions aimed at blocking the IL17-associated pathway in T cells and senescent stromal cells.

Still, macrophages have undeniable effects on regulating the FBR and biomaterial-mediated tissue repair.^{24,28,29} Targeted and timely interventions in macrophage function represent a key area for future strategies to modulate the inflammatory response. For example, manipulation of specific macrophage signaling pathways, such as CSF1/CSF1R^{24,259} and CXCR7/CXCR4/CXCL12,²⁷⁴ have been shown to ameliorate fibrous capsule development around biomaterials. The inflammasome of macrophages represents another valuable opportunity for therapeutic targeting. The inflammasome comprises a cytosolic multiprotein complex that assembles in response to the presence of danger signals and triggers the production of pro-inflammatory mediators such as IL1 β and IL18.³³ In a given moment, this complex can persist and develop a chronic inflammatory process, or it can take an alternative direction toward resolution of inflammation and tissue remodeling.²⁷⁵ Gaining further insight into the biology of this mechanism may unveil key regulators of the innate response that can be modulated to enhance biomaterial integration.

Relatedly, future studies should direct efforts to overcome the limitations of many biomaterials’ studies analyzing macrophage phenotype and overinterpretation of the M1/M2 paradigm. Most studies use a handful of typical M1 and M2 markers but fail to thoroughly characterize macrophage phenotype or extend the characterization to functional effects. In contrast, Sommerfeld et al. used single cell RNA sequencing to thoroughly characterize the phenotype of macrophages participating in the divergent responses to biologic (i.e., ECM scaffolds) or synthetic materials.¹⁵² The team identified a particular combination of surface markers that discriminated between unique macrophage phenotype subsets in fibrotic or pro-regenerative environments. In addition, these populations did not necessarily converge with canonical M1/M2 markers, which reinforces the need for further investigation in this regard.

5.4. New Strategies to Modulate Monocyte/Macrophage Behavior

Given the importance of macrophages in inflammatory disease and tissue repair, new strategies are needed to control their behavior. As an example, recent studies demonstrate how researchers have taken advantage of the natural behaviors of monocytes and macrophages to ameliorate excessive inflammation. When nanoparticles or microparticles are injected into the bloodstream, they are rapidly cleared from circulation by the reticuloendothelial system, which comprises circulating phagocytes (neutrophils and monocytes) and filtration organs like the liver, spleen, and kidney. While particle properties such as hydrophilicity, surface charge, size, shape, molecular weight, and even coating with biomimetic moieties affect their circulation time before they are cleared, all particles are eventually cleared from circulation within minutes to hours.^{276–280} While this rapid clearance is a major impediment to systemically delivered drug delivery systems, recently, some researchers have turned it into an advantage. Because monocytes phagocytose particles in the bloodstream and carry them to the liver and spleen, Getts et al. showed how this phenomenon can be leveraged to divert monocytes away from

sites of inflammation, instead trafficking to the liver and spleen, thus reducing the number of monocytes at the inflammatory site and ameliorating disease in mouse models of myocardial infarction, multiple sclerosis, colitis, peritonitis, and West Nile virus.²⁸¹ The authors showed that negatively charged (-40 mV) particles of $0.5\text{ }\mu\text{m}$ in diameter and comprised of polystyrene, PLGA, or microdiamond were internalized by monocytes and quickly carried to the spleen. The effect was found to be more pronounced for negatively charged particles than neutral particles and to be primarily mediated by the phagocytosis-related MARCO receptor on macrophages. Later, it was shown that the ability of the particles to promote monocyte redirection away from sites of injury also depended on PLGA molecular weight.²⁸² Finally, using a murine model of spinal cord injury, Park et al. extended this strategy to show that not only did negatively charged particles cause monocytes to redirect away from sites of inflammation, but the few remaining monocytes/macrophages at the injury site expressed higher levels of typical M2 markers compared to controls.²⁸³ While the mechanisms behind this finding remain to be determined, this study shows the potential for leveraging natural phenomena to ameliorate disease and promote tissue repair.

6. CONCLUSIONS

The host inflammatory response is a critical determinant of the success or failure of implanted polymeric biomaterials, with outcomes ranging from the foreign body response to biomaterial–tissue integration. Interdisciplinary studies at the intersection of biomaterials science and immunology have shown how biomaterials can be rationally designed to enhance outcomes by modulating immune cell behavior. It is now possible to encode biomaterials with certain properties, especially in terms of microarchitecture and structure that decrease fibrous encapsulation. The controlled release of bioactive factors can be used to recruit monocytes and convert them to pro-regenerative phenotypes that regulate other cells and promote angiogenesis and tissue repair. New tools are being developed to advance understanding of how diverse immune cells regulate the response to biomaterials in real time and with human-specific features. The role of the adaptive immune system in particular remains wide open for exploration. Collectively, while many aspects of the immune system are still poorly understood, it is increasingly possible to design biomaterials that harness this system to improve outcomes in regenerative medicine.

AUTHOR INFORMATION

Corresponding Author

Kara L. Spiller — School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, Pennsylvania 19104, United States;  orcid.org/0000-0001-7798-1490; Email: spiller@drexel.edu

Authors

Ricardo Whitaker — School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, Pennsylvania 19104, United States
Beatriz Hernaez-Estrada — School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, Pennsylvania 19104, United States; NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University

of the Basque Country (UPV/EHU), Vitoria-Gasteiz 01006, Spain

Rosa Maria Hernandez — NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz 01006, Spain; Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Vitoria-Gasteiz 01006, Spain;  orcid.org/0000-0002-3947-409X

Edorta Santos-Vizcaino — NanoBioCel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz 01006, Spain; Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Vitoria-Gasteiz 01006, Spain

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acs.chemrev.0c00895>

Author Contributions

[¶]R.W. and B.H.-E. contributed equally.

Notes

The authors declare no competing financial interest.

Biographies

Ricardo Whitaker has a bachelor's degree in Biomedical Engineering from the New Jersey Institute of Technology (NJIT). Ricardo is currently a Ph.D. student at Drexel University and is interested in nano-/microparticle-based drug delivery systems to control leukocyte behavior for regenerative medicine.

Beatriz Hernaez-Estrada is a Ph.D. student in Pharmaceutical Technology at the University of the Basque Country (UPV/EHU) (Spain) in the NanoBioCel group, under a fellowship given by the Basque Government. During her Ph.D., she received a travel award to conduct a six-month research stay at the Biomaterials and Regenerative Laboratory under the supervision of Dr. Kara Spiller at Drexel University, Philadelphia (USA). In 2020, she joined the group full-time as a research scientist. Her current research focuses on understanding the interaction between tissue-engineered constructs and macrophages.

Rosa María Hernández is Full Professor of Pharmaceutical Technology at the University of Basque Country, Spain. She received her Ph.D. (1992) from the University of Salamanca. She is Principal Investigator in several research projects and has supervised 21 Ph.D. thesis works and coauthored more than 200 scientific articles in high impact journals and 10 patents. Her research fields focus on several areas of drug delivery, including the micro- and nanoencapsulation of growth factors and peptides as an alternative therapy for neurodegenerative disorders and vaccine development. She is also interested in the development of cell-based and cell-free therapies for regenerative medicine.

Edorta Santos-Vizcaino is a lecturer and researcher at the University of the Basque Country (UPV/EHU). He received his Ph.D. with international mention and Extraordinary Award from the UPV/EHU in collaboration with Harvard University and CIMA (University of Navarra). He also received the 2013/2015 SPLC-CRS Best Ph.D. Thesis Award. He has extensive experience in cell encapsulation and drug delivery systems. His research interests include the biomedical application of different biomaterials, mesenchymal stromal cells (MSCs), and extracellular vesicles to exert an immunomodulatory and regenerative effect in the treatment of immune-mediated inflammatory diseases (IMIDs) and chronic wound healing, among others.

Kara L. Spiller is an Associate Professor and Director of the Biomaterials and Regenerative Medicine in Drexel University's School of Biomedical Engineering, Science, and Health Systems Laboratory. She received her Ph.D. in Biomedical Engineering from Drexel University, with her research in collaboration with Shanghai Jiao Tong University. She was a Fulbright fellow at the University of Minho, Portugal, and a postdoctoral fellow in biomedical engineering at Columbia University. Her research interests include the role of immune cells in tissue regeneration, the design of immunomodulatory biomaterials and drug delivery systems, and international engineering education.

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