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The foreign body response: emerging cell types and considerations for targeted therapeutics

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Abstract

The foreign body response (FBR) remains a clinical challenge in the field of biomaterials due to its ability to elicit a chronic and sustained immune response. Modulating the immune response to materials is a modern paradigm in tissue engineering to enhance repair, while limiting fibrous encapsulation and implant isolation. Though the classical mediators of the FBR are well-characterized, recent studies highlight that our understanding of the cell types that shape the FBR may be incomplete. In this review, we discuss the emerging role of T cells, stromal-immune cell interactions, and senescent cells in the biomaterial response, particularly to synthetic materials. We emphasize future studies that will deepen the field's understanding of these cell types in the FBR, with the goal of identifying therapeutic targets that will improve implant integration. Finally, we briefly review several considerations that may influence our understanding of the FBR in humans, including rodent models, aging, gut microbiota, and sex differences. A better understanding of the heterogeneous host cell response during the FBR can enable the development and design of immunomodulatory materials that favor healing.

Introduction

The canonical immune response to a biomaterial

Implantable medical devices have revolutionized modern medicine by restoring tissue function, replacing tissue, and improving quality of life.[1] Medical implants are made of biomaterials, biologically derived or synthetically produced materials designed to interact with biological systems. Biomaterials are used in various clinically relevant applications, including tissue reconstruction, implantable medical devices, and drug delivery. Most recently, modern medicine is using biomaterials for therapeutic delivery of immunotherapy treatments to target cancer,[2] vaccine development for infectious disease,[3] and engineering effective therapies to promoting immune tolerance during autoimmune conditions and graft tolerance.[4,5] However, the clinical potential of biomaterials heavily relies on their biocompatibility with host tissue and ability to effectively direct host immunity.

Whether of biologic or synthetic origin, all materials when implanted initiate a complex host immune response. The immune response is a self-defense mechanism that protects against foreign pathogens and maintains homeostasis. It diverges into two primary arms: innate immunity and adaptive immunity. While a fast-responding and non-specific inflammatory phenotype defines innate immunity, the adaptive response takes longer to form, is antigen-specific, and develops memory. The intimate crosstalk between the innate and adaptive immune response governs the canonical foreign body response (FBR). Classically, the FBR progresses through four overlapping stages: (1) blood-material interactions, (2) acute inflammation, (3) chronic inflammation, and (4) fibrotic encapsulation of the material.[6,7] In the initial stage, proteins from the blood adsorb to the material's surface and form a provisional matrix.[8]

Complement proteins, danger-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs) initiate the innate immune response. Innate immune cells, largely consisting of neutrophils, are then rapidly recruited to the site of implantation, where they generate an acute inflammatory microenvironment through degranulation and cytokine production. This inflammation then recruits monocytes and lymphocytes. Monocytes differentiate into macrophages with varied phenotypes, some of which fuse into foreign body giant cells (FBGCs) that surround the implant and attempt to phagocytose it.[9] Lymphocytes, such as T-helper cells, release cytokines that promote macrophage fusion. Stimulated by factors released from macrophages and FBGCs, fibroblasts proliferate and deposit an extracellular matrix (ECM) around the implant, ultimately enclosing it in a fibrous capsule.

Many processes within the FBR can significantly compromise the implant's function, whether through enzymatic degradation of the material itself or pain from fibrotic tissue formation that necessitates implant removal.[7] Though all materials can induce the FBR, its severity and clinical manifestation depend on various factors, including material properties, implant location, and host immune system. The immune response to biological or synthetic materials results in a diverging immune microenvironment.[10] Biologically derived materials are typically biocompatible when implanted in tissue, though they may prematurely degrade. Of note, xenogeneic biologics are highly immunogenic. Therefore, the use of natural biomaterials should consider immunogenicity, biodegradability, and its mechanical properties.[11] On the other hand, synthetic materials are more prone to induce pro-inflammatory signaling though their functional properties, such as physicochemical and mechanical, can be more easily engineered. It has been reported that synthetic materials provoke a stronger FBR and highly fibrotic microenvironments, such as

PLGA, PCL, Gel, and SN.[12] Therefore, in this review, we will primarily focus on the emerging cell types associated with synthetic materials for understanding the development of fibrotic encapsulation. Many groups have attempted to reduce the FBR and the resultant fibrosis by manipulating material properties, such as size, topography, chemical properties, and degradation rate.[13,14] Though the innate response has been heavily studied, the adaptive arm is emerging as an essential mediator in the FBR and offers a therapeutic target for preventing the FBR.

Engineering approaches for biomaterial design focus on attenuating the immune response to reduce chronic inflammation and mitigate the foreign body response. A thorough understanding of the role of emerging cell types in the FBR and cell-to-cell interactions at the biomaterial-host tissue interface is critical for developing effective strategies to reduce the clinical consequences of the FBR. This review outlines the role of adaptive immune cells in the FBR, stromal-immune cell interactions, and senescent cells in the FBR (**Graphical Abstract**). Additionally, we discuss the challenges that arise in studying the FBR and translating it to humans, such as various mouse models, aging, gut microbiota, and sex differences (**Figure 1**).

The role of T cells in the development of the FBR

The adaptive immune response is primarily governed by B and T lymphocytes, which can proliferate and perform effector functions to eliminate pathogens.[15] The activity of T cells in particular plays a critical role in response to tissue repair.[16–18] However, our understanding of how T cells modulate the FBR is an emerging field of research and largely varies based on biomaterial, tissue type, and model. Here, we will discuss the function of different T cell types in the context of the FBR.

Most T lymphocytes in adaptive immunity are $CD4^+$ and $CD8^+$ T cells. $CD4^+$ T cells are classically referred to as helper T cells. They perform diverse functions in our immune system, including influencing innate immune cells and regulating inflammation.[15] They are particularly important for resolving tissue repair.[16,17] Depending on the environment during activation, naïve $CD4^+$ T cells can differentiate into five primary subsets: Th1, Th2, Th17, Tfh, and Treg.[19] Each plays a unique role in adaptive immunity. During chronic inflammation, soluble mediators, such as cytokines and growth factors, are produced by T cells that direct the pro- and anti-inflammatory response.[15] The type of $CD4^+$ T cell response that develops is critical for fibrosis or tissue repair.[17,20] $CD8^+$ T cells, on the other hand, are often called effector T cells due to their effector functions, such as killing infected cells and producing anti-viral cytokines.[15]
$CD4^+$ and $CD8^+$ T cells recognize antigens in the context of a peptide bound to a major histocompatibility complex (MHC) molecule presented on an antigen presenting cell (APC). $CD4^+$ T cells recognize peptides bound to MHC Class II molecules while $CD8^+$ T cells recognize peptides bound to MHC Class I molecules.[21] The three signals required to activate naïve T cells to perform their effector functions are engagement of the TCR to the peptide-MHC complex on

an APC, co-stimulatory signal via CD28, and cytokine signaling from the bound APC.[22] Depending on the local environment and the state of the APC, T cells can develop a tolerogenic or immunogenic response to an antigen.[23]

The role of T cells in the FBR is just becoming appreciated. Early studies showed that *in vitro*, the presence of lymphocytes (largely $CD4^+$ and $CD8^+$ T cells) during the initial adhesion of monocytes led to a significant increase in the rate of monocyte adhesion and fusion,[24] which are crucial steps towards the fibrotic encapsulation of a foreign material implant. A follow-up study showed that macrophage interaction with lymphocytes on hydrophobic and hydrophilic/cationic surfaces led to increased production of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, IL-8, and MIP-1β.[25] In T-cell-deficient rats implanted with hexamethylenediisocyanate-crosslinked dermal sheep collagen (HDSC), Luyn *et al.* showed a delay in material degradation and an increase in collagen around the implant.[26] These studies demonstrate the significance of T cells in the FBR. However, it is still unclear to what extent these T cell responses are specific to an antigen derived from the implanted material. Careful material selection that activates various T cell subtypes may serve as a novel approach for tissue engineering applications. The following sections will document specific T cell subtypes studied in the context of the FBR (summarized in **Table 1**).

T-helper 1 (Th1) drive an inflammatory microenvironment in the FBR

Th1 cells are canonically involved in the clearance of intracellular pathogens. The differentiation of $CD4^+$ T cell subsets is driven by exposure to IFN-γ and IL-12 and activation of primary transcription factor T-bet.[27] The secretion of pro-inflammatory cytokine IFN-γ by Th1 cells is

intimately linked with macrophage polarization during infection and the early stages of wound healing.[28] During the acute inflammatory phase of wound healing, Th1 cells drive a largely inflammatory environment that recruits and polarizes macrophages to the M1 phenotype, driving a feed-forward loop with Th1 cell differentiation. The failure to transition to the type 2 reparative immune response via Th1-mediated response can lead to tissue scarring.

The role of Th1 cells in the immune microenvironment of the FBR is highly dependent on the material and the tissue. Phenotypic analysis of intracapsular T cells in peri-silicone mammary implant capsular tissue from women resulted in the presence of $CD4^+$ T cells that were predominantly Th1/Th17 type cells, as defined by their cytokine production.[29] Alternatively, in a murine subcutaneous biomaterial implantation model using nylon mesh, immunohistochemical staining of the implant tissue did not reveal positive staining for TNF-α and IFN-γ at 2-, 4-, and 10-weeks post-implantation. IFN-γ was also not observed at earlier timepoints at 3- and 7-days. This lack of positive staining suggests that type 1 inflammatory immune response did not occur with nylon mesh. However, the cellular source of these cytokines is unclear and can be secreted by many other cells besides Th1 cells.[30] Additionally, a model of polyether-polyurethane sponge implantation in IFN-γ deficient mice impaired angiogenesis, decreased neutrophil and macrophage infiltration, and reduced collagen deposition. Though the source of IFN-γ is redundant, IFN-γ drives a type 1 inflammatory response contributing to inflammatory angiogenesis and fibrogenesis and the development of the FBR.[31] Additionally, whole blood isolated from human volunteers was exposed to PLGA *in vitro,* and the cell culture supernatant was used for protein quantification to better characterize the acute immunological response to the material. IFN-γ was most apparent in *in vitro* culture of blood cells with PLGA. However, IFN-γ

was not increased in conditions where these cells were exposed to calcium sulphate/-carbonate (CS) or poly(methyl methacrylate) (PMMA).[32] Studies utilizing polyetheretherketone (PEEK), stainless steel, and titanium alloy cultured on macrophages found that more T cells polarized to Th1/Th17 than Th2.[33]

The initiation of an acute pro-inflammatory response immediately after biomaterial placement activates the immune system to rapidly recruit innate immune cells to attempt to repair tissue. The transition from acute to chronic inflammation during implant placement is observed during the FBR. Th1 cells and NK cells can produce the cytokine IFN-γ.[34] The production of this cytokine largely drives a profibrotic response due to its ability to polarize macrophages to an M1 phenotype and stimulating macrophages to release hydrogen peroxide (H_2O_2) and nitric oxide (NO) that leads to tissue damage.[34,35] The host inflammatory response is a critical feature of wound repair and material-tissue integration. Though it has been documented that Th1 cells have been found in peri-silicone mammary implant capsular tissue from women[29] and that IFN-γ plays a critical role during the formation of the FBR,[30–32] the role of IFN-γ-producing $CD4^+$ T cells in contributing to fibrotic encapsulation has not been fully elucidated. Future studies could be strongly informed by intracellular staining to identify IFN-γ-producing Th1 cells via flow cytometry. Furthermore, $CD4^+$ T cells can be isolated and functionally characterized using *in vitro* cultures for cytokine expression or gene expression.

T-helper 2 (Th2) is critical for tissue repair

Th2 cells mediate a type 2 immune response characterized by the production of cytokines IL-4, IL-5, and IL-13, most commonly observed in allergic inflammation and anti-helminth parasitic

infections.[36] Upon TCR engagement, co-stimulation, and IL-4 receptor signaling coupled with activation of transcription factor GATA3, the Th2 cell differentiation program is activated.[37] The type 2 immune response is canonically associated with tissue repair and regeneration through resolving type 1 and type 17-driven inflammation, thereby restoring homeostasis.[38] However, type 2 responses that are not appropriately regulated can lead to pathological fibrosis.[38] There are limited studies on the role of Th2 cells in the context of the FBR. Depending on the biomaterial implantation site and anatomical location, type 2 immunity can impair or promote the FBR. The production of IL-4 regulates macrophage function, including macrophage fusion during the FBR and resolution of inflammation via M2 macrophages.[7,39] Others have concluded they serve as an anti-inflammatory polarization mediator for macrophages that promotes myoblast fusion in muscle repair.[40,41] Understanding the role of Th2 cells in the biomaterial-tissue microenvironment is further complicated by the redundant cellular sources of IL-4, including activated mast cells, basophils, and eosinophils.

Several studies broadly investigated type 2 immune responses to various biomaterial implants. In a rat model implanted with polyether urethane (PEU), IL-4 was detected via ELISA peaking on day 14 (as opposed to days 4 and 7). Also, IL-13 gene expression increases around hydrophobic and cationic surfaces while decreasing around hydrophilic surfaces, though IL-4 was unchanged. [42] To explore the impact of IL-4 on treating pelvic organ prolapse, a polypropylene mesh implant surface was engineered to release IL-4 as a surface-localized cytokine delivery system. This delivery system increased M2 macrophages at the implantation site, decreased M1-type macrophages, reduced fibrotic encapsulation around the implant, and improved integration.[43] Similarly, another group used poly(lactic-co-glycolic acid)-multistage silicon particles (MSV)

composite microspheres that released IL-4 in the tissue microenvironment. *In vivo* characterization revealed increased expression in anti-inflammatory genes such as *Il10, Mrc1*, and *Arg1* and increased $CD206^+$ macrophage presence. Th2 responses are most notably associated with biological scaffolds. Sadtler *et al*. showed that pro-regenerative remodeling with biomaterials requires Th2 cells and M2 macrophages.[44] Xu *et al*. found that polysaccharide-based materials induced Th2 skewing.[45] Monitoring specific type 2 T-cell immune responses to biomaterial can be informed by various functional assays including secretion of cytokines via enzyme-linked immunospot (ELISPOT) or flow cytometric analyses of intracellular cytokines.[46]

Some studies demonstrated the relevance of IL-4 producing Th2 cells in the FBR. In T cell-deficient BALB/c mice implanted with Elasthane 80A (PEU), silicone rubber (SR), and polyethylene terephthalate (PET), IL-4 was not detected, suggesting that Th2 cells are a significant producer of IL-4 in this microenvironment. Macrophages isolated from the biomaterial surface of T-cell deficient mice had significantly decreased adherence when treated with PET. However, the lack of IL-4 did not impact the formation of FBGCs.[47] In a study using a synthetic biomaterial PCL, Chung *et al.* showed a decrease in IL4-producing $CD4^+$ T cells compared to saline controls via intracellular staining for flow cytometry, ultimately contributing to a fibrotic immune microenvironment.[20] Using several clinically relevant synthetic biomaterials, these studies suggest an important material-dependent role for Th2 cells in regulating macrophage phenotype in the FBR and fibrosis.

The role of Th2 cells in the mechanistic development of the FBR is less clear and can be either inflammatory or regulatory. The presence of Th2 cells and their production of IL-4 promotes

macrophage polarization to an M2 phenotype during tissue repair. Additionally, Th2 cytokines can instruct the recruitment of fibroblast, endothelial, and epithelial cells, transcending their role in immunity.[38] It has been widely reported that the type 2 immune signature can induce beneficial wound healing and promote muscle and liver regeneration.[38] However, some groups have reported over-proliferation and pathogenic function of Th2 lymphocytes in a fibrotic microenvironment, suggesting the pathogenic Th2 cells can also contribute to the FBR.[48] For example, Martin *et al*. found that biomaterial constructs that are prepared with xenogeneic serum components elicit a robust type 2 response with increases in eosinophils, $CD4^+$ T cells, and type 2 cytokines, such as IL-4, IL-5, and IL-13, which ultimately impair tissue repair.[49] Elucidating the functional heterogeneity, kinetics, and evolution of Th2 cells in the FBR microenvironment can help clarify whether the type 2 immune response is pathogenic or protective. Additionally, it is crucial to consider the metabolic programs of Th2 cells. The activation of Th2 cells requires the mammalian target of rapamycin complex 1 (mTORC1)-dependent and regulatory-associated protein of mTOR (RAPTOR)-dependent metabolic programming.[50] The interactions between type 2 immunity and metabolism in the context of the FBR have yet to be explored. Targeting the metabolic requirements of type 2 immunity in the FBR can be an attractive strategy for improving fibrotic outcomes. Lastly, the antigen specificity of Th2 cells in the local injury site is unclear. TCR repertoire sequencing at fibrotic sites is needed to determine the antigen specificity of Th2 cells and the contributing stimuli, such as bacteria-specific, biomaterial-specific, or self-antigens. Nevertheless, type 2 immunity is critical for tissue repair and regenerative outcomes in several tissues. Further elucidating the role of Th2 cells in the context of the FBR is an important research goal.

T-helper 17 (Th17) cells have varying kinetics in response to biomaterial implants

Th17 cells play a significant role in our immunity by protecting barrier tissues and defending against extracellular pathogens.[51] Classically, naïve $CD4^+$ T cells require TGF-β and IL-6 to differentiate into Th17 cells, which are defined by their expression of the transcription factor retinoid orphan receptor gamma T (RORγT) and their production of IL-17, IL-22, and IL-23.[52] Th17 cells are regulated by Tregs, which play an immunosuppressive role that balances the pro-inflammatory actions of Th17 cells. An imbalance between Th17 cells and Tregs can lead to dysregulated inflammation and is linked to the pathogenesis of a variety of conditions, including inflammatory disorders,[53] autoimmune disorders,[54,55] and tissue fibrosis.[56–59] Though Th17 cells are linked to fibrotic pathologies, their role in the FBR and implant fibrosis has only recently emerged as a new area of study.

Recent years have demonstrated evidence of a Th17 immune response to biomaterial implants in humans. Wolfram *et al.* discovered that $CD4^+$ T cells inside the fibrotic capsule surrounding a silicone breast implant in humans displayed a Th1/Th17 phenotype based on the production of cytokines such as IL-17, IL-6, IL-8, TGF-β1, and IFN-γ. The inflammatory Th1/Th17 intracapsular cells were unable to be suppressed by Tregs.[29] Similarly, Chung *et al.* found that $CD4^+$ T cells in the tissue surrounding human silicone breast implants were skewed towards a Th17 phenotype based on IL-17 production.[20]

Studies in mice have also demonstrated the presence of Th17 cells in response to various material implants. Some studies illustrated an acute increase in Th17 cells, while others observed chronic Th17 responses. Wu *et al.* found a higher proportion of Th17 cells in mice receiving an

implant of mesoporous silica particles, relative to the control group at 2 weeks, but found no differences at 4 weeks.[60] Hotchkiss *et al.* profiled the T-helper cells around titanium implants in the femur of mice and found, in contrast to the previously cited studies, a reduced presence of Th17 cells around the implant relative to sham at 3 days. However, there were fewer differences by day 7.[61] The results from Wu *et al.* and Hotchkiss *et al.* suggest the importance of profiling Th17 kinetics around an implant, as these cells may play a more prominent role depending on the time post-implant. Chung *et al.* performed a time course analysis of IL-17 production from Th17 cells around a polycaprolactone (PCL) implant in mice, demonstrating that IL-17 production from Th17 cells plays a more prominent role in later time points (3 and 6 weeks post-implant) than earlier ones (1-week post-implant).[20]

Studies on how Th17 cells interact with other key players in the FBR, and the contribution of Th17 cells to fibrotic encapsulation of the implant, are also beginning to emerge. Hotchkiss *et al.* demonstrated that macrophages might promote Th17 differentiation in titanium materials. Direct co-culture of naïve $CD4^+$ T cells with macrophages on a titanium surface increased Th17 differentiation relative to indirect co-culture (conditioned medium from macrophages).[61] In a follow-up study comparing four common materials used in orthopedic implants, Avery *et al*. found that co-culture of macrophages and T cells on stainless steel, titanium alloy, or polyetheretherketone (PEEK) substrates induced higher levels of T cell differentiation towards pro-inflammatory Th1/Th17 subsets relative to a titanium substrate. The inflammatory profile *in vivo* generally corresponded to these results, with the stainless steel and PEEK inducing higher levels of infiltrating neutrophils, pro-inflammatory macrophages, and $CD4^+$ T cells than the titanium and titanium alloy.[33] Though they did not investigate the downstream fibrotic

consequences of increased Th17 differentiation, Chung *et al.* demonstrated the functional outcome of Th17 cells in the FBR. That study directly linked IL-17 production from Th17 cells, innate lymphoid cells (ILCs), and gamma delta (γδ) T cells, to the fibrosis around PCL particles in mice. IL-17A and IL-17RA knockout mice displayed reduced fibrosis via αSMA staining, Picrosirius red staining, and collagen thickness relative to wild-type mice. The Th17 cells also participated in a feed-forward loop with senescent cells surrounding the PCL implant, maintaining the fibrosis. Additionally, the study demonstrated that the IL-17 production from $CD4^+$ T cells in the PCL implant was antigen-specific, via a bone marrow chimera experiment involving infusion of CD45.1 and OTII-$Rag^{-/-}$ CD45.2 bone marrow into C57BL/6 CD45.2 mice. The specific antigen that the $CD4^+$ T cells responded to, however, was not identified.[20]

In summary, Th17 cells have been observed at both acute and chronic timepoints in the FBR to biomaterial implants in mice and humans. A limited number of studies have demonstrated the role of Th17 cells in influencing other FBR-associated cell types or promoting a fibrotic outcome. These studies illustrate that Th17 cells are not passive bystanders in the FBR, warranting further investigation. In particular, given the interplay between Th17 cells and Tregs in homeostatic immunity and various pathologies, it would be worthwhile to explore their interaction in the FBR and how it contributes to implant-related outcomes. Additionally, future studies can validate whether the essential role of Th17 cells in biomaterial-induced fibrosis is specific to PCL[20] or applies to other clinically relevant biomaterials. If they are essential for fibrosis around various implants, researchers could probe the Th17 cells further to identify where they are being recruited from, whether they are recognizing any material-specific antigens, and how these biomaterial-induced Th17 cells differ phenotypically (e.g. transcriptionally,

epigenetically, or metabolically) or functionally (e.g. cytokine secretion, interaction with other immune cells) from the classic Th17 cells associated with protective mucosal immunity. These studies can provide insight into therapeutic targets for reducing the FBR and fibrosis.

T-regulatory (Treg) cells are phenotypically heterogenous in the FBR

Tregs play a fundamental role in tissue homeostasis and immunosuppression. They maintain homeostasis and peripheral tolerance via the production of anti-inflammatory cytokines, expression of co-inhibitory molecules, modulation of antigen-presenting cells, and depletion of growth factors from the microenvironment.[62] The differentiation and activation of naïve $CD4^+$ T cells to Tregs depend on the activation of transcription factor forkhead box P3 (FOXP3) and the presence of cytokine TGF-β.[63,64] Within the tissue repair microenvironment, Tregs frequently crosstalk with M2 macrophages via secretion of IL-10 to skew the microenvironment to anti-inflammatory/anti-fibrotic and prevent the overactivation of T effector cells.[28] More recently, Tregs are also implicated in mediating tissue repair processes like angiogenesis in a tissue- and context-specific manner.[65]

The role of Tregs in the FBR is controversial due to evidence for pro-regenerative[66] and fibrotic [67,68] outcomes. Artsen *et al*. collected and characterized the tissue surrounding polypropylene mesh fibers removed from women that underwent urogynecological surgery. Though TGF-β1 increased in the mesh tissue, the group saw significantly fewer Tregs in areas of fibrosis compared to non-fibrotic areas in the same patients.[66] On the other hand, Tennyson *et al*. also characterized the T cell response to surgically excised polypropylene meshes from vaginal tissue and saw increased Treg cells. Additionally, and in agreement with previous studies, the fibrous

tissue contained increased amounts of TFG-β, which correlated with increased collagen I and III.[67] Dievernich *et al*. analyzed tissue sections from seven polypropylene meshes used for abdominal wall hernia repair and found more Tregs than any other T cells in the FBR.[68] Differences in tissue type, time of implantation/excision, material origin, or standardization of immunohistochemistry quantification may partially explain these differences. However, the role of Tregs in the FBR is still inconclusive.

Studies have reported plasticity in Treg phenotypes, where their immunosuppressive activity is lost, and they produce pro-inflammatory cytokines. Similar phenomena appear in the FBR literature. In a volumetric muscle loss (VML) model, $CD3^+$ T cells were isolated and sequenced from the inguinal lymph nodes that drain the injury site. Animals treated with the synthetic material, PCL, showed T-regulatory cells displaying an inflammatory gene expression profile characterized by up-regulation of *Tnf* gene expression.[69] In peri-silicone mammary implant capsular tissue isolated from women, immunohistological staining revealed that T-regulatory cells were highest in capsules with the mildest symptoms of clinical capsular contracture. However, more Tregs were found in the intracapsular tissue compared to the peripheral T cells collected from the blood of the same patients. *In vitro* work with Tregs isolated from fibrotic capsules became less immunosuppressive when they came from patient samples immunohistologically graded with high fibrosis.[29] Therefore, it is reasonable to conclude that Tregs may function to control capsular fibrosis.

While Treg lymphocytes control and regulate the inflammatory response in the FBR, some studies have reported increased Treg cells in biomaterial implant sites. Whether these Tregs are

heterogenous in function is less clear. Future studies should seek to understand the heterogeneity of Tregs, their temporal and spatial characteristics, and their phenotypic subsets using *in vitro* cultures and single-cell RNA sequencing.

Gamma delta (γδ) T cells are largely unexplored in the FBR

Like Th17 cells, γδ T cells play a significant role in barrier defense, residing mainly in the skin and mucosal tissues.[70] They protect against various bacterial infections.[71–73] Unlike Th17 cells, however, γδ T cells are more innate-like and express a γ/δ TCR, which can respond to non-peptide antigens such as lipids and soluble proteins.[70,74] γδ T cells can produce a variety of inflammatory cytokines such as IFN-γ, IL-17, and TNF-α,[75] and they can be further divided into IFN-γ producing and IL-17 producing functional subsets.[76] γδ T cells may also play a pathogenic role in conditions such as inflammatory bowel disease,[77] rheumatoid arthritis,[78] multiple sclerosis,[79] and tissue fibrosis.[80–82]

Very little has been published on γδ T cells' role in responding to biomaterial implants. Chung *et al.* observed an increased γδ presence in the fibrotic capsule around human breast implants. That study demonstrated that IL-17 production from γδ T cells (as well as other cell sources) played a role in the fibrotic outcome of synthetic material implants in mice.[20]

Though only one study has directly investigated γδ T cells in the context of the FBR, we can postulate the role they may play based on current knowledge about γδ T cell functions. γδ T cells can play a homeostatic and protective role, particularly in the skin, but they can also promote local inflammation in various infections and injury conditions.[83] For instance, in an intestinal

ischemia-reperfusion injury model, γδ T cells were shown to contribute to the acute leukocyte influx following injury.[84] In a muscle injury model, γδ T cells increased the initial inflammatory influx by increasing mainly neutrophil recruitment.[85] Given that a biomaterial implant typically induces an injury and inflammatory cascade, it is possible that the γδ T cells found near the implant site are increasing the inflammation by recruiting other immune cells. Interestingly, the acute inflammation induced by γδ T cells in these different injury models had opposing downstream effects. In the context of the ischemia-reperfusion model, γδ T cells promoted distant organ injury, but in the context of the muscle injury model, they promoted muscle tissue regeneration via muscle stem cell and progenitor cell proliferation.[84,85] Since the FBR can progress to an unresolved, chronic inflammatory environment around the biomaterial, a boost in the initial inflammatory response to the biomaterial may result in increased fibrosis around the implant.

On the other hand, some γδ cell subsets serve a regulatory, anti-inflammatory role.[86] In both a burn wound model and an acute lung injury model, γδ T cells reduced myeloid cell infiltration at the wound site.[87,88] Therefore, it is also possible that the γδ T cells are reducing inflammatory cell recruitment to the implant site. Regardless of their pro- or anti-inflammatory role, γδ cells' role in the FBR is likely biomaterial-dependent. Future studies using TCRδ-knockout mice in various implant models will help elucidate whether γδ T cells are necessary for fibrosis around an implant. It will also be useful to identify additional functions of γδ T cells besides IL-17 production that may be contributing to fibrosis, such as production of other cytokines like IFN-γ, recruitment of immune cells, or dampening of the inflammatory response. Lastly, phenotyping

the γδ T cells via flow cytometry, RNA sequencing, and cytokine arrays will help researchers better understand their role in the FBR.

$CD8^+$ T Cells in the FBR are biomaterial-dependent

The classic roles of $CD8^+$ T cells in the immune system are anti-viral and anti-cancer immunity.[89,90] Activated $CD8^+$ T cells release inflammatory cytokines such as IFN-γ and TNF-α to amplify the immune response to a pathogen. They can also directly perform cytotoxic functions by releasing cytotoxic granule contents or by inducing apoptosis via surface ligand interactions with infected cells.[91] $CD8^+$ T cells have been linked to the pathogenesis of many autoimmune diseases[92] and some cases of tissue fibrosis.[93–95]

$CD8^+$ T cells have been observed around a biomaterial implant, but their role in the FBR has been largely unexplored. James *et al.* observed scattered $CD8^+$ T cells in the fibrous capsule surrounding both silicone and cellulose acetate filter implants in rats at 1-week post-implant.[96] Sadtler *et al*. showed that biologically derived scaffolds like collagen, cardiac ECM, and bone ECM skewed the ratio of $CD4^+$:$CD8^+$ T cells towards a lower proportion of $CD8^+$ T cells at 1-week post implant.[44] In humans, $CD8^+$ T cells increased in polypropylene meshes that were removed for exposure,[97] suggesting a possible degradative function of $CD8^+$ T cells in the FBR to this polypropylene material. Similarly, Artsen *et al.* observed $CD8^+$ T cells surrounding polypropylene mesh fiber implants in women, with Tregs in close association with the $CD8^+$ T cells.[98]

Studies have also suggested that the material properties of the implant may play a role in the duration and magnitude of the $CD8^+$ response. James *et al.* showed that by 2 months post-implant in rats, the $CD8^+$ T cell presence decreased in the silicone and impermeable cellulose acetate filter implants but not in the porous cellulose acetate filter implant, suggesting that chronic $CD8^+$ responses depend on the porosity of the material.[96] Doloff *et al.* demonstrated a link between $CD8^+$ responses and implant surface roughness, with the $CD8^+$ subset increasing more significantly in the rough surface (90 um) silicone implant relative to the smoother surface (0 um and 4 um) implants.[99] *In vitro* studies showed that hydrophobic surfaces such as PET were more selective for $CD8^+$ T cell interactions.[100]

Though the literature is limited, it suggests that $CD8^+$ T cells are a part of the FBR to a biomaterial implant and that modification of material properties may tune their presence. Whether $CD8^+$ T cells contribute to fibrosis around an implant or are simply recruited as a part of the inflammatory cascade is still inconclusive. Further studies utilizing $CD8^+$ T cell-depleted mice can be used to clarify the functional role of these T cells in the FBR.

Emerging stroma-immune interactions may govern the foreign body response

The microenvironment of the biomaterial-tissue interface is a heterogenous population of cells, including stromal cells such as fibroblasts, vascular endothelial cells, and pericytes. Their phenotype in the context of the FBR depends on the stage of repair. Successful tissue remodeling can be attributed to regulated ECM deposition by fibroblasts and the formation of new vasculature, or angiogenesis, at the injury site. While angiogenesis in tissue repair is characterized by functional and interconnected vessels, pathological angiogenesis in tumors and

chronic inflammatory diseases produces immature and disorganized vessels.[101] In the context of tissue repair and fibrosis, differentiating healthy from abnormal angiogenesis and the way this influences immune cell infiltration is critical.

Fibrous capsules around biomaterial implants contain avascular zones, impairing tissue repair due to limited cellular penetration. Researchers have proposed that revascularization with biomaterial implantation can improve biomaterial integration. The genetic deletion of an angiogenesis inhibitor protein, TSP2, resulted in an increase in blood vessel density within the collagenous capsule, irregularly shaped collagen fibers, and abnormal aggregates of fibroblasts surrounding a polydimethylsiloxane (PDMS) implant.[102] Staining of PECAM-1 showed that local delivery of antisense cDNA inhibiting TSP2 led to an increase in neovascularization, suggesting its molecular role in inhibiting vascularization in the FBR as well as its connection to fibroblast phenotypes.[103] The surface properties of materials are now being engineered to promote angiogenesis. One group engineered nanostructured degradable PCL mesh with reparative mesenchymal stem/stromal cells from women's endometrium to treat pelvic organ prolapse. This mesh promoted angiogenesis via increased gene expression in *Vegfa, Fgf1, Ctgf, Ang1,* and *Pdgfa* and increased blood vessel formation via H&E staining of the tissue-biomaterial interface. [104] Taken altogether, the literature suggests that improving vascularization during biomaterial placement can dramatically improve tissue repair and material integration.

The formation of fibrotic capsules during the FBR is highly dependent on immunological signals secreted from immune cells that activate quiescent fibroblasts. Fibroblasts are heterogenous populations of cells with diverse phenotypes. The release of TGF-β, vascular endothelial growth

factors (VEGF), and platelet-derived growth factors (PDGF) by macrophages, platelets, endothelial cells, and/or adipocytes recruit local fibroblasts to the FBR.[105] These fibroblasts function to remodel the ECM environment by producing collagens, fibronectin, and proteoglycans to heal tissue.[106] However, during fibrotic encapsulation of biomaterials, fibroblasts contribute to excessive collagen deposition resulting in mechanically stiff scar tissue. Macrophage phenotypes primarily regulate fibroblast behavior and the crosstalk between fibroblasts-macrophages occurs mainly through juxtacrine and paracrine signaling and is reviewed elsewhere.[107] Modulating the behavior of these interactions is also being exploited for biomaterial surface design[108–110] and drug delivery systems[111–113] However, the impact of other immune cells on fibroblast phenotypes in the FBR, such as T cells, will be an area of future investigation.

Constructive tissue remodeling during biomaterial placement depends on the coordinated efforts between stromal and immune cells. The secretion of chemokines and upregulation of cellular adhesion molecules from endothelial cells and fibroblasts at the site of biomaterial implantation recruits immune cells from circulation and local tissues. The chronic activation of endothelial cells by cytokine signaling from immune cells, or vice versa, can sustain or exacerbate the inflammatory process, particularly in the context of the FBR.[114] One approach for improving integration is manipulating the involvement of immune cells, particularly M1 and M2 macrophages, during neovascularization.[115] Using infrared-excited nonlinear microscopy to visualize the 3D structure of the FBR to calcium phosphate-coated medical grade poly(E-caprolactone) (mPCL-CaP), Dondossola *et al*. showed myeloid cells at the scaffold-tissue interface producing vascular endothelial growth factor (VEGF) and promoting immature

neovessel networks. Selective elimination of the FBGCs using clodronate coupled with neutralizing VEGF-A reduced neovascularization and fibrosis.[116] Similarly, in another model of the FBR using subcutaneously implanted degradable cross-linked dermal sheep collagen discs, disrupting a chemokine-glycosaminoglycan (GAG) interaction to prevent leukocyte recruitment resulted in reduced macrophage infiltration and angiogenesis.[117] This suggests how proteoglycan interactions can be targeted to disrupt immune cell recruitment and angiogenic signals to reduce inflammatory cascades in the FBR. Inhibition of colony stimulating Factor-1 Receptor (CSF-1R) in a mouse model implanted with alginate, ceramic glass, and polymer polystyrene spheres led to a complete loss of fibrosis while preserving macrophage function for wound healing.[9] This connection was supported in a zebrafish model where a mutation in the colony-stimulating factor-1 receptor (CSF-1R) and subsequent treatment with hydrocortisone reduced inflammation. This treatment improved revascularization, thereby reducing the development of fibrosis.[118] These studies demonstrate that modulating immune cell behavior can impact angiogenesis during biomaterial implantation. Successful biomaterial integration is dependent on a delicate balance of angiogenesis and immune cell infiltration. However, the formation of abnormal blood vessels can impede repair. A better understanding of these mechanisms and how to fine-tune materials to promote functional and interconnected vessels can limit the FBR.

The role of stromal cells in the FBR has been long appreciated. Fibroblasts are critical during the development of fibrosis through their excessive deposition of ECM. Further, the secretion of cytokines and growth factors from fibroblasts recruit lymphocytes. However, the interaction between certain stromal cells, host factors, and their immune counterparts is just becoming apparent, and future work in co-culture experiments can inform these interactions. Additionally,

the role of pericytes and perivascular fibroblasts, which are essential for vascularization, are beginning to be highlighted. One paper demonstrated the role of implanted synthetic diblock copolypeptide hydrogels (DCH) in neural tissue and showed increases in pericytes surrounding the deposit of DCH with a cationic interface.[119] Interestingly, electrospun polymer scaffolds have shown success in reducing the FBR, while still increasing fibroblast recruitment, offering new strategies to utilize synthetic implants for consistent manufacturing.[120] How these cells may be communicating with immune cells to promote or limit the FBR will be an important area of study in the future. Advanced imaging techniques highlight a new era for understanding vascularization within the FBR and the spatio-temporal heterogeneity of vascular-immune interactions. Single-cell transcriptomic analysis can be used to understand the cell-cell communication via ligand-receptor interactions and how this crosstalk can impact the pathological process of fibrotic encapsulation. Targeting these cellular interactions and their mediators can help to identify therapeutic targets to promote successful material integration.

Senescent cells respond to implanted biomaterials

Senescent cells play a role in embryonic development and tissue homeostasis.[121,122] They are classically defined as cells that (a) have undergone durable cell cycle arrest, (b) express anti-proliferative molecules such as p16 and p21, (c) secrete senescence-associated secretory phenotype (SASP) molecules (most commonly, IL-6, IL-1, IL-8, MMP-1, MMP-3, MMP-13), and (d) have increased β-galactosidase (β-gal) activity in the lysosome.[123–125] It is widely documented that senescence increases with aging, and senescent cells are linked to age-related degenerative and cancerous pathologies.[125]

Whether senescence is primarily beneficial or adverse for health is not well-defined, although it appears to be related to the length of time the cells linger in the body. Senescence can be an anti-cancer mechanism, allowing pre-neoplastic cells to withdraw from the cell cycle.[126,127] Yet, long-term senescent cell presence can lead to chronic inflammation and potential reversion to a cancerous stem-like state.[128] Senescence is also linked to the promotion of tissue repair and wound healing. In contrast, over-accumulation of senescent cells can impair regeneration, potentially due to the chronic presence of inflammatory and pro-fibrotic SASP factors.[129]

Recent literature suggests a link between implants and senescent cell presence. For example, $p16^+$ senescent cells can infiltrate the tissue surrounding human breast implants.[20] Mesenchymal stem cells derived from patients with hip and knee joint implants displayed a phenotype associated with senescence early after isolation, upregulating genes such as *SAA1, SAA2, IL-1β, IL-6*, and *IL-8*.[130] Using computational tools, Cherry *et al.* found senescent pericytes and cartilage-like fibroblasts in scRNAseq datasets of mouse and human FBR.[131]

Several studies suggest that material-dependent properties play a role in influencing the senescent phenotype in cells. In a study comparing chitosan, polyvinyl alcohol (PVA), and poly (2-hydroxyethyl methacrylate) (pHEMA), Tsai *et al.* found that only senescent fibroblasts cultured on chitosan had reduced β-gal activity and increased proliferative capacity. The ability of chitosan to inhibit cell senescence was linked to its number of amino groups and the ionization degree of the amino groups.[132] Similarly, another study found that the charges and functional groups of a biomaterial played a role in inhibition of cell senescence, with the NH_2/positively charged PAAm-based material inhibiting senescence the most relative to polypropylene acid

(PAAc) and polyethylene glycol (PEG)-based materials. In this study, senescence was measured via β-gal staining and protein levels of p16, p21, and p53.[133] Arisaka *et al.* investigated the material property of molecular mobility in polymer chains by modifying the mobility of polyrotaxane polymers. Increasing mobility of the polyrotaxane surface reduced the senescent phenotype in endothelial cells aged on these surfaces, measured by proliferation, spreading size, and β-gal staining.[134] A study of the novel ceramic material baghdadite ($Ca_3ZrSi_2O_9$) found that baghdadite decreased senescence-related gene expression in passage 7 (P7) human osteoblasts and negated the pro-senescent effects of P7 osteoblast secretomes.[135] In another approach to material modification, Zavan *et al.* investigated the effect of VEGF coating on titanium dental implants on senescent mesenchymal stem cells. The cells cultured on VEGF-enriched implants decreased β-gal expression relative to control implants.[136] Interestingly, in all these studies, the favorable outcome of the cell-material interactions appeared to be inhibition of cellular senescence. Given that senescent cells can also have beneficial effects such as promoting tissue repair and wound healing,[129] a potential new avenue of exploration could be to modulate the kinetics of senescent cell phenotype rather than promote complete inhibition of senescence.

In contrast to investigating the effects of biomaterials on senescent cells, others studied the effect of senescence on the biomaterial response. Holt and Grainger found that senescent-like macrophages, compared to non-senescent macrophages, demonstrated reduced phagocytic capabilities.[137] Phagocytosis is a key function of macrophages in the FBR. Phagocytic macrophages clear out debris and apoptotic cells, which regulates the initial inflammatory influx induced by an implant.[138] Reduction of phagocytic capability in macrophages is likely to increase the presence of pathogen-associated and damage-associated molecular patterns (PAMPs and

DAMPs) in the tissue surrounding the implant and extend the timeline of chronic inflammation. Chung *et al.* showed that $p16^+$ senescent cells played a significant role in the fibrosis around PCL particles in mice, as senolytic treatment reduced αSMA staining and fibrotic gene expression at the implant site.[20]

In conclusion, studies have found senescent cells surrounding or infiltrating biomaterial implants in mice and humans, but there is limited understanding of their role in the FBR. Only one study demonstrated that senescent cells were critical in developing murine fibrosis around a PCL implant.[20] The literature suggests that the senescent phenotype depends on the implant's material properties. For example, the link between senescent cells and fibrosis found by Chung *et al.* may be limited to PCL implants. Further studies are needed to clarify the mechanisms (e.g., production of SASP factors, direct interactions with other cell types) senescent cells use to influence fibrosis. Senolytic treatment can be used to determine whether senescent cells contribute to the FBR and fibrosis around various biomaterials. Given that the length of time with which senescent cells linger in the body appears to be linked to their beneficial or adverse effects, it will also be crucial to profile the kinetics of senescent cells after a biomaterial implant. Determining when cells begin to adopt a senescent phenotype and how long these senescent cells remain in the tissue surrounding the implant will help researchers better understand which time points are optimal for therapeutic intervention.

Challenges for targeting the FBR in humans

Gut microbiota

The gut microbiota poses a unique challenge in understanding human biological processes, as it is influenced by various host and environmental factors. Likewise, the gut microbiota influences the function of many tissues and biological processes in the body. This multivalent functionality makes it difficult to isolate the gut microbiota as a causative variable for a single outcome in experimental animal studies and clinical studies. In animal studies, mice can have different gut microbiota depending on (a) the vendor selling them, (b) where they are housed (on the level of the institution, facility, room, and even location on a single rack), (c) the feeds they eat, (d) the bedding in the cage, and (e) how often their cages are changed.[139–142] For example, mice from different vendors showed different levels of Th17 cells in the lamina propria, leading to the discovery of a single bacterium (SFB) responsible for this discrepancy.[143] In humans, the number of potential confounding variables is even greater. The composition of the gut microbiota varies significantly from person to person (depending on age, diet, sex, geography, method of birth, and previous history of injury and infection)[144,145] and varies temporally within each person.[146]

In addition to the many factors influencing the gut microbiota, the microbiota itself affects various factors relevant to the FBR. First, the gut microbiota shapes overall host immunity throughout our lifespan.[147–149] It also affects the immune cells that are both classically known and recently emerging as mediators of the FBR. Kennedy *et al.* summarizes the effects of a dysbiotic gut microbiota (via broad-spectrum antibiotics treatment) and a lack of gut microbiota (via germ-free mice) on innate and adaptive immune cells. Compared to wild-type mice, both antibiotic-treated and germ-free mice have systemically decreased neutrophils, inflammatory monocytes, and macrophages in immune tissues like the bone marrow, blood, and spleen. They also differed in $CD4^+$ T cell subsets and γδ T cells, though the studies focused more on effects on

GI-related tissues such as the small intestine and colon rather than systemic tissues like the blood and spleen.[150] One study even demonstrated that in antibiotic-treated and germ-free mice, the fibrotic capsule surrounding a silicone implant was reduced relative to specific pathogen-free (SPF) mice.[151] Therefore, it is likely that different gut microbiota compositions from person to person will influence the development and outcome of the FBR.

Additionally, the gut microbiota can influence the systemic inflammatory state of the body, an essential factor in modulating the chronic inflammation involved in the FBR. Yoon *et al.* showed that increased diversity of the gut microbiota reduced the neutrophil-to-lymphocyte ratio in the blood, a marker of systemic inflammation.[152] Brandsma *et al.* and Jiao *et al.* demonstrated that pro-inflammatory gut microbiota can increase systemic inflammation (pro-inflammatory cytokine levels) in different disease contexts (atherogenesis and insulin resistance, respectively).[153,154] Increased plasma pro-inflammatory cytokine levels after ischemic injury are linked to specific gut microbiota compositions, such as aged microbiota[155] or the Bacteroidetes phylum.[156] Such studies demonstrate that certain gut microbiota compositions may be linked to an increased baseline state of systemic inflammation, potentially predisposing people to a heightened inflammatory response to a foreign material implant.

Lastly, the gut microbiota is linked to the function of a variety of peripheral tissues, including the brain,[157] skin,[158] liver,[159] and skeletal muscle.[160] It is also linked to tissues that are common locations for medical implants. For example, many patients require joint implants such as titanium-based hip and knee implants. The gut-joint axis has been studied in the context of various joint-related conditions, such as osteoarthritis and rheumatoid arthritis, where the

composition of the gut microbiota has been shown to impact inflammation around the joint.[161] Treatment with certain probiotic bacteria has been shown to mitigate osteoarthritis-associated pain and cartilage degeneration in a mouse model[162] and improve clinical osteoarthritis index scores in patients.[163] Therefore, it is likely that the gut microbiota composition plays a role in the FBR to joint implants and other peripheral tissues.

Sex differences

Though sex differences lead to systemic changes that may influence the FBR, this factor has not yet been studied in the context of a biomaterial implant. Crucially, sex differences affect the innate and adaptive immune cells that contribute to the FBR.[164] Females have neutrophils and macrophages with higher phagocytic capacity[165] and higher counts of $CD4^+$ T cells relative to age-matched males.[164,166–169] Males have overall fewer T cell counts relative to females,[170] but how the T cells differ in terms of cytokine production of IL-2, IL-4, and IL-10 is inconclusive.[171] Given the emerging role of T cells in the FBR, females may have a stronger immune reaction to biomaterial implants due to their increased overall T cell counts and $CD4^+$ T cell counts. In support of this, Xu *et al*. investigated the immunological response to several biological biomaterials and found alginate and agarose increased naïve $CD4^+$ T cells and led to higher Th1 differentiation in females relative to males.[45] Whether higher levels of $CD4^+$ T cells promotes or reduces fibrosis likely depends on the ratio of the different subsets of T cells, as Th17 cells and γδ T cells can promote murine fibrosis[20] while the role of Th2 cells, Tregs, and $CD8^+$ T cells is still unclear. Additionally, the increased phagocytic capacity of neutrophils and macrophages in females may help clear out debris in the tissue surrounding the implant but may also contribute to higher levels of frustrated phagocytosis when macrophages fuse into FBGCs. It is important to

consider the reproductive status of the individual when investigating sex differences in immune response, as sex hormone levels change rapidly for menopausal women while the change is more gradual in males.[172] Post-menopausal women have lower total lymphocytes relative to fertile women[173] but higher levels of IL-2 production from lymphocytes.[174] Therefore, the immune cell changes due to sex can change over time.

One mechanism through which sex differences in immunity occur is through sex hormones.[172] Estrogen skews the immune system towards a Th2 response and B cell-driven antibody response, while testosterone augments the Th1 immune response and increases activation of $CD8^+$ T cells.[172,175] Overactive type 2 immune responses can drive fibrosis,[176] so females with a baseline immune system skewed towards type 2 immunity may develop an increased fibrotic response to a biomaterial implant relative to males. However, studies have demonstrated that type 1 cytokines (e.g. IFN-γ) and $CD8^+$ T cells play a role in the FBR (see **Th1** and **$CD8^+$ T cell** sections), so it is possible that males are equally predisposed to developing a fibrotic response to an implant.

These sex-driven variations in the immune profile are likely to contribute to variations in the FBR. Most studies, however, only study the FBR in the context of one sex. Some studies are performed only in female mice,[20,99] while others use male mice[60,61] without explaining why a specific sex was chosen. Given that sex differences can affect immune responses, an essential outcome in future studies will be to compare the FBR in both sexes.

Aging

Though modern medicine has expanded lifespan, the immune system undergoes age-related changes that function as an accelerator for age-related pathologies, including a failure to heal effectively.[177] As a result, there is increased demand for medical implant devices, including pacemakers, glucose monitors, catheters, and orthopedic implants. Despite their role in supporting or replacing damaged tissue, the FBR and the fibrotic encapsulation of biomaterials ultimately results in device failure and subsequent extraction surgeries to replace them.[14] This poses significant endangerment to the patient's health and a burden on the healthcare system. Therefore, there is a need for understanding the aging factors that impact device performance in order to improve their long-term biocompatibility with aging physiologies.

As we age, the function of our immune system dramatically declines, impairing both innate and adaptive immunity. This dysregulation impacts circulating immune cells, increases the systemic presence of inflammatory mediators, and delays migration and responses to infection or injury.[177–180] Furthermore, an aging immune system is characterized by an increase in low-grade inflammation, called inflammaging, that may be partially responsibility for declining immunity. More detailed age-related changes in immunity have been reviewed elsewhere.[177,181,182] Overall, aging is a key modifier of the immune system and has a significant impact on tissue healing capacity. Despite the cellular changes with age and the prevalence of biomaterial implantation in aging individuals, our understanding of the effect of aging on the FBR is limited.

Most studies comparing the FBR in young and aged animals have assessed innate immune cells, most notably macrophages. One *in vivo* study compared the response to polypropylene mesh implants in 2-month versus 18-month-old mice. The group showed delayed infiltration of

macrophages and increased pro-inflammatory M1-like macrophages.[43] In a separate study, *in vitro* analyses of alveolar macrophages isolated from young and aged rats were exposed to titanium dioxide micro- and nanoparticles. Increased pro-inflammatory mediators, TNF-α and NOS, characterized age-dependent functional changes in macrophages.[183] However, a similar study used bone marrow macrophages from young and aged mice, challenged the macrophages with titanium particles, and showed different macrophage phenotypes. Interestingly, the group did not observe the same changes in inflammatory mediators and expression of transcription factor nuclear factor-kB (NF-KB).[184] Depending on the tissue source from which they are derived, macrophages can show varying responses to biomaterials. Recent work has detailed the role of T lymphocytes in the response to a biological scaffold and found that aging induces senescence and increases IL-17-related signaling that impairs tissue repairs and therapeutic responses to biological scaffolds.[185] In one study looking at sources of xenogeneic biological scaffolds, scaffolds isolated from young pigs mounted a robust regenerative response characterized by an M2 macrophage response and improved tissue remodeling in rats, compared to biologic scaffolds isolated from >52-week-old animals.[186] Xu *et al*. found that alginate and chitosan impaired extracellular matrix remodeling transcriptional signaling while increasing apoptotic pathways in an *ex vivo* model of blood-biomaterial interface from aged humans.[45]

Apart from aging-related changes in immunity, aging has detrimental changes on other physiological processes, such as angiogenesis and fibroblast phenotypes, that contribute to delayed wound healing. There have been several studies documenting the impact of aging on various wound healing models. It has been shown that aged fibroblasts contribute to delayed wound healing due to decreased fibroblast proliferation and migration, and ineffective ECM

remodeling.[187] In another study looking at fibroblast phenotypes in a full thickness skin wound model, histone deacetylase 6 (HDAC6) was attributed to delayed angiogenesis, impaired fibroblast migration, and decreased collagen deposition in aging mice.[188] In a bone formation study, Olivares-Navarrete *et al*. looked at the *in vitro* and *in vivo* age-dependent response on osteoblast maturation to titanium implants in rats. The group showed reduced osteoblast maturation and bone formation in aged animals.[189] It is reasonable to hypothesize that age-associated alterations in growth factors, increased endothelial senescence, and decreased endothelial cell dynamics may contribute to the FBR during device placement.[190]

Despite the rise in medical device implantation in the aging population, little is known regarding the immune response to materials in aging given that nearly all studies are performed in young mice. It is clear that there are immunological differences between young and aged animals. Current aging studies can benefit from standardizing the aging mouse model as many laboratories and research work define the age of aged mice differently leading to inconsistences in result interpretation. Future studies are needed to better understand the role of adaptive immunity in the FBR, the origin of cells participating in the FBR, and the impact of material corrosion on the host response. Approximately 95% of the elderly population live with at least one chronic condition (NCOA), impacting their systemic immunity further. The impact of these comorbidities on the FBR to medical devices and biomaterials will be an area of future study. Such studies would inform better next-generation design of biomaterials and therapeutic strategies for modulating the host immune response to the FBR.

Rodent models

The use of mouse models in biomedical research is grounded in genetic and physiological similarities to humans. Animal models are used extensively to study the mechanism of human disease and test the efficacy of drugs. Although invaluable, mouse models have faced scrutiny due to failure to reproduce data and treatments not translating into humans. Several differences are well-characterized, including metabolic rate, life history, gut microbiota, susceptibility to pathogens, and cognitive development.[191] However, it is important to recognize that animal models have enabled significant advances in understanding human biology, health, and immunology. Before the clinical application of any biomaterial, biocompatibility and the FBR should be assessed and characterized in animal models.

In vivo models for assessing the FBR typically utilize small animal rodents, both wild-type and genetically modified. Implants in mouse or rat models are usually given subcutaneously or intraperitoneally. In a study comparing five rat strains (AO, BN, F344, LEW, and PVG) to three mouse strains (129 SVEV, BALB/c, and C57BL/6), Khouw *et al*. applied hexamethylene diisocyanate cross-linked dermal sheep collagen (HDSC) subcutaneously on the animals' backs.[192] Interestingly, F344 and LEW rat strains had increased T cells compared to AO and PVG rats.[192] Overall, all mouse strains showed decreased giant cell formation and more stroma when compared to rats.[192] Additionally, mice showed limited phagocytosis of the HDSC material compared to rats.[192] Therefore, it is imperative to consider the species and strain-specific context of the response to the FBR to interpret results and understand the biocompatibility of newly designed materials.

The mouse strain also impacts the FBR. King *et al*. implanted alginate/poly-L-lysine(PLL)/alginate capsules into BALB/c and C57BL/6 mice. BALB/c mice showed reduced inflammatory responses via IL-1β and TNF-α gene expression in peritoneal macrophages. Furthermore, C57BL/6 mice showed more fibrous encapsulation using histological examination that was more similar to humans.[193] Another source of immunological variation in mouse models is in housing conditions. These conditions include specific-pathogen-status (which describe the microbiological status of mouse colonies free of a defined list of pathogens), bedding, light/dark cycle, group or single housing, and diet that can shape the immune response to any insult via mechanisms such as the gut microbiota. (See **Gut Microbiota** section for more details.) However, appropriate controls can help interpret data.[194]

The design and biocompatibility of biomaterials would not be possible without animal models. Though there are several limitations to consider when choosing a rodent model, its specific species, and its housing conditions, proper controls can improve the interpretation of results. Using *in vivo* models will continue to drive our understanding of the immune system's role and cellular interactions in the FBR and develop strategies to improve the desired host-material interaction.

Cell-cell networks

Many studies focus on the role of one or several cell types of interest in response to a biomaterial implant. Some, however, focus on interactions between two cell types, such as macrophages and lymphocytes[24] or Th17 cells and senescent cells.[20] These studies demonstrate that the interaction between different cell types can enhance the fibrotic response to a foreign material implant, thus

highlighting the significance of investigating cell-cell networks to understand the FBR. The emerging cell types discussed in this review may interact with the classical cell types (neutrophils, monocytes/macrophages, FBGCs, and fibroblasts) involved in the FBR and participate in undiscovered signaling networks within the local tissue environment. *In vitro* studies are useful first steps in understanding how these interactions contribute to the FBR. Still, these studies require researchers to know which cell types to probe in order to study their interactions. On the other hand, investigating cell-cell networks *in vivo* presents its own set of challenges. *In vivo* environments have many cell types that interact at different times and across various regions throughout the tissue. This multiplicity makes it difficult to conduct controlled experiments concluding whether observed effects are due to one specific interaction or multiple interactions.

New technologies have emerged to study these complex networks. However, it is important to interpret these data with the appropriate controls and orthogonal methods of validation. Spatial transcriptomics, for instance, can map gene activity throughout an entire tissue and determine where the activity is localized. This technique can deduce at the transcriptome level the interactions between different cell types at various locations of the biomaterial implant. Additionally, researchers can use computational algorithms on single-cell transcriptomic data to reconstruct intercellular signaling networks and characterize biomaterial-specific responses.[131] These techniques are critical for deepening our understanding of stromal-immune and senescent-immune cell interactions in the FBR. For instance, the computational algorithm described in Cherry *et al.* could probe the signaling network between $p16^+$ senescent cells and Th17 cells in

Chung *et al*.[20,131] Therefore, as technologies advance, the field can progress toward studying cells in the context of a network of interactions rather than cells in isolation.

Conclusion and future perspectives

In summary, every implant will induce a unique FBR depending on material properties and patient-specific host factors. Though this review focuses on the FBR to synthetic materials, biologically derived materials such as naturally derived polymers and decellularized tissues will generate different immune profiles and T cell responses[195] (e.g. allergic response to bovine collagen,[196] Th2 response to tissue-derived ECM scaffolds,[44] pro-regenerative $CD4^+$ T cell response to acellular adipose tissue[197]). New technologies can probe the immune landscape, its stromal interactions, and emerging cell types such as senescent cells, to determine response to biomaterial implants. Big data approaches such as single-cell RNA sequencing and spatial transcriptomics provide rich data sets that elucidate key cell types, their cellular interactions, and transcriptional pathways in the FBR. The degree of antigenic specificity of the T cell response to biomaterial implants is also emerging as an important consideration. Understanding which antigens lead to which T cell responses and implant outcomes can help researchers design immunomodulatory materials that may influence biomaterial-directed adaptive immunity. For instance, biomaterial design strategies can engage T cell responses via the inclusion of immunostimulatory ligands that promote a tolerant or immunogenic response. These design approaches can be engineered by applying several compositional characteristics, such as epitope content, size, and multivalency, to shape the response to materials.[198] To drive therapeutic advancements in the field, we can utilize both new technologies and standard experimental tools to gain a mechanistic understanding of how antigens, molecular mediators, cells, and pathways

drive dysregulated responses to biomaterial implants. Discovering detailed mechanisms through a holistic approach to targeting the FBR in patients is equally crucial.

Many factors influence an individual's FBR, including age, sex, and gut microbiota composition. These factors are interdependent and interplay continuously throughout an individual's lifetime. Therefore, a therapeutic target for one individual may not be effective for another. Given that the foreign body response is an immunological process, additional consideration should be given to individuals with an underlying immune condition and/or illness.[199] For example, it has been shown that individuals with fibromyalgia, chronic pain syndrome, and various autoimmune disorders are at higher risk of having an adverse foreign body reaction to synthetic implants.[199,200] Underlying conditions such as type 1 and type 2 diabetes predispose individuals to immune system dysfunction and may accelerate the inflammatory response towards implants.[201] Knowing these host-related factors that may influence the immunity to biomaterial implants can continue to drive novel immune-evasive or immune-interactive engineering strategies for biomaterials.

Though we are far from creating patient-specific data sets, the first step towards a personalized treatment approach is to perform studies that investigate the broad influence of these host-related factors (e.g. age, sex, gut microbiota) on the FBR. This approach can be synergized with material design principles that can reduce the overall inflammatory and fibrotic response (by modulating size, shape, charge, surface topography, and surface coating/functionalization),[202] as well as more targeted approaches that aim to modulate T cell activation (by considering epitope content of the material via predictive algorithms[198,203] and modulating surface chemistry to reduce

lymphocyte adhesion[204]). Through this multi-disciplinary approach, we can move further towards the goal of mitigating fibrotic encapsulation of implants and reducing a strong, inflammatory immune response in a large patient population.

Conflict of interest statement

J.H.E. is an inventor on intellectual property related to biological scaffolds and inhibiting fibrosis. J.H.E. holds equity in Unity Biotechnology and Aegeria Soft Tissue. J.H.E. is a member of the scientific advisory boards of ACell Inc., Camden Nexus Fund, Histogenics, Tessera Therapeutics, HapInScience, and Font Bio. J.H.E. is a consultant for Vericel.

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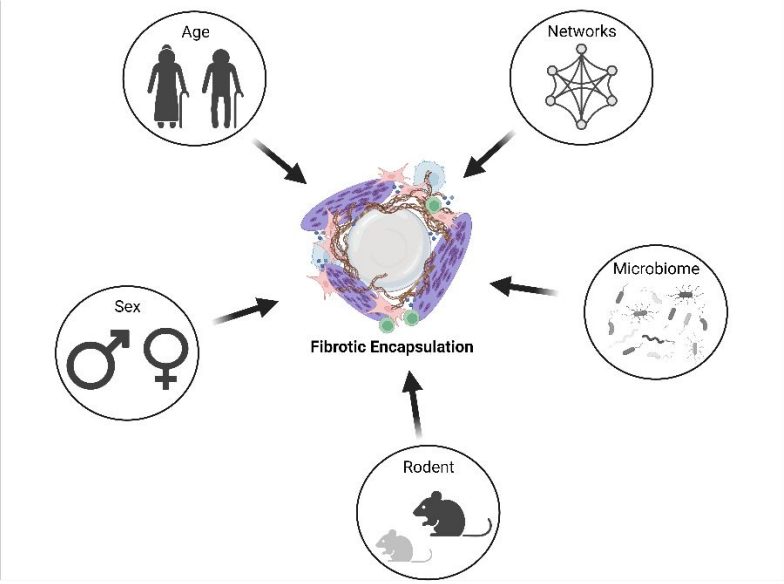

Figure 1. Considerations for assessing the FBR
During fibrotic encapsulation to a synthetic biomaterial, dense extracellular matrix is deposited by fibroblasts. The implant is surrounded by foreign body giant cells (FBGCs), lymphocytes, cytokines, and senescent cells. Understanding and characterizing the FBR microenvironment can be confounded by several variables, including age, sex, gut microbiome, rodent model, and cell-cell interactions. Created with BioRender.com.

	Cytokine production	Role in FBR
Th1	IFNγ, IL-12	• Early pro-inflammatory stages of the FBR[28] • Macrophage polarization to an M1 phenotype[28] • Inflammatory angiogenesis and fibrogenesis[31] • Development of fibrotic capsular tissue[29]
Th2	IL-4, IL-5, IL-13	• Resolving type 1 and type 17 inflammation[38] • Macrophage polarization to an M2 phenotype[7,17,22,39,46] • Macrophage fusion[7,39] • Recruit stromal cells[38]
Th17	IL-17a, IL-17f, IL-22, IL-23	• Found near implant site[29,60,61] • IL-17 production[20,29] • Increased Th17 differentiation after interaction with macrophages[33,61] • Interact with senescent cells to contribute to fibrosis around implant[20]
Treg	IL-10, TGF-β, IL-33	• Cross-talk with M2 macrophages[28] • Treg plasticity[29,69]
γδ	IFNγ, IL-17a, IL-17f, TNFα	• IL-17 production[20] • Contribute to fibrosis around implant[20]
CD8	IFNγ, TNFα	• Found near implant site[17,66,96,99] • Increased response based on roughness of implant surface[99]

Table 1. Summary of the role of T cell subsets and their cytokine signaling in the FBR.