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Review

Manipulating immune activity of macrophages: a materials and mechanics perspective

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Macrophage immune cells exist on a plastic spectrum of phenotypes governed by their physical and biochemical environment. Controlling macrophage function to facilitate immunological regeneration or fighting pathology has emerged as a therapeutic possibility. The rate-limiting step in translating macrophage immunomodulation therapies has been the absence of fundamental knowledge of how physics and biochemistry in the macrophage microenvironment converge to inform phenotype. In this review we explore recent trends in bioengineered model systems that integrate physical and biochemical variables applied to macrophage mechanosensing and plasticity. We focus on how tuning of mechanical forces and biomaterial composition orchestrate macrophage function in physiological and pathological contexts. Ultimately, a broader understanding of stimuli-responsiveness in macrophages leads to informed design for future modulatory therapies.

Highlights

Macrophages exist on a plastic spectrum of phenotypes, responding to physical and biochemical stimuli.

Physical forces such as fluid flow amplitudes titrate mechanosensitive macrophage plasticity without altering macrophage recruitment.

Tissue-scale and extracellular matrix (ECM)-scale strains alter macrophage response to inflammatory stimuli.

ECM-derived peptides trigger variable macrophage release of immunomodulatory cytokines by engaging adhesive ligands.

Geometry and topography of biomaterial-bound bioactive factor presentation also variably polarize macrophages.

Macrophages are governed by their environment

Macrophages are phagocytic myeloid immune cells that eliminate pathogenic microbes and dead cells [1–4]. These cells exist on a plastic spectrum of phenotypes, including canonical proinflammatory (M1) and pro-regenerative (M2) states (Box 1) [5,6]. Macrophage plasticity has been reviewed extensively in recent years [5,7–9]. Macrophages alter their biochemical and metabolic pathways to reflect functional rewiring in response to changes in microenvironmental stimuli [10–12]. This review focuses on *in vitro* studies modeling the macrophage microenvironment, shedding light on how microenvironmental cues alter macrophage behavior.

The macrophage microenvironment is highly complex, with molecular, cellular, and tissue-level forces driving macrophage behavior. Looking from an external perspective, mechanical forces as well as biological and physical cues impact the macrophage microenvironment [13]. Forces of the macrophage microenvironment come from native tissue movements, **extracellular matrix (ECM)** (see Glossary) interactions, and cell–cell-based forces [14–16]. The ECM is composed of fibrillar materials (collagens), structural glycoproteins (laminin), and other proteoglycans that act as reservoirs for biomolecules and adhesion ligands [16]. By virtue of its composition, the ECM directly contributes to mechanical properties of tissues. Alterations in ECM composition, as well as applied forces from native tissue mechanics, drive macrophage plasticity.

To respond to changes from external stimuli, macrophages sense changes in mechanical or physical forces through cell adhesion receptor/ligand interactions, cytoskeletal components, and mechanosensitive ion channels [14,15,17] (Figure 1). Integrins govern a large part of macrophage function by mediating cell–cell and cell–ECM adhesion [18]. ECM composition thus directly affects integrin–ligand interactions by altering presentation of ECM ligands. Downstream of integrin signaling, the actin cytoskeleton also mediates macrophage mechanosensing [14]. In response to externally applied or sensed physical forces, the cellular actin structure undergoes remodeling to

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Box 1. Biological and historical context of macrophages and their phenotypes

Macrophages are highly plastic antigen-presenting cells with the ability to adapt to their external microenvironment. Traditional classification of macrophages for *in vitro* studies are: (i) M1, or classically activated, and (ii) M2, or alternatively activated [83]. M1 macrophages attain an inflammatory profile following exposure to lipopolysaccharide (LPS) and interferon γ (IFN-γ) [84,85]. M2 macrophages are activated by interleukin-4 (IL-4) and/or IL-13 to adopt a pro-regenerative or anti-inflammatory state [84,86]. M2 macrophages can also be further divided into several subtypes based on their function and key surface markers (M2a, M2b, and M2c) [87,88]. Recent studies are straying away from this traditional nomenclature as macrophage phenotypes *in vivo* are typically beyond the realm of M1 and M2. In most cases, the phenotype depends on pathology and the surrounding microenvironment, creating a heterogeneous population [89,90]. For example, a study conducted on human skin samples with discoid lupus erythematosus discovered co-expression of M1 and M2 macrophage-related proteins on $CD163^{+}$ macrophages. Other terminology often used to define macrophage state reflects the activator used for *in vitro* studies. For example, LPS and IFN-γ-activated macrophages are labeled M(LPS+IFNγ), while IL-4-activated macrophages (traditionally M2a) are called M(IL-4) [91]. Additionally, M2 subtypes activated by tumor growth factor β (TGFb) (M2c) and IgG+LPS (M2b) were called M(TGFb) and M(IC+LPS), respectively. Importantly, the complexity of macrophage phenotypes is yet to be fully understood *in vivo*. While we appreciate the complexity of macrophage phenotypes in vivo, for simplicity within published literature, this review uses proinflammatory or pro-regenerative/anti-inflammatory terminology as descriptors for all cited studies.

Macrophages are also critical in cancerous environments. Macrophages of solid tumor microenvironments are called **tumor-associated macrophages (TAMs)** [92]. TAMs orchestrate angiogenesis, metastasis, and ECM remodeling [93]. For example, proinflammatory macrophages typically enhance antitumor immunity while pro-regenerative macrophages are pro-tumoral in function [94].

It is also important to note that macrophages do not exist independently of other immune cells. Specifically, the antigen presentation of macrophages is pertinent to activate adaptive immune cells, including T cells and sometimes even B cells. The interactions between macrophages and other immune cells also dictate macrophage behavior. This crosstalk has been fantastically reviewed in recent publications [95,96].

promote downstream signal transduction. When physical forces alter tension in the cell membrane, mechanosensitive ion channels also activate downstream signaling [17]. **Pattern recognition receptors (PRRs)**, such as **Toll-like receptors (TLRs)**, and cytokine receptors are also directly implicated in dictating macrophage behavior [19,20]. PRRs and other membrane-associated receptors will activate downstream transcription in response to specific stimuli. Together, sensory input relayed by cell adhesion receptors, the cytoskeleton, ion channels, or membrane-level receptors causes phenotypic and functional alterations in macrophages (Figure 1). The intricacies of the molecular mechanisms employed by macrophages to initiate downstream changes have been reviewed extensively in recent years [11,15,21].

With numerous mechanical, physical, and biological factors at play in the macrophage microenvironment, building systems at the intersection of materials and mechanics are key to increasing our understanding of macrophage function. Traditional engineering approaches studying cellular response to forces rely heavily on 2D-based systems and animal models. These have been instrumental in our early understanding of cellular mechanotransduction, including the understanding of macrophage mechanosensing [14,22]. Recent advances in bioengineered platforms, however, facilitate cellular interactions with dimensionality, that is, they provide spatial variations in mechanosensing or ligand presentation that better mimic conditions *in vivo* [23–25]. By engineering novel systems, researchers can study the nuanced relationships between physics and macrophage activation.

Understanding that macrophages respond uniquely to their microenvironment, this review describes engineering approaches to study macrophage mechanoresponse. Approaches that mimic mechanical and physical stimulation for macrophages are examined. We close by articulating two key aspects of future work for studying macrophages: incorporating phenotype plasticity over time and integrating equitable learning of macrophages considering variables such as age, sex, and demographics. Strengthening the understanding of a macrophage's response

Glossary

Extracellular matrix (ECM): a complex network of structural and functional components and glycoproteins that function synergistically with cells to inform cell fate.
JAK/STAT: Janus kinase/signal transducers and activators of transcription pathway, an essential signaling pathway that controls various biological processes, including hematopoiesis and immune cell development through regulation of cytokine expression.
NF-κB: nuclear factor κ-light-chain-enhancer of activated B cells, a transcription factor that is critically involved in inflammasome activation and cytokine release.
Pattern recognition receptors (PRRs): proteins capable of recognizing molecules frequently found in pathogens or molecules released by damaged cells.
Piezo 1: a mechanosensitive ion channel protein that is activated by sensing mechanical forces.
Rho activator (RhoA): a member of the Rho family of small GTPases which function as molecular switches in signal transduction cascades. Rho proteins promote reorganization of the actin cytoskeleton and regulate cell shape, attachment, and motility.
ROCK: Rho-associated coiled-coil-containing protein kinase, a small GTP-binding protein that operates via downstream mediators to regulate various aspects of cell shape, motility, proliferation, and cell death.
Toll-like receptors (TLRs): crucial receptors that recognize foreign organisms so the body can distinguish between 'self' and 'non-self'.
Tumor-associated macrophages (TAMs): macrophages that can be recruited or are tissue-resident in tumors. These macrophages can be antitumoral or protumoral in function.

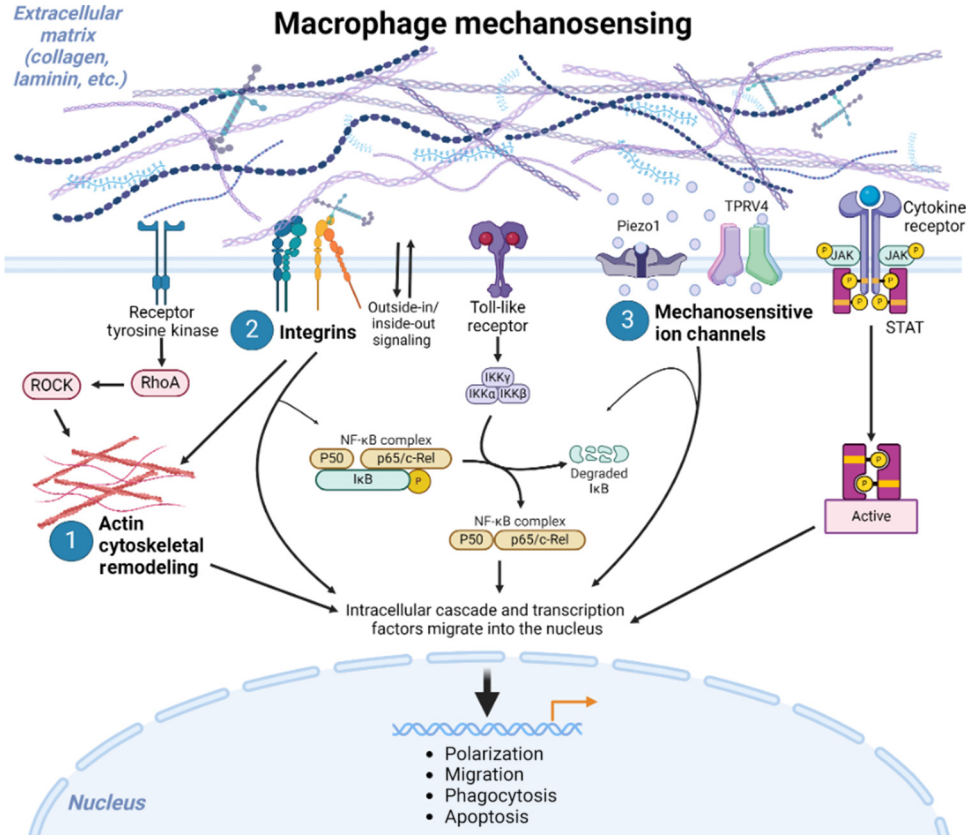

Figure 1. Macrophage mechanosensing. Three main mechanisms are implicated in translating mechanical cues into cellular responses in macrophages: (i) actin cytoskeletal components, (ii) integrins, and (iii) mechanosensitive ion channels. The actin cytoskeleton can independently mediate macrophage mechanosensing through Rho activator (RhoA)/ROCK signaling while also being affected downstream of integrin signaling. In response to physical force changes, the actin structure will undergo remodeling and promote downstream signal transduction. Integrins are crucial in governing macrophage function by mediating cell adhesion to extracellular matrix components and to other cells. Alterations in the matrix architecture and cytoskeletal stiffness are sensed by integrin–ligand interactions through outside-in and inside-out signaling. Mechanosensitive ion channels, such as Piezo1 and TRPV4, are opened when physical forces alter the tension in the cellular membrane. Following any course of macrophage mechanosensing, intracellular signals are then sent to the nucleus through numerous activation pathways where transcription factors induce different polarization states or functional alterations including phagocytosis, migration, or apoptosis. Activation pathways are complex in nature and inherently have a lot of overlap in methods of activation. For example, the **RhoA/ROCK** pathway and the resulting actin structure is primarily activated by receptor tyrosine kinases, but can also be activated by integrins. Further, many other molecular mechanisms are implicated in macrophage behavior through pattern recognition receptors (PRRs), such as **Toll-like receptors (TLRs)** and cytokine receptors. Important pathways like nuclear factor κB (NF-κB) and JAK/STAT are activated via membrane-associated receptors in response to external stimuli. NF-κB is another pathway example that can be activated by numerous molecular mechanisms such as integrins and mechanosensitive ion channels. Abbreviation: IKK, IκB kinase. Figure created with BioRender.

to its microenvironment will help in developing better immunomodulatory platforms for therapeutic solutions to diseases.

Exploring engineering approaches to understand macrophage response

External application of mechanical forces

To advance the study of macrophage mechanobiology, researchers endeavor to apply physical forces found *in vivo* as external stimuli. One such stimulus is interstitial flow, originating from fluid

movement through the ECM of tissues, often driven by pressure differences. Interstitial flow, a pronounced physical cue in tumors, is also an interesting avenue to understand impact on macrophage function. Song and colleagues created a microfluidic device where an interstitial fluid flow gradient was established between a microvessel channel and a tumor aggregate channel (Figure 2) [26]. Biochemical cues from cancer cell aggregates reprogram macrophages into a pro-tumoral state. In this study, interstitial fluid flow enhanced macrophage pro-tumoral activation, reciprocally promoting tumor cell invasion. Independently, Li and colleagues used a collagen

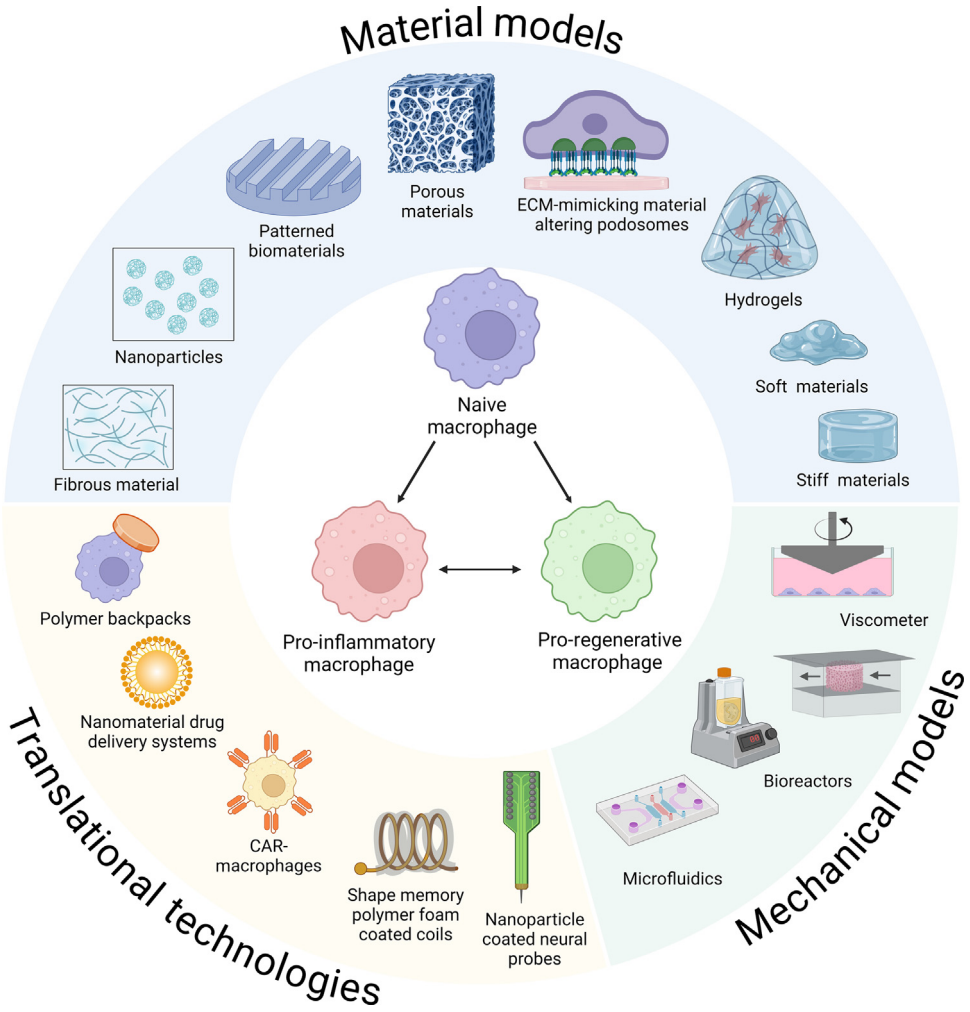

Figure 2. Model systems and translational technologies dictating macrophage behavior: naïve macrophages are influenced by their environment to adopt a proinflammatory state, a pro-regenerative state, or a mix of both. Macrophages can also be re-educated to go from a proinflammatory to a pro-regenerative state or vice versa. This review covers both material- and mechanical-based models that demonstrate how forces and environmental interactions can influence macrophage behavior. Depending on the forces applied, such as shear stress, compression, and tension, or the properties of the materials, including stiffness, architecture, or source, macrophages will respond accordingly. With the information gained from both material- and mechanical-based models, researchers can design translational technologies for clinical applications to dictate macrophage function. Some recent examples of translational technologies are shape memory-polymer-foam-coated coils for aneurysms, polymer backpacks, neural probes with nanoparticles, chimeric antigen receptor (CAR)-macrophages, and other nanomaterial drug delivery systems. Abbreviation: ECM, extracellular matrix. Figure created with BioRender.

I-based engineered tumor microenvironment platform to verify that interstitial flow directs macrophage migration and pro-regenerative plasticity through βI integrin signaling [27]. Compared with cultured platforms without flow, with interstitial flow macrophages were significantly stimulated towards a pro-regenerative state, underscoring the importance of studying the physical aspects of immune modulation, especially as researchers tailor immunotherapy platforms.

In addition to low levels of interstitial flow (<0.01 Pa) [28,29] within tissues, the most prevalent form of flow in the body is fluid shear stress, arising from blood flow in the vasculature (0.035–7 Pa) [29,30]. Using perfusion bioreactors that create flow within cancer-cell-laden collagen sponges, Manfredonia and colleagues demonstrated that fluid flow itself did not regulate macrophage recruitment to a 'tumor site' [31]. In the context of tissue regeneration and pretreatment, Han and colleagues showed that preconditioning scaffolds with perfusion *in vitro* drives pro-tumoral polarization *in vivo* [32]. Natural ECM-based scaffolds were seeded with adipose-derived stromal cells and exposed to perfusion before *in vivo* implantation in mouse models. Stimulation with perfusion caused the adipose stromal cells to secrete immunomodulatory cytokines, including interleukin 10 (IL-10). In both perfusion studies, the mechanical stimulus itself was not responsible for increased macrophage recruitment, rather it reprogrammed macrophages into a pro-tumoral state directly or indirectly.

An additional application of shear force is understanding the long-term effects of microgravity on macrophages in the context of spaceflight. In the work by Ludtka and colleagues with a rotating wall vessel bioreactor [33], macrophages were exposed to simulated microgravity where gravity is counterbalanced by hydrodynamic shear, centrifugal, and Coriolis forces [34]. On microgravity exposure, macrophage populations co-expressed proinflammatory and pro-regenerative-associated markers due to altered mechanosensing capabilities of the cells. Rotating wall vessel bioreactors have numerous applications, but this unique operation to simulate spaceflight conditions highlights the need to further study macrophage response to microgravity (Figure 2). This example also underscores the complex nature of macrophage phenotypes, with the mixed expression of markers indicating macrophage state.

The distinct advantage in using bioengineering approaches to study macrophage activation is the ground-up complexity one can build into the system, as demonstrated using the example of atherosclerosis. Cholesterol accumulation results in obstruction of blood vessels, altering shear stress. Immune-mediated dysfunction in this process is well documented [35]. Jui and colleagues provide a preliminary approach to studying macrophage response to shear stress using cone and plate viscometers (Figure 2) [36]. Their work demonstrated that both shear stress magnitudes (3.5 Pa) and duration (48 h) cause macrophages to adopt proinflammatory signatures. Building nuance to expand the understanding of macrophage mechanobiology, Menon and colleagues created a microfluidic vessel constriction model [31]. Macrophages were circulated within the device through channels that were constricted via pneumatic activation, subjecting them to shear stresses similar to those in atherosclerosis. Flow disturbances around atherosclerotic constrictions resulted in increased macrophage adhesion, which could result in local amplification of inflammation. Both these examples highlight how bioengineering approaches mimicking shear stress provide unique opportunities to decode macrophage mechanobiology.

In addition to fluid shear stress, solid stresses are often present in the macrophage microenvironment as a result of compressive and tensile strain at the tissue and cell levels. Exogenous application of strain via cyclic uniaxial stretching resulted in macrophages expressing higher levels of the integrin CD11b, while simultaneously reducing the expression of the mechanosensitive ion channel **Piezo-1** [37]. Macrophages were exposed to strain on a custom-built device that

used servomotors to stretch a macrophage-seeded silicone substrate [38]. These studies demonstrated ultimately that external application of strain alters how macrophages respond to inflammatory stimulation via interferon-γ (IFN-γ). Understanding that the physical stimulus synergizes with biochemical cues to regulate macrophage function is applicable to studying numerous biological processes where inflammatory behavior is present.

ECM-scale strain is not just vital for macrophage activation, but also to direct macrophage migration via integrin binding and stretch-sensitive ion channel activity. In the context of the fibrotic lung, fibroblasts create pathological strain fields that recruit macrophages to amplify local inflammation [39]. Translation of this fundamental knowledge of macrophage response to strain in the context of the lung has led to the recent development of a mechanosensitive therapy to mitigate ventilator-induced lung injury. Here, a nanoparticle carrier delivered a mechanosensitive microRNA to mitigate macrophage-induced inflammation in the lung [40].

Zooming out from ECM-scale strains to strains from tissue-level movements, Schroder and colleagues used a glass plate to create compression and a flexible membrane to create tension to mimic orthodontic tooth movement [41]. This work demonstrated that macrophages independently respond to compressive or tensile strain. Both types of force applications enhanced proinflammatory signatures of macrophages. With a proinflammatory signature, macrophages can impact fibroblasts, osteoblasts, and immune cells by triggering an immunological response. In the context of orthodontics, this response leads to increased bone resorption allowing for orthodontic tooth movement.

Tissue-level movements are also present in skeletal tissues where macrophages play an important role in healing and bone remodeling. Fahy and colleagues seeded monocytes in agarose gels that were dynamically modulated using a multiaxial load bioreactor that can apply compression, shear, or a combination of the two [42]. These applied forces and skeletal-tissue-like matrix mimicked the tissue environment of a bone fracture. Shear and compression independently and concurrently resulted in increased proinflammatory cytokine production necessary for bone regeneration. With combinatorial models like this, researchers can create nuanced mechanical environments for bone loading to better study mechanically regulated healing pathways.

While each of these models mimicked shear stress or strain forces, they are unique in how they apply that force based on their pathological application. Importantly, their results all differ due to different force patterns, frequency, amplitude, etc., as expected to be found in the *in vivo* environment. Despite context-dependent results, these examples provide clear evidence that bioengineering approaches allow us to study nuances in macrophage behavior. Understanding how shear stress and strain forces directly impact macrophage function is key to advancing our knowledge of immunomodulatory platforms in the diagnosis and treatment of disease.

Physical and biochemical cues from material microenvironments

The 3D structure of the ECM adds complexity in biochemical signaling and biophysical forces sensed by macrophages [18,43]. When monocytes travel to tissue-resident sites, they will respond differently to macrophages conditioned in the tissue microenvironment due to ECM composition, matrix stiffness, etc. In the tissue microenvironment, material source, ECM ligand presentation and spatial variations therein, topography, and stiffness all dictate macrophage function (Figure 2). Researchers have reconstructed aspects of the ECM using natural or synthetic materials to better understand how the ECM modulates macrophage function. These approaches include a range of formulations from the use of whole ECM proteins, which retain natural conformation and biochemical cues, to isolation of specific immunomodulatory domains, which offer precise tunability of biochemical and physical cues.

Decellularized ECM (dECM) is a versatile natural material used widely to study macrophage immunomodulation. The composition, architecture, and retained bioactive cues of the dECM depend on the organ source, as well as on processing technique [44]. For example, dECM scaffolds derived from porcine brains promoted pro-regenerative macrophages *in vitro*. Implantation of these scaffolds for spinal cord injuries continued to promote the pro-regenerative phenotype, leading to positive outcomes in neuronal outgrowth [45]. Retained bioactive factors within the dECM, such as osteopontin, actively contribute to macrophage reprogramming. Interestingly, solubilizing dECM from the brain elicits a proinflammatory phenotype [46], which leads to the speculation that processing and spatial presentation of dECM from specific tissues of interest can determine macrophage response. Furthering this point, intact or solubilized dECM from the spinal cord or small intestinal submucosa promoted pro-regenerative phenotypes in many regenerative healing contexts [47,48]. Collectively, these studies suggest that the tissue source and, thus, processing of bioactive factors isolated from various dECM sources informs macrophage functional response. Characterizing which factors are retained versus which are lost in processing is important when considering dECM material-based immunomodulation.

In addition to processing, hydrogel pore size of dECM can dictate macrophage phenotype (Figure 2). For example, hydrogel pore size informs macrophage plasticity in the scenario of a fungal (*Candida albicans*) infection [49]. Cicuédez and colleagues demonstrate that macrophages encapsulated within dECM components from porcine adipose tissues respond to pore size by altering their phenotype. A larger pore size induced pro-regenerative macrophages and promoted increased phagocytosis [49]. Thus, understanding how material porosity affects macrophage phenotype is of critical consideration, especially considering encapsulation of macrophages and optimization for material design.

The concept of porosity dictating macrophage function is also seen in collagen–chitosan scaffolds. Yin and colleagues found that porous collagen–chitosan scaffolds promoted a transient inflammatory phenotype at earlier timepoints which then switched to a regenerative state at later timepoints [50]. The plastic re-education of proinflammatory macrophages to pro-regenerative ones subsequently enhanced angiogenesis both *in vitro* and *in vivo*. Larger pore sizes supported macrophage phenotype switching, likely due to increased cellular invasion. This is an example of employing porosity to harness macrophage function and improve vessel regeneration.

Along with porosity, micron-scale geometry and topographical features (surface roughness) are also incorporated in biomaterial design. Fibrillar polycaprolactone (PCL) scaffolds with various pore sizes were used to assess how pore size and elongation induce certain macrophage phenotypes [51]. As the box-shaped pore decreased from 100 μm to 40 μm, macrophages increased expression of anti-inflammatory or pro-regenerative markers and cellular elongation. The reduced pore size provided the mechanical support and spatial cues for changes in morphology through confinement geometry. These studies emphasize how using biomaterial approaches with changes in physical features alter macrophage function.

Modulating biomaterial surface roughness is another cue to improve tissue integration and prevent macrophage inflammation. Chen and colleagues modified polydimethylsiloxane (PDMS) structures with microgrooves of varying depth and width to understand macrophages in fibrosis [52]. After comparing different microgroove combinations, groove depths and widths of 50 μm promoted the highest level of macrophage adhesion compared with the other combinations of depth and width. Thus, roughness as a topographical variable can be employed to direct macrophage orientation, migration, and polarization.

Changing topography can also alter stiffness of biomaterials, making stiffness an important criterion when emulating physical properties in materials (Figure 2). With increasing substrate stiffness, macrophages can be reprogrammed from the proinflammatory to pro-regenerative state as demonstrated in a study using polyacrylamide hydrogels [53]. Comparing the hydrogel stiffness levels with those of collagen fibers, osteoid tissue, and pre-calcified bone, it was found that lower collagen fiber-like stiffness was favorable to drive proinflammatory phenotypes. By contrast, the higher osteoid-like stiffness initiated anti-inflammatory or pro-regenerative reprogramming. All stiffness levels induced actin cytoskeletal, morphological, and oxidative changes to macrophages due to activation of the **NF-κB** pathway (Box 2). Specifically, with low stiffness, enhanced reactive oxygen species (ROS) production activates the NF-κB pathway, giving rise to proinflammatory signatures in macrophages. Thus, transcription factor activity in macrophages is mechanically regulated given differential macrophage responses with each substrate stiffness.

Combinatorial materials can also dictate macrophage phenotype through a combination of physical and biochemical cue combinations. For example, by combining PCL with silk fibroin (SF) and carbonate hydroxyapatite, Jia and colleagues leveraged the pro-regenerative potential of macrophages for bone regeneration [54]. While PCL/SF maintained mechanical properties, carbonate hydroxyapatite promoted pro-regenerative macrophages via **JAK/STAT** pathways (Box 2). STAT5, a member of the JAK/STAT family, suppresses inflammatory cytokine expression and promotes expression of pro-regenerative marker Arg1. Therefore, using combinatorial materials,

Box 2. Mechanisms of macrophage polarization

Macrophage polarization to a proinflammatory or pro-regenerative state occurs via extracellular signals that cause downstream alterations in transcription factors and cytoskeletal architecture (Figure 1 in main text) [97,98]. These intracellular changes culminate in and promote macrophage phenotypes. Several intracellular signaling pathways play key roles in dictating macrophage polarization, such as nuclear factor κB (NF-κB), activation of Janus kinases (JAK) and signal transducers and activators of transcription (STAT) (the JAK/STAT pathway), and the actin-related pathway Rho activator (RhoA) and Rho-associated coiled coil-containing protein kinase (ROCK) or RhoA/ROCK.

JAK/STAT

The JAK/STAT pathway can be modulated by regulatory proteins that control the intensity and duration of the cytokine responses [99,100]. Suppressors of cytokine signaling (SOCS) proteins are negative feedback regulators that directly interact with JAKs by binding to the cytokine receptor or JAK [101,102]. SOCS1 has been used to understand its role in the regulation of activation states of monocyte-derived macrophages [55,103,104]. Downregulation of SOCS1 expression was shown to upregulate the JAK/STAT pathway to promote proinflammatory macrophages. Blocking STAT1 with SOCS1 increased proinflammatory behavior upon IFN-γ activation. Therefore, STAT1 is a key transcription factor involved in proinflammatory macrophage polarization. However, STAT6 has been considered to be the pathway to activate pro-regenerative macrophages upon IL-4/-13 stimulation [55,105,106]. Using molecules targeting the JAK/STAT pathway can be beneficial to modulating macrophage polarization [107].

NF-κB

NF-κB transcription factor is critically involved in inflammasome activation and cytokine release [108]. Among the major mediators of NF-κB pathway are reactive oxygen species (ROS) [109]. By enhancing IκB phosphorylation, ROS activates the NF-κB pathway participating in proinflammatory activation. ROS are also shown to regulate pro-regenerative states by activating extracellular signal-regulated kinase (ERK) signaling [110,111]. Activation of NF-κB in macrophages by TLRs can drive macrophage polarization depending on pathological conditions [112,113].

RhoA-ROCK

The actin cytoskeleton is a crucial determinant of macrophage function by mechanotransduction [114]. The actin cytoskeleton is highly dynamic and is regulated by the ROCK pathway. Rho activator (RhoA) activates ROCK which increases binding of myosin to actin, thereby promoting macrophage contractility. Without RhoA, macrophages tend to migrate faster, leading to rapid recruitment to the source of inflammation [115]. RhoA pathway interference has also been shown to promote proinflammatory states and inhibit pro-regenerative states [116]. In the context of organ transplant rejection, inhibition of the RhoA/ROCK pathway has been demonstrated to prevent chronic rejection by inhibiting macrophage infiltration [117,118].

researchers activated the STAT5 pathway inducing pro-regenerative reprogramming. Upregulation of the pro-regenerative state in turn promoted osteoblast differentiation enabling better bone regeneration. This study took a combinatorial approach wherein retention of mechanical strength with chemical cues culminated in a different phenotypic state in macrophages.

Biochemical modifications through ligand presentation trigger a wide range of cytokine/chemokine secretions. Using intracellular peptides or ECM-derived peptides allows for engineering biochemical properties in biomaterial design [55,56]. ECM-derived peptides function through integrin–ligand signaling, either blocking or binding their cognate receptors [57,58]. For example, using peptides to promote adhesion such as fibronectin-derived RGD has modulated macrophage function through integrin interactions [59,60]. Translating this knowledge to bone regeneration, Wu and colleagues functionalized RGD to the endogenous molecule phosphatidylserine [61]. RGD–integrin engagement enhanced macrophage adhesion, phagocytosis, and pro-regenerative macrophages, ultimately promoting bone growth. The Moore group investigated integrin–ligand signaling through collagen-derived peptide DGEA [62]. DGEA functionalized to polyethylene glycol (PEG) hydrogels inhibited proinflammatory macrophage function by blocking integrin α2β1. These studies highlight the importance of leveraging integrin–ligand signaling to govern macrophage behavior. Which integrins participate in macrophage alterations and what mechanisms are employed are not yet fully understood.

Understandably, due to differences in integrin activation, peptides derived from different segments of ECM proteins may impact macrophages differently. Specifically, laminin-derived IKVAV and YIGSR demonstrate distinct biological functions in macrophages. For example, PEG–IKVAV hydrogels promoted anti-inflammatory macrophage function [55]. In another study, a YIGSR–fibrinogen conjugate reduced proinflammatory polarization over time in a wounded submandibular gland of female mice [63]. Without YIGSR, matrix formation was disorganized compromising healing. While the fibrinogen scaffold offered physiologically relevant properties, YIGSR was important in attenuating inflammatory macrophage behavior. The studies with IKVAV and YIGSR highlight nuances with using peptides from the same ECM protein to modulate macrophage function. Ultimately, the field can now appreciate that macrophage function is governed by integrin signaling and can be attenuated in the presence of specific ligand cognate pairs.

A direct translation employing biochemical cues is seen in engineering diabetic wound dressings with the use of protocatechuic aldehyde. Fu and colleagues developed an all-natural immunomodulatory hydrogel to re-educate proinflammatory macrophages to pro-regenerative macrophages in a diabetic wound model [63]. Protocatechuic aldehyde, isolated from *Salvia miltiorrhiza,* has a unique phenolic aldehyde structure. When combined with fish gelatin and photocross-linked with gelatin methacrylate (GelMA), to bolster hydrogel strength and adhesiveness, protocatechuic aldehyde has demonstrated antibacterial and anti-inflammatory properties. This combined material promoted wound closure and reduced macrophage-mediated inflammation. Moreover, the materials increased macrophage scavenging function which increased clearance of microbes. The hydrogel preserved crucial biochemical cues while presenting the protocatechuic aldehyde, a biochemical cue, necessary to reduce inflammatory signatures of macrophages in diabetic wounds.

These biomaterial models highlight the impact of mechanical and biochemical alterations in governing macrophage function. Tuning properties like stiffness or porosity, independently or in conjunction with biochemical modifications, underscore the wide range of design criteria for attenuating macrophage plasticity. These biomaterial approaches are also adaptable to several pathophysiologies. Such engineering approaches that consider the range of mechanical and biochemical cues in governing macrophage function will continue to increase our understanding of macrophage function and plasticity in response to microenvironmental cues.

Translational technologies in macrophage immunomodulation

So far, we have extensively discussed how macrophage function is altered with mechanics and materials. This knowledge naturally cascades into technology development that tunes macrophage immunomodulation in diverse translational settings using mechanics and materials [13]. Genetic reprogramming and *in situ* bioactive factor delivery (e.g., cytokines or antibodies) remain at the forefront of current biotechnologies aimed at macrophage re-education in diverse disease contexts. Many of these innovative technologies do not explicitly consider the mechanics or material repercussions on macrophage polarization discussed in earlier sections. Technologies that consider these variables explicitly are certainly few and far between.

For example, neural probes and implantable bioelectronics are examples of mechanics and material-informed technologies. Milli-scale fluid shear stresses generated during neural probe implantation and micromotion at the electrode/tissue interface often amplified proinflammatory immune responses, leading to compromised device function [64]. A combinatorial mechanics/material approach involved creating electrodes with alternative materials that matched soft neural tissue stiffness (and therefore flexible); this approach significantly reduced the proinflammatory activity of macrophages [65]. A purely material-inspired approach in this space involved modified surface roughness and area of neural probes with nanoparticle and bioactive factor coatings to reduce inflammation [66]. Similar material-inspired approaches are also seen in shape memory polymer foam-coated coils in aneurysm occlusion devices. Enhancing surface roughness of smooth and bare platinum coils significantly reprogrammed macrophages into a pro-regenerative phenotype, resulting in enhanced healing of aneurysms [67–69].

In a different pathological context of tumor microenvironments, material stiffness in the growing tumor contributes to suppressed macrophage activity towards tumor clearance. This knowledge has led to the development of many new drugs that target matrix stiffness for tumor therapy, including enhancing or reprogramming macrophages to adopt tumoricidal states [70]. An emerging technology in macrophage immunomodulation is the development of biodegradable polymer 'backpacks' that stimulate proinflammatory programming through continuous delivery of cytokines [71]. In its current form, this technology does not consider macrophage mechano-response, but utilizes biomaterials in a creative way to adhere to macrophages to modulate their proinflammatory activity. The backpack technology has also been adopted in healing contexts other than cancer, driving pro-regenerative activation to improve healing in traumatic brain injury [72].

In a technology inspired by both mechanics of the microenvironment and materials, bicalcium phosphate ceramics were coupled with micro-vibration stimulation to enhance bone healing. The ceramic biomaterial matched bone composition while the vibration induced cyclic loading; ultimately both drove pro-regenerative programming of macrophages, and improved bone repair defects [73].

The integration of macrophage immunomodulation into translational medicine is paving the way for novel, more effective treatments, underscoring the critical role of macrophages in disease modulation and highlighting their potential as therapeutic targets.

Concluding remarks and future perspectives

As evidenced by the work covered in this review, macrophage function and phenotypic state are influenced by the mechanics, physics, and biochemistry of their surrounding microenvironment. Externally applied mechanical forces, as well as biological and physical cues, play important roles in signaling pathway activation, polarization, and function. To continue understanding macrophage behavior, advanced engineering approaches must incorporate these relevant microenvironmental

Outstanding questions

How can biofabrication methods advance to probe mechanosensing with increased nuance to flow stress and matrix elasticity?

How can learning from externally applied mechanical forces be translated into modulating macrophage phenotypes?

Is mechanical preconditioning a viable avenue to inform macrophage function?

How can we evolve bioengineered model systems to enable temporally longitudinal evaluation of macrophage function?

How can biomaterial systems be leveraged to introduce micron-scale complexity while enabling favorable macro-scale physical interactions to direct macrophage function?

Are there opportunities to combine externally applied mechanics with biomaterials towards a therapeutic product?

aspects. As these models evolve over time, it is important to consider additional variables such as temporal regulation and biological profiles of cell sources.

Macrophage plasticity allows for a specific phenotypic response to microenvironmental stimuli dependent on tissue location or disease state with respect to time. As an example of shifting phenotypes over time, in the first week following myocardial infarction, Mouton *et al.* demonstrated time-dependent changes in macrophages [74]. Day 1 macrophages exhibited proinflammatory signatures, day 3 macrophages showed increased phagocytosis, and Day 7 macrophages displayed a pro-repair signature. This work highlights the need to consider macrophage phenotype plasticity over time, a phenomenon that has also been extended to additional studies of macrophages in pathological conditions. Macrophage polarization relies heavily on external stimuli. The incorporation of time would also be relevant in the study of how monocytes in a suspension state sense mechanical cues and biochemical signals to differentiate into macrophages. By incorporating 4D technology with the aspect of time, the understanding of macrophage behavior over time will provide greater insight into trends of desired macrophage response.

Biological profiles are another key aspect that should be incorporated in studying macrophage function. This includes age, sex, genotype, and demographic information of the macrophages being examined. These variables are important in determining the immune response. Specifically, macrophages isolated from older ages experience metabolic dysregulation and altered cytokine function [75]. Biological females have a dynamic hormonal profile that directly influences immune response [76,77]. Genetic determinants, like ancestry identified markers, impact a patient's risk of numerous diseases [77]. Moreover, emerging evidence suggests a causal link between experiences of discrimination and monocyte immune function [78]. The current state of global healthcare is a one-size-fits-all approach. Recent advancements make for compelling arguments that incorporation of age, sex, genotype, demographic information, etc. is necessary to identify and successfully treat immunological disorders [79–82].

Promising future avenues continue to grow in macrophage mechanobiology. Leveraging advanced 3D systems and biomaterials that better mimic the mechanical and biochemical properties of native tissues allows for a more realistic study of macrophage behavior. However, the inherent complexity of *in vivo* conditions presents ongoing difficulties in creating fully representative *in vitro* systems. Future challenges – such as clinical translation, temporal incorporation, and micro- versus macroscale complexity – all require continued innovation to address (see Outstanding questions). As the macrophage mechanobiology field grows, adapting recent, novel models and materials will be imperative to answer some of these complex macrophage questions.

This review highlights recent model systems created to analyze macrophage behavior through mechanical, physical, and chemical alterations. The studies emphasize the important role of microenvironmental cues in macrophage response. With this work, we sought to answer: how exactly do external stimuli dictate macrophage function and their resulting behavior in healthy and disease states? Advancing technologies and materials allow new ways to investigate and harness macrophages in immunotherapeutic platforms. Our knowledge around macrophages is evolving dynamically, with future possibilities of integrating mechanical and physical complexity into *in vitro* models. Such integration will certainly make a positive impact on macrophage modulation for therapeutic gain.

Author contributions

E.M. and S.A.R. conceived the thematic elements of the review. A.J.C. and A.J. wrote the first draft, as equal authors, which was edited critically by E.M. and S.A.R. All authors agreed upon the final version of the manuscript. E.M. and S.A.R. share corresponding and senior authorship of this manuscript.

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Declaration of interests

E.M. and A.J. are named inventors on a patent application related to some of the work presented in this review. S.A.R. and A. J.C. declare that they have no competing interests.

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