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Matrix biophysical cues direct mesenchymal stromal cell functions in immunity

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Abstract

Hydrogels have been used to design synthetic matrices that capture salient features of matrix microenvironments to study and control cellular functions. Recent advances in understanding of both extracellular matrix biology and biomaterial design have shown that biophysical cues are powerful mediators of cell biology, especially that of mesenchymal stromal cells (MSCs). MSCs have been tested in many clinical trials because of their ability to modulate immune cells in different pathological conditions. While roles of biophysical cues in MSC biology have been studied in the context of multilineage differentiation, their significance in regulating immunomodulatory functions of MSCs is just beginning to be elucidated. This review first describes design principles behind how biophysical cues in native microenvironments influence the ability of MSCs to regulate immune cell production and functions. We will then discuss how biophysical cues can be leveraged to optimize cell isolation, priming, and delivery, which can help improve the success of MSC therapy for immunomodulation. Finally, a perspective is presented on how implementing biophysical cues in MSC potency assay can be important in predicting clinical outcomes.

Graphical Abstract

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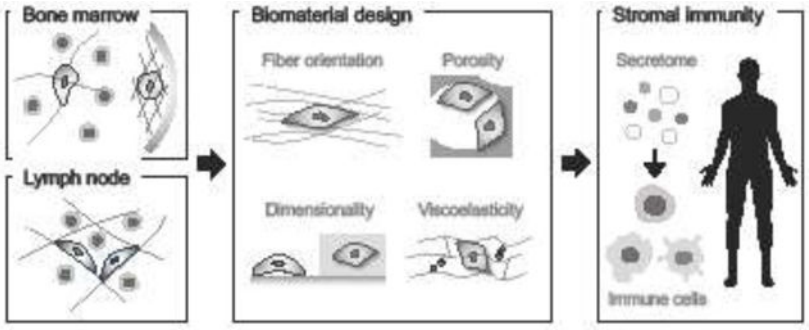
Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



Keywords

Biomaterials; Mechanotransduction; Immunomodulation; Mechanomedicine; Mesenchymal stromal cells

1. Introduction

Mesenchymal stromal cells (MSCs) were first identified in bone marrow in the 1970s [1, 2] as cells with multilineage potential that can differentiate *in vitro* into bone, cartilage and fat [3, 4]. As resident cells in bone marrow where blood and immune cells are produced, MSCs were also postulated to regulate immune cell functions. In early 2000s, preclinical *in vivo* studies showed that injection of MSCs promotes skin graft survival [5] and reduces graft-versus-host disease (GvHD) by suppressing T-cell activation [6]. Since then, the number of clinical trials to test therapeutic efficacy of MSCs in various acute and chronic inflammatory pathologies has grown to over one thousand [7]. In addition to T-cells, MSCs were shown to regulate other immune cell lineages, including natural killer cells [8], B-cells [9], dendritic cells [10, 11], and macrophages [12]. The predominant mode of action by which MSCs modulate immune cells is thought to be by the stimulation of MSCs with inflammatory signals from microenvironments, followed by downstream production and paracrine secretion of immunomodulatory factors [13].

Most of the studies on the role of MSCs in immunity were based on either MSCs on plastic culture *in vitro* or direct adoptive transfer of MSCs *in vivo*. However, MSCs in tissue microenvironments receive different types of signals, ranging from soluble to insoluble cues. Indeed, advances in recombinant protein engineering have enabled investigations into roles of soluble cues such as inflammatory cytokines in regulating immunomodulatory functions of MSCs. However, the contribution of insoluble cues, especially the extracellular matrix (ECM), to MSC-mediated immunomodulation has only begun to be appreciated recently. The insoluble cues from microenvironments can be classified further into biochemical and biophysical components. While proteomic approaches have defined biochemical components in the ECM, which are implicated in regulating MSC functions [14], advances in biomaterial design have enabled investigations into how matrix biophysical cues impact MSC functions by controlling mechanical properties of the matrix independently of biochemical cues [15]. Most studies have so far focused on the role of matrix mechanics in mechanotransduction and multilineage differentiation of MSCs. However, emerging studies

have highlighted roles of matrix biophysical cues in regulating immunomodulatory functions of MSCs.

In this review, we will first summarize the current understanding on the role of MSCs in hematopoietic system and discuss how biophysical signals from the microenvironment regulate the ability of MSCs to modulate the immune system. We will also discuss potential factors and challenges that may impact the success of MSC therapy in immunomodulation and how biomaterial strategies can help implement MSC-based mechanomedicine for immunomodulation.

2. Stromal cells of mesenchymal origin in hematopoietic and immune organs

Various immune cells that serve specific functions in the immune response are generated and matured [16] in a number of hematopoietic and immune organs, including bone marrow, lymph nodes, spleen and thymus [17]. In this section, we focus on bone marrow and lymph nodes, where stromal cells of mesenchymal origin are most well-characterized in terms of their roles in immunity. These cells play essential roles in maintaining immunity in large part by secreting cytokines to communicate with immune cells.

In bone marrow where immune cells are produced from hematopoietic stem cells (HSCs) and progenitors, most MSCs are present as pericytes in the vasculature [18], but some MSC subpopulations are also localized near the endosteal surface [19]. Subcutaneous implantation of $CD146^+$ MSCs alone is sufficient to create a new bone marrow ossicle, suggesting that MSCs play critical roles in generating hematopoietic microenvironments [20]. MSCs serve as niche cells for HSCs [21–23], since the number of quiescent HSCs is reduced when MSCs are genetically depleted [24]. In addition, MSCs regulate trafficking and production of myeloid lineages in bone marrow. MSCs secrete CC-chemokine ligand (CCL)-2 in response to systemic inflammation to promote monocyte egress from bone marrow into circulation *in vivo* [25]. MSCs are also able to generate regulatory dendritic cells from HSCs through Notch signaling [26, 27]. Moreover, MSCs support the survival of neutrophils in bone marrow [28, 29]. In terms of lymphoid lineages, MSCs are known to maintain a pool of B cell progenitors [30], while their role in producing T cell and natural killer cells still remains to be elucidated.

In lymph nodes, various stromal cell types of mesenchymal origin have been identified and are collectively called fibroblastic reticular cells (FRCs) [31]. FRCs are known to play important roles in coordinating adaptive immunity. Lymph nodes are formed during embryonic development when hematopoietic lymphoid tissue inducer cells interact with mesenchymal precursors that differentiate into lymphoid tissue organizer cells, which eventually give rise to different types of FRCs [32]. Among FRC subsets, T cell-zone reticular cells enwrap a network of the extracellular matrix and form a porous conduit network, which undergoes dynamic swelling and regulates lymph flow during inflammation [33]. During this process, these reticular cells secrete chemokines, including CCL19 and CCL21 to recruit T cells and dendritic cells [34, 35]. In contrast, B cell-zone reticular cells promote B cell survival, thereby maintaining humoral immunity by secreting B-cell

survival factors, such as B-cell activating factor and CXC-chemokine ligand (CXCL)-13 [36]. Follicular dendritic cells have been identified within the B cell areas of the lymph node cortex and play critical roles in the maintenance of germinal centers [37]. While other types of stromal cells have been discovered, such as marginal reticular cells [38] and pericyte FRCs [39], their direct roles in mediating immunity remain to be determined. The summary of the effects of stromal cells on regulating various types of immune cells in marrow and lymph nodes, and potential mediators in these processes are shown in Table 1.

3. Biophysical properties of bone marrow and lymph node microenvironments and their biological implications in stromal cell-mediated immunomodulation

Understanding biophysical properties of tissue microenvironments will inform the design of biomaterials with physiologically relevant cues that can be used to better understand and control cellular functions. Bone marrow and lymph nodes represent compartments where fluid and solid environments interface with each other, thereby providing opportunities to understand the effect of diverse biophysical cues on stromal cells of mesenchymal origin and functions in the context of immunomodulation. The biophysical cues in tissues can be generally classified into (1) intrinsic cues that are encoded within tissues under steady-state, such as viscoelasticity, and (2) extrinsic cues that undergo change in response to movement, such as strain, pressure, and fluid flow.

Bone marrow as a reservoir of diverse biophysical cues.

From a biophysical point of view, bone marrow is a soft tissue that interfaces with rigid bone [40]. Various macroscale measurements including rheology and indentation show that overall elastic modulus (E) values can vary significantly from one marrow sample to another [41]. Recent analysis by atomic force microscopy (AFM) revealed biophysical complexity of the marrow environment at the microscale [42]. The E of marrow is ~0.1 kPa, while the regions closer to the inner bone surface after washing away marrow show different peaks at 2, 30, and 100 kPa, which likely represent E values of nascently secreted matrix, osteoid matrix organized into fibers [43], and the mineralized matrix [44], respectively. Adding to this complexity, matrix compositions are known to vary across different marrow regions where collagen-I is localized in the endosteal area, collagen-IV and laminin are near vessels, and fibronectin is localized throughout marrow [45]. To date, the majority of MSC subpopulations have been identified as pericytes that interface with the vasculature within the marrow region [21, 22, 46], which could provide a strategic advantage for MSCs to regulate immune cell trafficking between marrow and blood via paracrine signaling, as shown in the context of systemic infection [25]. In contrast, some MSC subpopulations have been identified near the endosteal region and contribute to bone homeostasis [19], although the contribution of MSCs in this region in immunomodulation remains unclear. A recent study shows that softer matrices increase the ability of MSCs to respond to inflammatory signals and synthesize paracrine molecules to regulate monocyte production and trafficking [47], which is consistent with the notion that vascular region properties promote immunomodulation. In addition to elastic modulus, it is known that marrow [48]

exhibits viscoelastic properties. A general viscosity gradient has been reported from the ~40cP at distal to ~400cP at central regions of the marrow [49], although microscale viscoelasticity in marrow remains to be characterized. Indeed, a previous study shows that substrates with a higher damping factor increase the ability of MSCs to promote hematopoietic recovery after injury [50]. Future investigations are warranted to understand combinatorial roles of matrix biophysical cues and matrix compositions [51] in MSC-mediated immunomodulation.

Bone protects marrow from exogenous strain and pressure. However, marrow is highly vascularized and hence subject to regulation by blood flow, which is altered in response to cardiac output as a result of habitual movement. Marrow receives fluid primarily from the periosteal arteries, which connect to the central arteries and arterioles within the marrow. Within marrow, fluid collects within the central sinus and leaves by eventually connecting with venous circulation. Between these extremes, sinusoidal circulatory systems made up of capillary systems exist to evenly distribute nutrients and collect blood flow for venous circulation [52]. Fluid within the arterial regions exhibits a higher shear rate at ~2000 s^{-1}, with a steady decrease in velocity towards venous regions to a shear rate at ~120 s^{-1} [53]. Intriguingly, MSC subpopulations near the arterioles are known to support lymphoid-biased HSCs and lymphoid progenitors [54], and are activated upon exercise via the mechanosensitive ion channel Piezo1 [55]. In contrast, MSC subpopulations near the sinusoids regulate the production of myeloid lineages, where granulocyte progenitors and monocyte/dendritic progenitors are localized at spatially distinct regions near the vessels [56]. Whether fluid shear directly impacts the ability of MSCs to regulate lineage decisions for adaptive versus innate immune cells in marrow remains to be investigated. Interestingly, *ex vivo* application of wall shear stress is known to promote anti-inflammatory effects of MSCs by upregulating prostaglandin E_2 [57]. Consistent with this observation, exercise is known to reduce inflammatory cell production in an MSC-dependent manner *in vivo* [58]. Together, the *in vivo* studies suggest that blood flow is an important determinant of MSC-mediated immunomodulation in marrow.

Lymph node as a dynamic fibrous network.

A lymph node is a secondary lymphoid organ that is strategically localized between vascular and lymphatic branching points to function as filters for antigens and to promote infiltration of immune cells during immune response [59]. These filters are characterized by the conduit network in the parenchyma, which is a porous mesh of bundled and aligned matrix fibers enwrapped by FRCs [60]. The matrix fibers in the conduit network primarily consist of collagen-III and collagen-I [61, 62], which are mainly produced by FRCs [63]. While chronic tissue inflammation often results in aberrant matrix deposition and crosslinking, fibrosis rarely occurs within the lymph node [64] despite the frequent occurrence inflammatory episodes there. Interestingly, a recent study shows that matrix deposition by FRCs is reduced during inflammation to enable swelling of the conduit network [65]. In addition, the contractility of FRCs is also reduced as they interact with mature dendritic cells that present antigens [66, 67]. During lymph node swelling, FRCs are known to play dual roles in controlling T-cell responses. On one hand, FRCs, just like bone marrow-derived MSCs, secrete molecules to restrain T-cell proliferation in response

to interferon-γ (IFNγ) [68]. On the other hand, interleukin-6 (IL6) from activated FRCs promotes T-cell fitness [69]. While microscale biophysical properties of the conduit network remain to be further characterized, these studies highlight the potential of the lymph node as an inspiration for a unique material system with dynamic properties at the interface of network swelling, stromal-stromal cell adhesion, matrix remodeling, and stromal cell-mediated immunomodulation.

Interstitial fluid drains into lymph vessels and enters lymph nodes via afferent lymphatics. Lymph transport lacks connection with the circulatory system, but instead relies on smooth muscle contraction, which operates under a low contraction rate (10–20 contractions per min) and low pressure (2–18 cmH_2O) [70]. As a result, lymph flow is generally slow, but exercise or external massage can increase the velocity [71]. In addition, lymph flow will likely increase in some cancer or fibrotic conditions where interstitial fluid pressure becomes higher and the interstitial matrix undergoes stiffening, thereby increasing pressure gradients to drive the flow [72]. Like MSCs under fluid flow in marrow [55], FRCs in the conduit network are highly sensitive to fluid flow via Piezo1, which is essential for lymphocyte migration and antibody responses *in vivo* [73]. Slower fluid flow is known to increase the secretion of the chemokine CCL21 from FRCs, and blocking the flow through the lymph node inhibits CCL21 expression, suggesting that lymph flow is essential for the ability of FRCs to recruit mature dendritic cells and naïve T-cells [74]. While lymph flow likely drives conduit network swelling, which can subsequently stretch FRCs, these studies also highlight the direct role of fluid flow in regulating stromal cell-mediated immunity.

4. Roles of cellular mechanotransduction in regulating fundamental processes related to immunomodulation

Immune cells are known to communicate with each other through receptor-antigen interaction on the cell surface or secretion of humoral mediators, such as cytokines. In understanding how mechanotransduction impacts the ability of stromal cells to communicate with immune cells, it will be important to consider fundamental mechanisms behind how biophysical forces impact biological processes that mediate intercellular communications, including receptor activation and exocytosis/endocytosis, all of which can be influenced by biophysical regulation of the plasma membrane (Fig. 1).

At the molecular level, external force is sufficient to increase affinities of B-cell receptors [75] and T-cell receptors [76] to antigens. Consistent with these results, 2D substrate rigidity is known to increase T-cell activation [77, 78], B-cell activation [79], and dendritic cell activation of T-cells in an actin polymerization-dependent manner [80]. Thus, in cell suspension without the matrix or on 2D substrates, it appears that increased biophysical forces enhance activation and surface presentation of immune receptors. However, in 3D environments, a recent study showed that tumor necrosis factor (TNF) receptor clustering on the plasma membrane of MSCs is enhanced in a softer 3D matrix in response to TNFα, thereby increasing the downstream production of monocyte factors [47]. Unlike 2D environments, spatial confinement is an important determinant of cell spreading in 3D [81–83], which may also impact plasma membrane dynamics, and subsequently receptor

activation. Thus, the effect of matrix degradation and viscoelasticity needs to be considered in future studies to understand how 3D matrix mechanics impacts the activation of cytokine receptors in MSCs as well as the juxtacrine interactions between MSCs and immune cells.

Earlier studies showed that cell spreading on rigid plastic requires the addition of cell membrane, thereby driving biological processes that lead to increased membrane fusion, such as exocytosis [84, 85]. These studies have led to a physical model where cells with increased intracellular tension activate biological processes that decrease tension to maintain homeostasis by adding more membrane, as occurs in exocytosis [86]. This model seems to be consistent with the finding that receptors undergo more internalization on softer 2D substrates through activation of endocytosis [87], while lysosomal secretion by exocytosis is increased by stiffer substrates [88]. However, a recent study showed that MSCs increase secretion of immunomodulatory factors on softer substrates in an actomyosin-dependent manner [89], although it is possible that this phenomenon is due to changes in protein synthesis, rather than exocytosis *per se*. Indeed, a recent study shows that matrix stiffness does not impact constitutive protein secretion, but increased production of secreted proteins in softer substrates is due to increased transcription in response to inflammatory activation, though it occurs in a myosin-II independent manner [47]. In addition, increasing homotypic cell-cell interactions of MSCs by encapsulation in scaffolds with a larger porosity [90] or by forming spheroids [91] promotes paracrine factor secretions. While cell-cell interactions enhance tissue-scale tension [92], which could then result in increased exocytosis [86], the contribution of cell-cell interactions to protein exocytosis/endocytosis *vs.* synthesis remains to be dissected. Future studies will likely address roles of matrix mechanics in regulating different membrane vesicle trafficking mechanisms that impact protein secretions, most notably, extracellular vesicles, which have emerged as carriers of therapeutic cargo [93] that can transport through the matrix [94].

5. Biomaterial strategies to study roles of matrix biophysical cues on MSC-based immunomodulation

Many previous studies used biomaterial strategies to reveal the sensitivity of blood and immune cells to biophysical cues, a topic which has been reviewed extensively [40, 95–97]. In general, material biophysical cues are known to mediate fundamental processes of immune response, including deformation, adhesion, and trafficking of cells [98, 99], cell-cell interactions [76, 100], and antigen affinity [101, 102]. Recent studies have shown that some of these regulatory processes for blood and immune cells can also be relevant to understanding biophysical regulation of MSC-based immunomodulation. Emerging studies show that biophysical properties of biomaterials, including topography, porosity, dimensionality, and viscoelasticity may play important roles in MSC-based immunomodulation (Fig. 2).

Matrix fibers are visible at the interface between inner bone surface and marrow, where they appear to exhibit different orientations [42]. To test the biological significance of matrix fiber orientation in stromal cell-mediated immunity, topography of substrates for cell adhesion can be controlled by various microfabrication approaches. In the context

of studying stromal cell functions, soft lithography, electrospinning, and stereolithography-based printing have been employed. Soft lithography has been widely used to generate patterns of adhesive substrates at the microscale, including microgrooves and microposts [103]. In general, a mask with desired micropatterns is used to fabricate a photoresist mold, on which softer materials, such as polydimethylsiloxane (PDMS) can be casted. Patterned PDMS substrates can be precisely tuned in terms of mechanical properties and functionalization with matrix molecules so that they can be used to interface with MSCs. While this approach has been used extensively to study mechanical regulation of MSCs [104, 105], it remains to be leveraged to understand biophysical regulation of stromal cell-based immunomodulation. Recent studies employed different approaches to fabricate more physiologically relevant microenvironments with controlled substate topography to study their impact on stromal cell-based immunomodulation. Electrospinning can be used to generate fibrous matrices with independent control of architecture and mechanics [106]. Using this approach, it was shown that MSCs increase the secretion of immunomodulatory factors when cultured on aligned fibers than on random fibers in a Yes-associated protein (YAP) and focal adhesion kinase (FAK) dependent manner [107], suggesting the potential importance of matrix fiber orientation in regulating stromal cell-mediated immunity. In contrast to electrospinning, stereolithography-based printing offers more precise control of where matrix fibers can be placed in a given space, at the microscale [108]. This approach can also expand the repertoire of materials that can be used to control topography beyond PDMS-based materials, such as hydrogels that can be cured by light. By leveraging this approach, it was shown that varying the placement and intrinsic properties of discrete matrix signals differentially impacts cell volume and YAP-based mechanotransduction of MSCs, which could in turn impact MSC-based immunomodulation [109].

Tissues need to be porous to accommodate immune cell production and trafficking. However, hydrogels are generally nanoporous. Porosity of nanoporous hydrogels is often inversely correlated with stiffness, although stiffness of alginate hydrogels can be tuned independently of porosity based on an egg-box model [110]. Increasing porosity in hydrogels will likely provide cells with less spatially confined environments, which could facilitate cell spreading, migration and intercellular interactions. The most convenient way to achieve this goal is to use freeze-drying of crosslinked hydrogels where ice crystals create the macroporous voids, followed by reconstitution and seeding of cells [111]. Using this approach, a previous study showed that MSCs in macroporous alginate-based hydrogels increase the production of growth factors, such as vascular endothelial growth factor (VEGF) due to N-cadherin mediated cell-cell interactions as opposed to MSCs in nanoporous alginate hydrogels, while the microscale material stiffness is kept constant [90]. Consistent with this observation, MSC spheroids in alginate gels show higher VEGF secretion compared to dissociated cells [91]. However, a freeze-drying approach results in a broad pore size distribution. To overcome this limitation, encapsulation of degradable microgels has recently been used to form monodisperse pores in hydrogels independently of intrinsic material properties—in this context, the pore-forming microgels have been created either by an aerosol-based method [112] or a droplet microfluidic approach [113]. In addition, electrospinning or printing can be used to fabricate fibrous matrices with defined porosity [114]. These recent approaches will help advance our understanding of

how microscale porosity impacts MSC-mediated immunity in conjunction with dynamic fluid flow as seen in lymph nodes.

Some stromal cells in tissues are present either on matrix fibers (2D) as in the conduit network, while others can be surrounded by the matrix as in marrow (3D). Substate dimensionality is generally controlled by seeding of cells on top of pre-formed materials (2D) or encapsulating cells in materials (3D) [115]. It is also possible to create an intermediate ('2.5D') condition where cells are sandwiched between two hydrogel layers [116], which can simulate the condition where cells are placed at the interface between two different regions, such as marrow and inner bone surface. A previous study showed that MSCs secrete less pro-inflammatory cytokines at the basal level in 3D compared to 2D in a non-hydrogel polymer scaffold [117]. Supporting this observation, another study reported that the expression of tryptophan 2,3-dioxygenase, which is associated with an immunosuppressive effect, is reduced when MSCs are encapsulated in 3D alginate-based hydrogels [118]. However, whether these effects are due to substrate dimensionality *per se* or other factors such as increased spatial confinement in 3D compared to 2D remains to be investigated by using strategies to selectively control porosity. With recent advances in temporal control of material properties [119], biophysical regulation of stromal cell-mediated immunity can also be studied over time (a '4^{th} dimension'). The '4D' material systems will be useful to understand how immunomodulatory properties of MSCs may change under temporal pathological conditions where tissues undergo stiffening over time such as in fibrosis and cancer, and the potential roles of mechanical memory [120] in regulating this process.

To simulate microscale variations in mechanical properties of natural marrow (Section 3), it is possible to leverage hydrogel systems with tunable matrix viscoelasticity. Matrix elasticity is a function of polymer crosslinking and can be controlled independently of ligand density and porosity either by conjugating a ligand to a polymer backbone [43, 121, 122] or interpenetrating a natural polymeric ligand with a synthetic polymer that is used to control elasticity [123]. In general, softer matrices have shown to be beneficial in enhancing basal secretion of different cytokines and growth factors by MSCs [124] and the responsiveness of MSCs to TNFα to increase the production of monocyte regulatory factors [47]. Since most tissues exhibit viscoelastic behaviors due to energy dissipation, efforts have been made to develop hydrogels with tunable viscoelasticity, which can be characterized by stress relaxation (decreased stress under constant strain), creep (increased strain under constant stress) or loss tangent (ratio between viscous modulus and elastic modulus). This has generally been achieved by employing reversible chemical bonds to crosslink hydrogels. For instance, alginate hydrogels undergo faster stress relaxation when they are crosslinked with ionic bonds *vs.* covalent bonds [125]. Decreasing molecular weight of alginate and introducing steric hinderance by PEG conjugation can further accelerate stress relaxation [81]. For non-ionic hydrogels, variation of monomer ratios [126], host-guest chemistry [127] and dynamic covalent crosslinking [128] have been used to introduce viscoelasticity. A recent study leveraged an interpenetrating network of collagen-I and alginate to show that MSCs in an ionically crosslinked hydrogel produce a higher level of anti-inflammatory factors in response to inflammatory challenge than MSCs in a covalently crosslinked

hydrogel [129]. Together, these studies highlight the importance of both elastic and viscous material properties in regulating MSC-mediated immunomodulation.

6. Relevance of material biophysical cues to isolation and delivery of MSCs for immunomodulation

Understanding biophysical regulation of cell-matrix interactions may inform better strategies to isolate and deliver stromal cells of mesenchymal origin for immunomodulation (Fig. 3). Endogenous MSC populations in marrow are rare (~0.05%) [130]. Thus, most investigators have been using MSCs that are derived from plastic adherence followed by culture in low glucose medium to remove any adherent hematopoietic cells and to expand MSCs. This process can take more than one month to obtain sufficient cells for downstream applications. However, it is known that MSCs have mechanical memory [120]. Thus, prolonging culture time on plastic may impair the ability of MSCs to sense softer substrates, which might negatively impact the corresponding sensitivity of inflammatory activation [47] or the constitutive production of immunomodulatory factors [89]. To address this issue, it may be beneficial to expand MSCs in natural or biologically-inspired substrates. For instance, collagen-based scaffolds were shown to promote MSC seeding and survival after isolation [131], to facilitate MSC proliferation [132], and to preserve MSC phenotypes [133]. Culturing MSCs in spheroids within Arg-Gly-Asp (RGD)-conjugated alginate hydrogels could also increase MSC survival [91].

While MSCs modulate immune cells, they are not resistant to immune clearance. In fact, most donor MSCs are cleared from the host within 24~48 hours [134], unless they are delivered directly to the tissue of origin as shown by long-term engraftment studies in bone marrow [47, 135]. In addition, it is likely that MSCs delivered in circulation respond to shear stress, which can impact their survival and functions [57]. While MSCs are known to express low Class I Major Histocompatibility Complex (MHC) and no class II MHC, both receptors can undergo upregulation upon inflammatory activation, which in turn could trigger a host defense mechanism, including foreign body response (FBR) to remove donor MSCs after administration [136]. In addition, MSCs express a low level of CD47, ‘marker of self’, compared to blood cells [137], thereby making them susceptible to potential phagocytosis by macrophages [138]. While some biomaterials are also susceptible to FBR, MSCs themselves are known to reduce FBR of biomaterials by reducing macrophage activation [139]. It is also possible to modify the surface of biomaterials to reduce FBR as shown by introducing zwitterionic groups [140] or triazole analogs to alginate hydrogels, the latter of which prolongs allogeneic islet transplantation in primates [141]. However, the utility of these approaches in prolonging delivery of MSCs remains unclear. Interestingly, coating individual MSCs with a thin alginate gel by droplet-based microfluidics prolongs the residence time of MSCs after intravenous injection [142], which is further enhanced by modifying the alginate coating with adsorption of poly-l-lysine [143]. Whether these observations are also applicable to other routes of administration will likely depend on the immune milieu of the administration site. The ability to precisely tune material properties of the gel coating around single MSCs [144, 145] will not only help control the biodistribution of MSCs, but also allow local cue specification to donor MSCs for optimal efficacy.

7. Potential roles of biomaterials in evaluating MSCs as therapeutic products

Like other therapeutic products, MSCs as therapeutic cells must be evaluated in terms of potency, cooperativity, and efficacy (Fig. 4). In particular, potency assays that capture the relevant biological activity of therapeutic products are essential requirements to submit an investigational new drug application to U.S. Food and Drug Administration to use an MSC product as an immunotherapy [146]. Investigations into MSC mechanotransduction by using biomaterial design have elucidated the effects of microenvironmental properties on MSC phenotypes. However, conventional MSC potency assays use standard tissue culture plastic, which does not capture the sensitivity of MSCs to matrix biophysical cues in different physiological or pathological conditions. To best consider the effects of microenvironmental cues on the therapeutic potential of MSCs, it will be important to more completely define these effects in terms of the established framework of pharmacodynamics for small molecules and biologics [147] (potency, cooperativity, and efficacy), and consider how biomaterial design can impact each parameter.

To determine the potency of MSCs as therapies, *in vivo* dose response studies can be performed to determine the cell dose that results in a half-maximum therapeutic response in an animal model. Since the primary mode of action by which MSCs modulate immune cells requires the exposure to inflammatory signals in the host [13], one way to predict *in vivo* dose response is to test the sensitivity of MSC products to inflammatory activation *in vitro* [148]. In pharmacology, the potency of receptor activation is influenced by intrinsic affinity or conformation of receptors, which is indeed influenced by substrate stiffness as demonstrated in immune cells [77–80]. Thus, understanding how different biomaterial properties impact the potency of inflammatory activation will be a key to better evaluating MSC potency for disease indications where mechanical properties of tissues vary. In addition, biomaterials may inform how a certain inflammatory ligand can be presented to MSCs for optimal immunomodulatory effects. For instance, some receptors, such as c-kit, can bind to ligands in an insoluble form at a lower concentration than the soluble form [149]. In this case, biomaterials can potentially be delivered along with MSCs to incorporate and present ligands from the host to MSCs to increase the potency of inflammatory activation.

Cooperativity indicates whether the intended effect is increased gradually with an increasing dose or as an “on/off” switch at a specific threshold dose, which is indicated by the slope of a dose response curve. The on/off response was previously reported in the context of MSC therapy where a systemic cytokine upregulation in the host could be observed only when a certain threshold dose of donor MSCs was used [150]. At the cellular level, positive cooperativity will likely suggest that the therapeutic activity of MSCs is amplified with a higher MSC dose due to homotypic interaction among MSC populations to enhance the production of immunomodulatory factors. In addition, positive cooperativity may indicate that when a single target immune cell is modulated by a single MSC, this causes other immune cells to become better influenced by other MSCs through heterotypic interactions. Thus, biomaterials that can tune cell-cell interactions [90] or spheroid formation [91] will

inform the cooperativity of a given MSC product. The ability to precisely direct single cells by biomaterials [109, 144], and assemble single cells into clusters in a bottom-up manner [151–153] will also improve our ability to predict MSC cooperativity.

Efficacy of MSCs refers to the maximum possible effect of a given MSC product. Unlike potency, efficacy is influenced by receptor density and clustering on the cell membrane. Thus, membrane fluidity, endocytosis and exocytosis are likely important determinants to determine the efficacy of MSCs. Indeed, a recent study showed that matrix stiffness impacts the maximum response of TNFα activation in MSCs by regulating TNF receptor clustering [47]. However, the same study showed that unlike TNF receptor activation, matrix stiffness does not impact IFN receptor activation. Thus, mapping the effect of different matrix biophysical parameters on paracrine secretions of immunomodulatory factors will be an important goal in the field, which can be facilitated by combining single cell RNA sequencing [154] with single cell encapsulation approaches [142, 144], both of which are based on droplet-based microfluidics. In addition, both secretion and recycling of paracrine factors will likely impact the maximum net release of immunomodulatory factors from MSCs. At the cellular level, the ability of donor MSCs to interact with the host and gain access to immune cells is likely an important determinant of MSC efficacy, since MSCs need to undergo substantial mechanical squeezing to permeate biological tissues, while immune cells can do so much more readily [155, 156]. To address this issue, biomaterial design can be combined with microfabrication approaches [157] to predict the ability of MSCs to traffic through spatial confinement and hence interact with immune cells.

8. Potential roles of material biophysical cues in improving MSC-based immunotherapy for clinical applications

One major class of clinical indications for which MSCs have been tested is immunological rejection due to allogeneic transplantation or autoimmunity. In the context of bone marrow transplantation, T-cells in allogeneic donor marrow recognize the host as foreign due to human leukocyte antigen mismatches and attack the host within 3 months, leading to acute GvHD [158]. The clinical use of MSCs for acute GvHD has been approved in some countries for patients that are resistant to anti-inflammatory steroids [159]. However, the clinical outcomes have been variable [160]. In fact, two distinct phase 3 clinical trials failed to show beneficial outcomes by MSCs in steroid-resistant GvHD patients [161]. Similarly, MSCs have been tested to treat autoimmune disorders, most notably, the Crohn’s disease, leading to approval in Europe to treat fistulas, a common complication of Crohn’s disease [162]. However, a phase 3 trial of MSCs in Crohn’s disease was not successful in the U.S [163]. While a number of factors may have contributed to variable clinical outcomes in these cases, one plausible possibility is that patients are administered anti-inflammatory corticosteroids, which are important clinical interventions to temporarily alleviate the symptoms, but also likely reduce the level of inflammation necessary to activate MSCs to produce paracrine factors that confer immune tolerance [164]. Priming MSCs on soft [47, 124], viscoelastic [129] materials with aligned fiber orientation [107] can potentially help increase the sensitivity of MSCs to inflammatory activation even when inflammation in the host is alleviated by steroid treatment. In addition, material strategies to prolong the

residence time of MSCs [142, 143] will enable the integration of attenuated inflammatory signals from the host by steroids.

MSCs have also been tested to treat acute tissue injuries. In particular, MSCs have shown to be efficacious in preclinical models of acute respiratory distress syndrome (ARDS) in part due to polarization of alveolar macrophages [165], which leads to increased phagocytosis [166] and restoration of vascular permeability [167] by secreting paracrine factors or extracellular vesicles. The number of clinical trials to test MSCs in ARDS have increased dramatically in the past year due to the COVID-19 pandemic [168], since ARDS is a major symptom of COVID-19 [169]. While MSCs were shown to be well-tolerated in phase 1 studies, their efficacy was shown to be unclear in a recent phase 2a clinical trial [170, 171]. Since pulmonary functions are significantly compromised in ARDS, it will be important to control the dose of MSCs to avoid occurrence of pulmonary embolism. Thus, material strategies to increase the sensitivity of MSCs to inflammatory activation or to delay the clearance of MSCs will likely help reduce the number of effective MSC doses needed for the treatment of ARDS.

In devising treatment strategies for chronic tissue injuries, it is not only important to consider inflammation but also subsequent aberrant tissue remodeling processes, eventually leading to fibrosis [172], which stiffens tissues due to increased collagen production and crosslinking [173]. For example, chronic GvHD leads to fibrosis in different organs, which contribute to long-term morbidity and mortality [174]. In addition, a subset of ARDS survivors can develop lung fibrosis later in life [175]. In this context, MSCs have shown to be potentially beneficial for treatment of myocardial infarction based on some phase 2 clinical studies, although a large randomized controlled trial remains to be completed [176]. A landmark preclinical study showed that the efficacy of MSCs in myocardial infarction requires the secretion of TNF-stimulated gene-6 (TSG6) [177], which has also been attributed to the efficacy of MSCs in preventing skin fibrosis in a preclinical model [178]. MSCs were also shown to be beneficial to prevent preclinical models of fibrotic lung injury and chronic obstructive pulmonary disease when administrated at early stages, but not later stages [179], highlighting the current limitations of MSC therapy in treating chronic tissue injuries. These limitations can be attributed by a couple of factors. First, inflammation subsides by the time fibrosis is diagnosed, thereby limiting inflammatory activation of MSCs to synthesize therapeutic factors once delivered to the host [13]. Second, significant biophysical changes in fibrotic microenvironments may influence donor MSCs to further adopt fibrotic phenotypes [180], thereby potentially limiting therapeutic efficacy or even exacerbating fibrosis as previously observed in a preclinical study of myocardial infarction [181]. To overcome these limitations, a recent study leveraged a conformal gel coating as a means to provide donor MSCs with locally specified biophysical and biochemical signals—using this approach, it was shown that MSCs singly coated with a soft gel that continuously presents recombinant TNFα facilitate the reversal of fibrotic lung injury in a preclinical model when delivered at later time points [145]. Thus, biomaterial design can be leveraged to design MSC-based therapeutics for chronic tissue injuries by providing control over how donor cells interact with specifically engineered environments versus those within the host.

9. Conclusion and Future Directions: Towards a single cell-level control for precision mechanomedicine

The contribution of matrix biophysical cues to MSC-based immunomodulation is an important yet largely unexplored area. Advances in biomaterials will enable better recapitulation of the physiological microenvironment to study MSC functions in various immune organs and disease contexts, as well as enable improvement of MSC priming, isolation, and delivery. With precise control of biophysical properties, emerging studies show that biomaterial systems can be tailored to direct MSC secretomes as potential therapeutics to treat immunological rejections and tissue injuries.

One major challenge of understanding the impact of matrix biophysical cues on MSC-based immunomodulation is heterogeneity of stromal cell populations, as recently demonstrated by single-cell RNAseq analysis [51, 182]. Supporting this challenge, a recent study showed that distinct MSC subpopulations exhibit differential mechanosensitivity to varied matrix elasticity, which influences their differentiation potential [183]. Since droplet-based microfluidic approaches have been used to profile cytokine secretions from single immune cells [184, 185], these approaches can potentially be combined with single cell encapsulation strategies to advance the field of single stromal cell mechanoimmunology. This line of approach can also be used to screen for individual MSC clones in engineered gel coatings with desired paracrine secretion activities, and subsequently deliver them to the host for therapeutic purposes.

The ability to miniaturize biomaterials down to the single cell level and specify their properties will likely advance the field of MSC-based immunotherapy. As shown in the airways [145], it will be possible to deliver MSCs along with locally specified synthetic microenvironments to tissues with a narrow space where immune cells reside via various routes of administration. Gel-coated MSCs can also be incorporated into larger tissue constructs or used as basic units to assemble into tissues, thereby potentially conferring immunomodulatory properties on engineered tissues. One of the biggest challenges in translating cell therapy is that mode of action is either poorly defined or considerably complex to immediately understand. However, as the clinical success of chimeric antigen receptor-T cell therapy has taught us [186], one way to overcome this challenge is to precisely define the input that confers a predictable therapeutic activity on engineered cells. Together with advances in synthetic biology [187], physical approaches to biomaterial design provide opportunities to specify microenvironmental cues around MSCs, to understand their impact on immunomodulation, and to use these cues directly to tailor MSCs for different disease indications.

The future of MSC-based immunotherapy remains optimistic and with a high ceiling for advancement by combining MSCs and biomaterials. With research efforts focused on investigating MSC mechanotransduction and developing novel biomaterial systems, the therapeutic potential of MSCs to control disorders of the immune system can be improved by leveraging biophysical cues.

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Statement of Significance

Stromal cells of mesenchymal origin are known to direct immune cell functions *in vivo* by secreting paracrine mediators. This property has been leveraged in developing mesenchymal stromal cell (MSC)-based therapeutics by adoptive transfer to treat immunological rejection and tissue injuries, which have been tested in over one thousand clinical trials to date, but with mixed success. Advances in biomaterial design have enabled precise control of biophysical cues based on how stromal cells interact with the extracellular matrix in microenvironments *in situ*. Investigators have begun to use this approach to understand how different matrix biophysical parameters, such as fiber orientation, porosity, dimensionality, and viscoelasticity impact stromal cell-mediated immunomodulation. The insights gained from this effort can potentially be used to precisely define the microenvironmental cues for isolation, priming, and delivery of MSCs, which can be tailored based on different disease indications for optimal therapeutic outcomes.

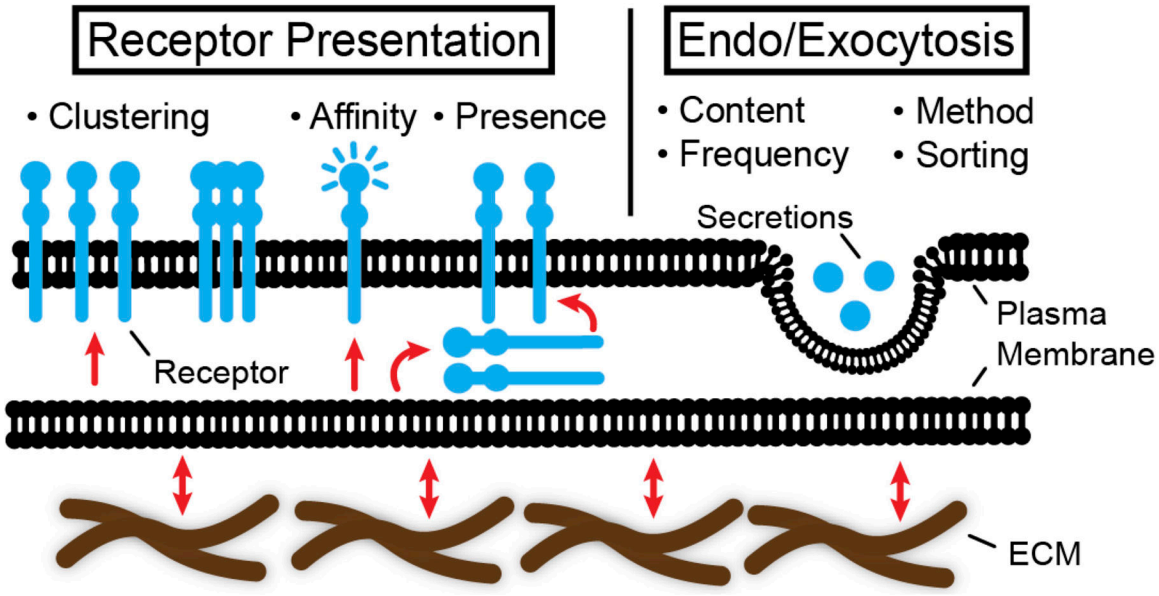

Figure 1. Fundamental mechanisms that impact biological processes related to stromal cell-mediated immunomodulation.

Biophysical properties such as elasticity, density and viscoelasticity of extracellular matrix (ECM) impact the immunomodulatory properties of stromal cells through outside-in signaling by regulating the receptor presentation, clustering, the affinity to ligands and endocytosis, as well as though inside-out signaling including exocytosis to control the release of secreted factors.

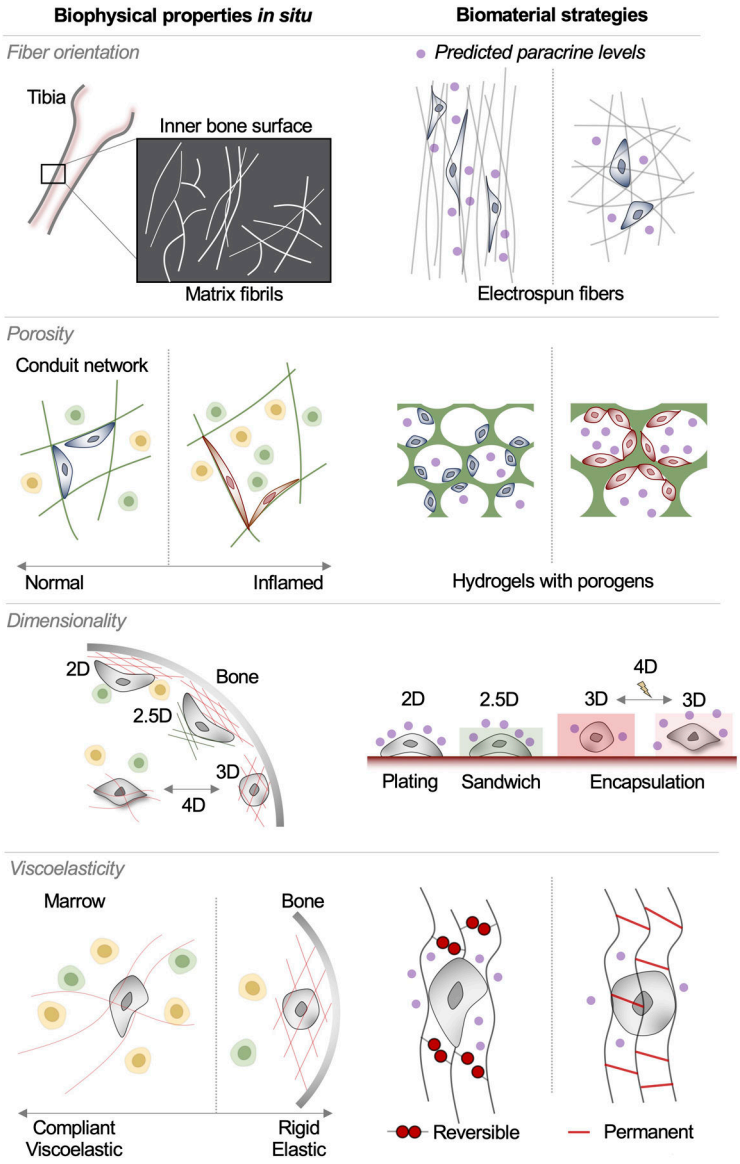

Figure 2. Biologically inspired design of materials to study biophysical regulation of stromal cell-mediated immunomodulation.

A deeper understanding of matrix organization and stromal cell-matrix interactions in immune organs, including bone marrow and lymph nodes, enables investigators to pursue appropriate biomaterial strategies to tune different matrix biophysical parameters, including fiber orientation, porosity, dimensionality, and viscoelasticity. Recent studies show the effect of these parameters on the level of paracrine secretions by stromal cells to mediate immunomodulation.

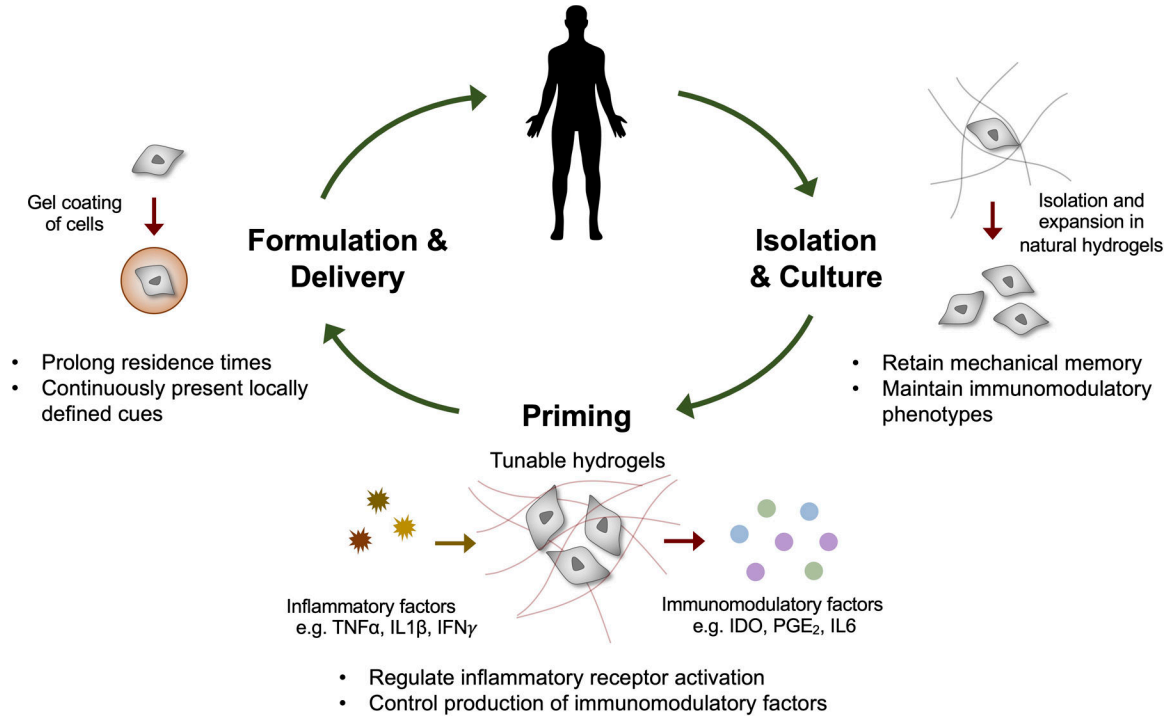

Figure 3. Biomaterials with tunable matrix biophysical properties improve the therapeutic potential of stromal cells.

With proper design of material properties, stromal cell isolation and culture can be improved. Biophysical cues also impact the immunomodulatory properties of stromal cells by regulating the receptor expression, sensitivity and the production of secreted factors by stromal cells. Encapsulating stromal cells in biomaterials can enhance *in vivo* residence of donor cells by preventing direct contact between donor cells and the host defense, and enable continuous presentation of specifically defined cues to donor cells.

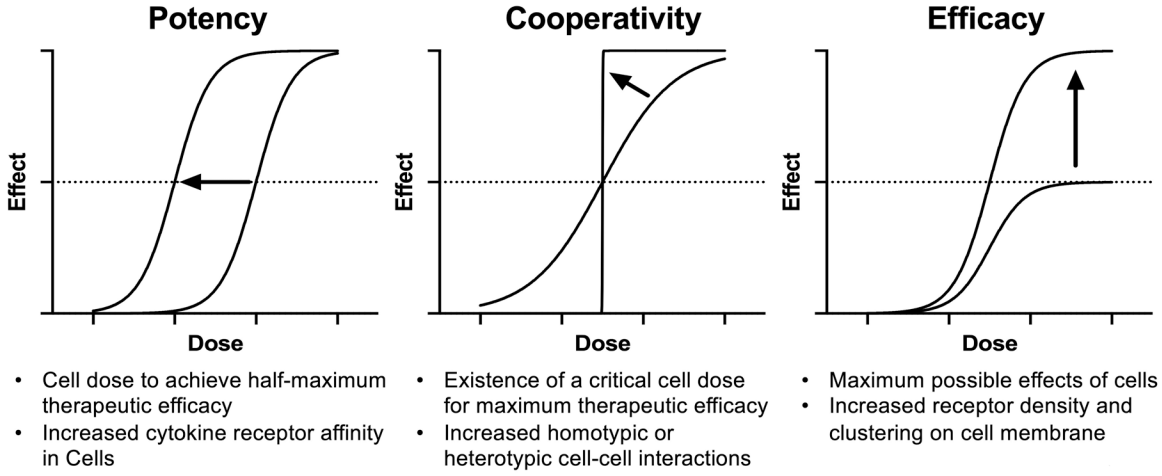

Figure 4. Pharmacodynamics of cells.

Dose response of cells is determined by three key parameters, including potency, cooperativity and efficacy as defined below each graph. Factors that can enhance each parameter are also described. Biomaterials can be designed to better inform each pharmacodynamic parameter of cells.

Table 1.

Examples of immune cell regulation by stromal cells of mesenchymal origin in bone marrow and lymph nodes.

Organs	Effector cells	Target immune cells	Mediators from stromal cells	Effects	Refs
Bone marrow	MSCs	Monocytes	CCL2	Essential for monocyte emigration from marrow upon infection	25
		Dendritic cells	Notch pathway	Facilitate dendritic cell differentiation from HSCs	26, 27
		Neutrophils	GM-CSF, IL6, IL8	Support neutrophil survival, recruitment, and phagocytic activity	28, 29
		B cells	CXCL12	Maintain a B cell progenitor pool in marrow	30
Lymph node	TRCs	T-cells, dendritic cells	CCL19, CCL21	From a conduit network to enwrap the matrix; Regulate lymph flow; Recruitment of T-cells and dendritic cells during inflammation	34, 35
	BRCs	B-cells	BAFF, CXCL13	Essential for B cell survival and humoral immunity	36
	FDCs			Essential for germinal center maintenance	37

MSCs: Mesenchymal stromal cells; FRCs: Fibroblastic reticular cells; TRCs: T-cell zone reticular cells; BRCs: B-cell zone reticular cells; FDCs: Follicular dendritic cells; CCL: CC-chemokine ligand; CXCL: CXC-chemokine ligand; IL: Interleukin; GM-CSF: Granulocyte and monocyte-colony stimulating factor; BAFF: B-cell activating factor.