eISSN 2005-5447
International Journal of Stem Cells Vol. 16, No. 3, 2023 https://doi.org/10.15283/ijsc22211

REVIEW ARTICLE

Applications of Bioinspired Platforms for Enhancing Immunomodulatory Function of Mesenchymal Stromal Cells

Ok-Hyeon Kim[1,*], Tae Jin Jeon[2,*], Young In So[2], Yong Kyoo Shin[3], Hyun Jung Lee[1,2]

[1]*Department of Anatomy and Cell Biology, College of Medicine, Chung-Ang University, Seoul, Korea*
[2]*Department of Global Innovative Drugs, Graduate School of Chung-Ang University, Seoul, Korea*
[3]*Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul, Korea*

Mesenchymal stromal cells (MSCs) have attracted scientific and medical interest due to their self-renewing properties, pluripotency, and paracrine function. However, one of the main limitations to the clinical application of MSCs is their loss of efficacy after transplantation *in vivo*. Various bioengineering technologies to provide stem cell niche-like conditions have the potential to overcome this limitation. Here, focusing on the stem cell niche microenvironment, studies to maximize the immunomodulatory potential of MSCs by controlling biomechanical stimuli, including shear stress, hydrostatic pressure, stretch, and biophysical cues, such as extracellular matrix mimetic substrates, are discussed. The application of biomechanical forces or biophysical cues to the stem cell microenvironment will be beneficial for enhancing the immunomodulatory function of MSCs during cultivation and overcoming the current limitations of MSC therapy.

Keywords: Mesenchymal stromal cells (MSCs), Immunomodulation, Biomechanical forces, Biophysical cues

Introduction

Multipotent mesenchymal stromal cells (MSCs) are obtained from various adult tissues, such as bone marrow (BM), adipose tissue, umbilical cord blood, Wharton's jelly, dental pulp, and skin (1). MSCs have been investigated as an attractive option for regenerative medicine and immune-mediated disorders due to their self-renewal ability, multilineage differentiation, and anti-inflammatory and immunomodulatory functions, which involve both paracrine and cell-to-cell contact mechanisms (2). Several studies have shown that the potent immunomodulatory properties of MSCs are due to the production of immune-modulating factors, such as indoleamine 2,3-dioxygenase (IDO), heme oxygenase-1 (HO-1), transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α)-stimulated gene protein-6 (TSG-6), cyclooxygenase-2 (COX-2), and prostaglandin E2 (PGE_2), which target various components of the immune system (3). These properties support the use of MSCs for cellular therapeutics, and accordingly, various tissue regeneration treatments with MSCs have been evaluated in clinical trials. Nonetheless, their clinical applications have not been satisfactory (4) due to poor engraftment, *in vitro* senescence, functional quiescence after transplantation *in vivo*, and the heterogeneity of MSC properties, which can be attributed to an incomplete understanding of the mechanisms regulating MSCs. Elucidating the anatomical and functional features of MSC niches in their native tissues and the interactions of MSCs with the extracellular matrix

Received: December 30, 2022, Revised: March 24, 2023,
Accepted: March 28, 2023, Published online: June 30, 2023
Correspondence to **Hyun Jung Lee**
Department of Anatomy and Cell Biology, College of Medicine, Chung-Ang University, 84 Heuksuk-Ro, Dongjak-gu, Seoul 06974, Korea
Tel: +82-2-820-5434, Fax: +82-2-813-5387
E-mail: pluto38@cau.ac.kr
*These authors contributed equally to this work.

(ECM) and surrounding cells is crucial to understanding the behavior of MSCs and to harnessing this knowledge for clinical applications.

The MSC niche has been defined as all of the elements immediately surrounding the stem cells when they are in their naive state (5). These elements include the non-stem cells in direct contact with the MSCs as well as the ECM and physical cues found in that locale (6, 7). As MSCs move away from this nurturing niche, they may encounter different environments, such as fewer cell-cell interactions and more ECM interactions, which regulate MSCs differentially. Features of the surrounding environment of MSCs, such as the microarchitecture, substrate rigidity, or oxygen level, affect and regulate MSC function and property. Maintaining the *in vivo* niche or enforcing this condition is considered to provide greater therapeutic potential and efficacy. Therefore, to better mimic *in vivo* conditions, nature-inspired platforms for cell culture have come to the fore (8). The extracellular microenvironment that controls the ability of MSCs includes its biological, chemical, and physical aspects. Although many studies have focused mainly on biological and chemical cues, the physical cues of the extracellular microenvironment are receiving increasing attention. The physical properties of the extracellular microenvironment include biomechanical forces and the intrinsic ECM.

Therefore, in this review, we summarize the application of nature-inspired platforms that mimic the biomechanical forces and the biophysical cues in the MSC niche. Additionally, the effects of the interaction between MSCs and their microenvironment on MSC immunomodulatory potential will be discussed.

Immunomodulatory Function of MSCs by Mechanical Force: Sensing and Reaction

MSCs have served as an archetypal model for understanding the properties of mechanical forces on cellular potential largely because of their ubiquitous nature, ease of *ex vivo* culture, and multipotentiality as bone progenitors (9). For example, BM-derived MSCs experience many mechanical forces, such as shear stress, hydrostatic pressure, and tensile forces, in their MSC niche (10, 11). These biomechanical forces can be transduced into internal biochemical signals in MSCs through their mechanosensors or responsive microdomains (12, 13). In the following section, we describe the immunomodulatory functions of MSCs in terms of their response to biomechanical forces, including shear stress, tensile stress, and hydrostatic pressure (Fig. 1).

Shear stress, tensile forces, and hydrostatic pressure as biomechanical forces

As a force tending to cause deformation of an object by slippage along a plane or planes parallel to the imposed stress, shear stress is known to exist in the blood vessel, lymphatic vessel, or lacunar-canalicular network of bone due to the fluid movement (Fig. 1) (14). These fluid flow patterns are not uniform in the biological system because shear stress depends on the velocity gradient between these layers and the viscosity of the liquid (15). Diaz et al. (9) demonstrated that vascular lumen mimicking fluid frictional forces (15 dyne/cm^2) elevated antioxidant and anti-inflammatory mediators from BM-derived MSCs, suggesting that biomechanical forces can improve the immunomodulatory function of MSCs by providing critical cues to MSCs residing at the vascular interface. Shear stress facilitates the opening of the stretch-activated calcium ion channels on the surface of MSCs, inducing an influx of intracellular calcium ions and activation of the focal adhesion kinase (FAK)-Akt signaling axis, including upregulation of COX-2, HO-1, and PGE_2 and downregulation of TNF-α secretion (16). This signaling ele-

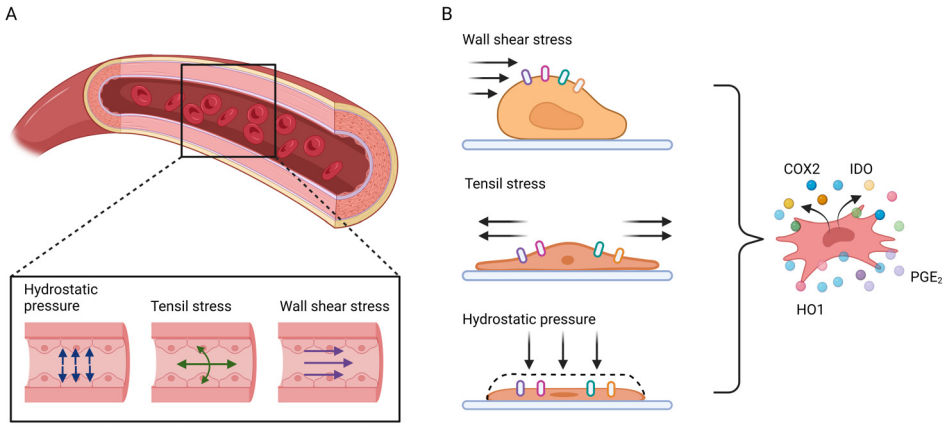

Fig. 1. Biomechanical forces in the MSC niche. Different types of biomechanical forces existing in the MSC niche are shown. (A) As blood vessels generate pulsatile flow, shear stress as a frictional force, hydrostatic pressure, and tensile force can be applied to the cells in the blood vessels or nearby. (B) Mechanosensors translate the biomechanical stimuli into biochemical signals inside cells.

vates the immunomodulatory function of MSCs to suppress an inflammatory response (16). Santos et al. (17) demonstrated the increased secretion of interleukin (IL)-6 by MSC spheroids exposed to spinner vessels equipped with ball impellers at 80 rpm, whereas a spinner flask at 40～50 rpm elevated the immunomodulatory properties of MSCs, which suppressed T cell proliferation (18). Further, artificial lymph node mimicking reactor enhanced anti-inflammatory cytokines (IL-1 and IL-12) and inhibited pro-inflammatory cytokines (TNF-α and IFN-γ) in rat MSCs (19). These findings indicate that an appropriate range of shear stress can trigger a cascade of inflammatory mediators critical in MSC immunomodulatory function.

As another biomechanical force, tensile stress is known to affect cell biology and gap junction intracellular communication (20). The blood vessel wall structure is built to withstand and propagate the forces applied by blood flow, pressure, and the surrounding tissues (Fig. 1A). Blood pressure measures the cyclic tensile forces acting radially and longitudinally on the vascular wall (21). Thus, vascular endothelial and smooth muscle cells are subject to tensile stress under pulsatile flow conditions (Fig. 1B). In addition, tensile forces are important physical cues that regulate MSCs within the stem cell niche (22). Intracellular tensile forces resulting from cytoskeletal reorganization play a critical role in regulating morphogenesis during development and enhancing the immunomodulatory properties of MSCs. When MSCs were cultivated on a custom-made polydimethylsiloxane (PDMS) membrane of 150 μm in thickness that was deflected to obtain a circumferential stretch of about 20% at a frequency of 0.2 Hz, the IL-6 and TNF-α levels were decreased, thus suggesting that the secretome from MSCs had changed (23). This secretion profile increased the anti-inflammatory and immunosuppressive outcomes of MSCs (23). By contrast, 10% or 12% uniaxial tensile strain on BM-derived MSCs increased IL-6 or IL-8 secretion as inflammatory cytokines, initiating osteogenic differentiation (24), which suggested that biomimetic forces modulate the autocrine signals of MSCs, such as IL-6 or IL-8. In summary, tensile stress differentially affects the function of MSCs dependent on the magnitude of the force or type of material to modulate MSC function.

Hydrostatic pressure is an important cellular cue (Fig. 1A) that regulates cell behaviors, such as differentiation, migration, apoptosis, and proliferation *in vivo* and *in vitro* (25, 26). The physiological hydrostatic pressure differs substantially by cell and tissue type. In some cases, changes in the hydrostatic pressure can be correlated with a pathological condition. For example, high hydrostatic pressure can induce vesicoureteral reflux and upper urinary tract deterioration (27). On the microscale, hydrostatic pressure can affect the cytoskeleton of cells. At sufficient amplitudes, hydrostatic pressure has been shown to induce the disassembly of the microtubule-based mitotic apparatus, resulting in cell-cycle arrest (28). Furthermore, hydrostatic pressure activates the noncanonical Hippo-YAP (Yes-associated protein)/TAZ (Transcriptional coactivator with PDZ-binding motif) pathway, enhancing clathrin-dependent endocytosis by regulating the cytoskeleton of cells (29). Transcriptional factors YAP and TAZ have been recognized as key mechanotransducers that sense mechanical stimuli and relay the signals to regulate the transcriptional programs for cell proliferation, differentiation, and transformation.

Sugimoto et al. (30) demonstrated that hydrostatic pressure induced Piezo-type mechanosensitive ion channel component 1 (Piezo1) channel activation as a mechanosensor, thereby promoting osteogenic differentiation through extracellular-regulated kinase (ERK) and p38 signaling in MSCs. Moreover, MSCs increased the expression of COX-2 when MSCs were cultivated in a custom pressure bioreactor at 10～300 kPa magnitude, 0.5～2 Hz, and up to 4 h of stimulation (31). Recently, bone mimicking mechanical forces drove physiological level of TNF-α secretion from mouse MSCs which was helpful to maintain stem cell homeostasis (32). Overall, these outcomes indicated that biomechanical forces regulate the differentiation and immunomodulatory properties of MSCs by stimulating mechanosensors on their membrane (Fig. 1B). A literature overview of the control of MSC immunomodulation by biomechanical forces is summarized in Table 1.

Biomimetic ECM

The MSC niche is a specialized microenvironment composed of MSCs, differentiated progenitor cells, non-stem supporting cells, and a non-cell part, the ECM (33). The ECM is a complex three-dimensional (3D) network of interlaced fibronectin, collagen, proteoglycans, multiple matrix protein macromolecules, growth factors, and bioactive factors that provide physical support for cells and modulate cell functional activity, proliferation, adhesion, and migration (34). Thus, various 3D microstructures have been designed to mimic the ECM of the native MSC niche. Multiple lines of evidence indicate that 3D aggregation of MSCs enhances their immunomodulatory properties. The formation of cell spheroids enhances cell-cell contact, and the mechanical properties of the extracellular microenvironment, such as matrix stiffness and elas-

Table 1. Literature overview of MSC immunomodulatory properties regulated by biomechanical forces

Biomechanical force	MSC origin	Bioreactor	Immunomodulation	References
Shear stress	Human	Microfluidics devices	Upregulated anti-inflammatory mediators (COX-2, HO-1, PGE_2) and downregulated TNF-α through Ca^{2+}, Akt, MAPK, FAK signaling	(16)
	Human	Spinner vessels	Upregulated IL-6	(17)
	Human	Spinner flask	Suppressed T cell proliferation	(18)
	Rat	ALN-reactor	Inhibited inflammatory markers (TNF-α, IFN-γ) and induced other cytokines (IL-1, IL-6, IL-12)	(19)
Tensile stress	Human	PDMS-based custom-made device	Upregulated inflammatory markers (IL-6, TNF-α)	(23)
	Human	Flexercell FX-4000^{TM} strain unit	Upregulated pro-inflammatory markers (IL-6, IL-8)	(24)
	Human	Custom-made pressure chamber	Upregulated Piezo1 and osteogenic maker genes expression through ERK, p38 signaling	(30)
	Mouse	Flexcell Fx-4000T tension unit	TNF-α endocytosis-derived MSC homeostasis	(32)
HP	Human	Custom pressure bioreactor	Upregulated COX-2, depending on HP magnitude and frequency	(31)

MSC: mesenchymal stromal cell, COX-2: cyclooxygenase-2, HO-1: heme oxygenase-1, PGE_2: prostaglandin E2, TNF-α: tumor necrosis factor-alpha, MAPK: mitogen-activated protein kinase, FAK: focal adhesion kinase, ALN: artificial lymph node, IFN-γ: interferon-gamma, IL-1: interleukin-1, PDMS: polydimethylsiloxane, IL-6: interleukin-6, IL-8: interleukin-8, IL-12: interleukin-12, Piezo1: Piezo-type mechanosensitive ion channel component 1, ERK: extracellular-regulated kinase, HP: hydrostatic pressure.

ticity, also affect MSC biology. The following section discusses the immunomodulatory properties of MSCs as affected by the ECM, such as stiffness and substrate surface functionalization, and various 3D culture systems using a spheroid culture or polymer scaffold (Fig. 2).

Spheroid as a 3D culture

Cell attachment to the ECM and the bidirectional signal transduction between the ECM and cells is mediated by a subset of integrins that specifically recognize the prevalently surface-displayed and evolutionary conserved arginine-glycine-aspartate (RGD) motif, which is present in fibronectin, fibrinogen, and various other proteins of the ECM. Integrin-mediated adhesion leads to the formation of loose masses composed of several cells, which increases intercellular communication and cadherin expression in these cells. Gradual accumulation on the surface of MSCs leads to increased cell density and multicellular spheroid formation (8).

Spheroid formation technologies can be divided into static models, including hanging drop, hydrogel, and concave micro-well aggregation, and dynamic models, including suspension cultures and spinner flask bioreactors (8). In several reports, the 3D spheroid culture enhanced the secretion of several immunomodulatory factors regardless of the spheroid formation technique. Bartosh et al. (35)

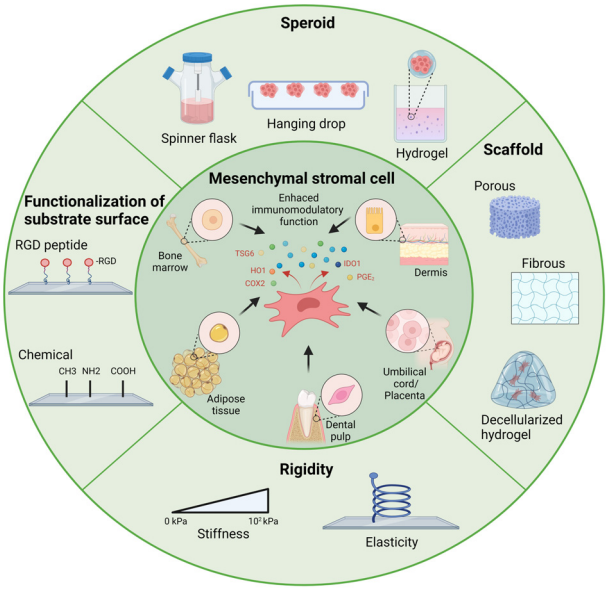

Fig. 2. Biophysical cues in the MSC microenvironment for enhancing immunomodulatory function. The application of a cell culture platform that mimics the microenvironment of the MSC niche, such as spheroid formation, scaffold, stiffness, and functionalization of the substrate surface, plays an important role in strengthening cellular immunomodulation function by secreting various immunomodulatory cytokines (IDO-1, PGE_2, HO-1, COX-2, TSG-6).

and others showed that 3D spheroids formed using the hanging drop technique enhanced the protein expression of tumor necrosis factor-(TNF)-stimulated gene-6 (TSG-6) and stanniocalcin-1 (STC-1), which have anti-inflammatory effects (35-37). Moreover, Camões et al. (38) showed that the systemic levels of the pro-inflammatory cytokines IL-6 and TNF-α in the exosomes of spheroids formed using a spinner flask culture system were reduced in the late stages of an *in vivo* wound healing model.

Scaffolds, substrate rigidity, and their functionalization as a biophysical cue

Scaffolds mimicking the properties of the MSC niches can provide a 3D environment for transplanted cells to support cell survival, adhesion, growth, and differentiation and enhance paracrine function, including immunomodulatory potential. A scaffold based on biodegradable biomaterials is preferred, except for bone and dental implants, because of the elimination of triggering a host immune response or toxic response. For this purpose, various techniques considering materials and shapes that can provide excellent biocompatibility and biomechanical properties have been considered (39). MSCs are typically delivered to a graft site using a decellularized ECM scaffold. Li et al. (40) showed that the MSCs cultured on a porcine decellularized liver scaffold system secreted more PGE_2 and less interferon-amma (IFN-γ) than traditional monolayer culture. In terms of biomaterials, highly tunable and biodegradable natural polymers, such as fibrin, collagen, and hyaluronic acid, possess biochemical cues or trophic factors that promote the recruitment of stem cells, suppress inflammation, and enhance tissue repair (41). Silk fibroin, a natural polymer derived from *Bombyx mori*, is a biocompatible material that can be processed into various forms, such as fibers, gels, films, and porous 3D microstructures. According to Kim et al. (42, 43), a meshed scaffold comprising silk fibroin nanofibers stimulated the expression of immune modulators, such as indoleamine-pyrrole 2,3-dioxygenase (IDO-1), COX-2, and PGE_2, and promoted the survival rate of the polymicrobial sepsis-induced mouse model. Synthetic polymers, such as polycaprolactone (PCL), poly (L-lactic-*co*-glycolic acid) (PLLA), and poly (lactic-co-glycolic acid) (PLGA), provide a 3D environment in the form of fibrous or porous sponges, plates, or membranes, and change the function of MSCs (44-46). Wan et al. (47) reported that human adipose-derived MSCs enhanced the expression of COX-2 and TSG-6 in aligned PLLA electrospun fibrous scaffolds compared to randomly fibrous scaffolds. In addition, Li et al. (48) demonstrated that a 3D porous scaffold using collagen, chitosan, and PLGA improved the stemness of MSCs compared to two-dimensional culture and upregulated the expression of *IL1A, IL1B,* IL-1 receptor antagonist (*IL1RN*), hepatocyte growth factor (*HGF*), and epidermal growth factor (*EGF*), which are immunomodulation-related genes.

As another physical cue, substrate stiffness affects not only the morphology, proliferation, and differentiation of MSCs but also their immunomodulatory function (48). However, there are conflicting reports associating stiffness with enhanced immunomodulatory function. Wong et al. (49) demonstrated that soft ECM (0.3～2 kPa) maximized the ability of TNF-α-primed MSCs to produce paracrine factors compared to a stiffer ECM (100 kPa). By contrast, Darnell et al. (50) reported that increasing the hydrogel stiffness from 3 to 18 kPa stimulated the expression of inflammatory modulators, such as IDO-1 and COX-2, in D1 mouse MSCs. Despite the contradictory findings, such studies indicate that the optimal range of stiffness of biomaterials can be a promising tool for enhancing the immunomodulatory properties of MSCs (51). Further studies are needed to suggest a range of strengths or structures that enhance immunomodulation.

Along with spheroids, scaffolds, differences in matrix stiffness, and different types of biomaterials, the functionalization of the substrate surface, such as surface chemistry and biomolecular properties, has been reported to provide a synergistic effect in improving the immunomodulatory function of MSCs (3). Roger et al. (52) demonstrated increased secretion of PGE_2 and interleukin-1 receptor antagonist (IL1RA, a protein encoded by the *IL1RN* gene) by MSCs cultured on grid-like thermoplastic polyurethane (TPU) plates compared to unstructured TPU surfaces. In addition, an RGD peptide, one of the key peptide sequences found in ECM proteins (as mentioned above), can enhance MSC adhesion when immobilized to a substrate, ultimately increasing osteogenic differentiation. It has been reported that RGD peptides can alter macrophage behavior and function by mediating macrophage adhesion and alleviating macrophage inflammation in response to biomaterials. Li et al. (53) applied a poly (dopamine) (DOP) coating to TiO_2 nanotubes (T/DOP) to functionalize with IL-4 (T/DOP/IL-4) and an RGD peptide and then covered T/DOP/IL-4 with a carboxymethyl chitosan hydrogel layer (T/DOP/IL-4/CG-RGD) to control IL-4 release and RGD peptide immobilization. This T/DOP/IL-4/CG-RGD surface on macrophages not only induced the conversion of macrophages to the anti-inflammatory M2 but also increased the expression of IL-10 compared to T/DOP/CG (53). The immunomodulatory functions of MSCs according to biomimetic ECM types are summarized in Table 2.

Table 2. Enhancement of MSC immunomodulation through various cell culture methods for each type of biomimetic ECM

Type of ECM	Method	MSC source	Immunomodulation	References
Spheroid	Hanging drop	Human bone marrow	Increased secretion of TSG-6, STC-1, LIF	(35)
	Hanging drop	Human bone marrow	High anti-inflammatory effect through TSG-6, TRAIL, IL-24 expression and PGE_2 secretion	(37)
	Hanging drop	Human umbilical cord	Increased expression of IL-6, MCP-1, LIF, G-CSF, SDF-1α, and decreased levels of TGF-β1, TGF-β2 in 3D culture conditions compared to 2D culture	(36)
	Hydrogel	Human bone marrow	Increased expression of *IDO1, GAL9, PTGS2, IL1RN, HGF, IL-10* in hydrogel spheroids compared to 2D	(38)
	Floating	Human bone marrow	Increased *HO-1, PTGES, COX-2* gene expression and PGE_2 secretion	(40)
	Spinner flask culture	Human umbilical cord	3D MSC-derived exosomes reduced the systemic levels of pro-inflammatory cytokines (IL-6, TNF-α) *in vivo* at late stage of wound healing; increased secretion of IL-10, TGF-β, TNF-α or upregulated IL-1α and VEGF-α *in vitro* compared to 2D MSC-derived exosome	(42, 43)
Scaffold	Decellularized liver scaffold	Human umbilical cord	Increased PGE_2 secretion by the 3D group and decreased IFN-γ	(44)
	Silk fibroin nanofiber	Human bone marrow	MSC on silk fibroin scaffolds elevated IDO1, COX-2, PGE_2 expression and reduced mortality in sepsis-induced animal models	(47)
	PCL/SF fibrous scaffold	Human bone marrow	M2 macrophage polarization and inhibited expression of cytokines (IL-1β, CXCL11, IL-10, IL1R2, TGF-β1)	(45)
	PLLA fibrous scaffold (PLLA fibrous scaffold, aligned fiber)	Human adipose tissue	Aligned fiber MSC enhanced the expression and secretion levels of TSG-6, COX-2 compared to random fiber MSCs, probably due to increased activation of YAP/TAZ signaling	(48)
	PCL fibrous scaffold (PCL fibrous random, mesh, aligned like fiber scaffold)	Rat adipose tissue	Increased gene expression and cytokine secretion of *COX-2, TSG-6, iNOS, PGE_2, TGF-β* in MSCs from random, aligned, and mesh-patterned scaffolds compared to microplates	(46)
	Porous scaffold (collagen, chitosan, PLGA substrate)	Human umbilical cord	Increased levels of *IL1A, IL1B, IL1RN, IL6ST, VEGF, HGF* genes and PTGS2, IL1RN, IL-1β proteins	(50)
	Porous scaffold (polystyrene)	Human bone marrow	MSCs in scaffold decreased IL-6, MCP-1 secretion and increased PGE_2, TSG-6 levels	(49)
Rigidity	Stiffness	Mouse MSC	Increased MSC protein production of inflammatory modulators (IDO1 and PGE_2) when hydrogel stiffness increased from 3 to 18 kPa	(51)
	Stiffness	Human bone marrow	TNF-α-primed MSCs in soft ECM (~2 kPa) mimicking bone marrow maximized the ability of MSCs to produce paracrine factors that induced chemotaxis upon inflammatory stimulation	(52)
	Elasticity	Human bone marrow	More VEGF secreted by MSC in stiffer gels, while PGE_2 secretion was highest in more compliant gels	(53)

Conclusions

In this review, various cell culture systems were proposed to enhance the immunomodulatory effects of MSCs. Enhancing the immunomodulatory capacity of MSCs may be critical in MSC-based cell therapy. However, several limitations will need to be overcome in order to realize the full clinical potential of MSCs. Despite various efforts to enhance the immunomodulatory ability of MSCs, there is no standardized cell culture system that embodies the microenvironment of MSCs. In addition, enhanced immunomodulation in the current system can be made through

Table 2. Continued

Type of ECM	Method	MSC source	Immunomodulation	References
Functionalization of substrate surface	Thermoplastic polyurethane plates with grid-like cavities or no structure	Human bone marrow	Enhanced secretion levels of immune modulators (PGE_2, IL1RA) by MSCs on grid-like structure	(44)
	RGD peptide	Human bone marrow	T/DOP-IL-4/CG-RGD surface induced the conversion of macrophages to the M2 and increased the expression of IL-10 compared to T/DOP/CG	(45)

MSC: mesenchymal stromal cell, ECM: extracellular matrix, TSG-6: tumor necrosis factor-alpha (TNF-α)-stimulated gene protein-6, STC-1: stanniocalcin-1, LIF: leukemia inhibitory factor, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand, IL-24: interleukin-24, PGE_2: prostaglandin E2, IL-6: interleukin-6, MCP-1: monocyte chemoattractant protein-1, G-CSF: granulocyte-colony stimulating factor, SDF-1α: stromal cell-derived factor 1 alpha, TGF-β1: transforming growth factor-beta 1, TGF-β2: transforming growth factor-beta 2, 3D: three-dimensional, 2D: two-dimensional, IDO1: indoleamine 2,3-dioxygenase, GAL9: galectin-9, PTGS2: prostaglandin-endoperoxide synthase 2/cyclooxygenase 2, IL1RN: interleukin-1 receptor antagonist, HGF: hepatocyte growth factor, IL-10: interleukin-10, HO-1: heme oxygenase-1, PTGES: prostaglandin E synthase, COX-2: cyclooxygenase-2, TNF-α: tumor necrosis factor-alpha, IL-10: interleukin-10, IL-1α: interleukin-1 alpha, VEGF-α: vascular endothelial growth factor-alpha, TGF-β: transforming growth factor-beta, IFN-γ: interferon-gamma, PCL: poly (ε-caprolactone), SF: silk fibroin, IL-1β: interleukin-1 beta, CXCL11: C-X-C motif chemokine ligand 11, IL1R2: interleukin-1 receptor type 2, PLLA: poly (L-lactic acid), YAP: Yes-associated protein, TAZ: transcriptional coactivator with a PDZ-binding domain, iNOS: inducible nitric oxide synthase, PLGA: poly (lactic-*co*-glycolic acid), IL6ST: interleukin-6 cytokine family signal transducer, IL1RA: interleukin-1 receptor antagonist (encoded by the *IL1RN* gene), RGD: arginine-glycine-aspartate, T: titanium, DOP: poly(dopamine), IL-4: interleukin-4, CG: carboxymethyl chitosan hydrogel layer, IL1A: interleukin 1 alpha, IL1B: interleukin 1 beta, VEGF: vascular endothelial growth factor.

epigenetic regulation, which does not last long. Thus, it could cause a need for a stylized MSC culture system for use as an international standard. In addition, the characteristics of MSCs, which require applying different culture systems for patient-specific immune therapy, MSCs will require more stabilization. Furthermore, the secretion of anti-inflammatory cytokines and other factors may paradoxically cause pathological immune responses depending on the situation involved in disease progression. For these reasons, in some cases, there is still a need for more standardized monitoring data of MSC transplantation in various diseases to relieve concerns.

Although further study is needed to tackle these hurdles, MSCs have considerable potential as alternative treatments for various diseases. Therefore, a thorough understanding of the mechanisms of MSCs in a standardized cell culture platform considering the MSC niche will improve the safety, efficacy, and results of MSC-based therapy.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF), funded by the Korean government [grant number 2020R1A2C2011617]; and by the Chung-Ang University Scholarship Grant in 2022.

Potential Conflict of Interest

The authors have no conflicting financial interest.

References

1. Reinisch A, Etchart N, Thomas D, Hofmann NA, Fruehwirth M, Sinha S, Chan CK, Senarath-Yapa K, Seo EY, Wearda T, Hartwig UF, Beham-Schmid C, Trajanoski S, Lin Q, Wagner W, Dullin C, Alves F, Andreeff M, Weissman IL, Longaker MT, Schallmoser K, Majeti R, Strunk D. Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood 2015;125:249-260
2. Lee S, Kim HS, Min BH, Kim BG, Kim SA, Nam H, Lee M, Kim M, Hwang HY, Leesong AI, Leesong MM, Kim JH, Shin JS. Enhancement of anti-inflammatory and immunomodulatory effects of adipose-derived human mesenchymal stem cells by making uniform spheroid on the new nano-patterned plates. Biochem Biophys Res Commun 2021;552:164-169
3. Chen Y, Shu Z, Qian K, Wang J, Zhu H. Harnessing the properties of biomaterial to enhance the immunomodulation of mesenchymal stem cells. Tissue Eng Part B Rev 2019; 25:492-499
4. Gan L, Liu Y, Cui D, Pan Y, Zheng L, Wan M. Dental tissue-derived human mesenchymal stem cells and their potential in therapeutic application. Stem Cells Int 2020;2020: 8864572
5. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 1978;4:7-25
6. Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of ni-

che, self-renewal and differentiation. Arthritis Res Ther 2007;9:204
7. Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regen Med 2019;4:22
8. Hazrati A, Malekpour K, Soudi S, Hashemi SM. Mesenchymal stromal/stem cells spheroid culture effect on the therapeutic efficacy of these cells and their exosomes: a new strategy to overcome cell therapy limitations. Biomed Pharmacother 2022;152:113211
9. Diaz MF, Vaidya AB, Evans SM, Lee HJ, Aertker BM, Alexander AJ, Price KM, Ozuna JA, Liao GP, Aroom KR, Xue H, Gu L, Omichi R, Bedi S, Olson SD, Cox CS Jr, Wenzel PL. Biomechanical forces promote immune regulatory function of bone marrow mesenchymal stromal cells. Stem Cells 2017;35:1259-1272
10. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol Rev 2011;91:327-387
11. Zhang Y, Yu J, Bomba HN, Zhu Y, Gu Z. Mechanical force-triggered drug delivery. Chem Rev 2016;116:12536-12563
12. Kim TJ, Joo C, Seong J, Vafabakhsh R, Botvinick EL, Berns MW, Palmer AE, Wang N, Ha T, Jakobsson E, Sun J, Wang Y. Distinct mechanisms regulating mechanical force-induced Ca^{2+} signals at the plasma membrane and the ER in human MSCs. Elife 2015;4:e04876
13. Sun Y, Chen CS, Fu J. Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment. Annu Rev Biophys 2012;41:519-542
14. Kornuta JA, Nepiyushchikh Z, Gasheva OY, Mukherjee A, Zawieja DC, Dixon JB. Effects of dynamic shear and transmural pressure on wall shear stress sensitivity in collecting lymphatic vessels. Am J Physiol Regul Integr Comp Physiol 2015;309:R1122-R1134
15. English K. Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol 2013;91:19-26
16. Lee HJ, Diaz MF, Ewere A, Olson SD, Cox CS Jr, Wenzel PL. Focal adhesion kinase signaling regulates anti-inflammatory function of bone marrow mesenchymal stromal cells induced by biomechanical force. Cell Signal 2017;38:1-9
17. Santos JM, Camões SP, Filipe E, Cipriano M, Barcia RN, Filipe M, Teixeira M, Simões S, Gaspar M, Mosqueira D, Nascimento DS, Pinto-do-Ó P, Cruz P, Cruz H, Castro M, Miranda JP. Three-dimensional spheroid cell culture of umbilical cord tissue-derived mesenchymal stromal cells leads to enhanced paracrine induction of wound healing. Stem Cell Res Ther 2015;6:90
18. Mizukami A, Fernandes-Platzgummer A, Carmelo JG, Swiech K, Covas DT, Cabral JM, da Silva CL. Stirred tank bioreactor culture combined with serum-/xenogeneic-free culture medium enables an efficient expansion of umbilical cord-derived mesenchymal stem/stromal cells. Biotechnol J 2016;11:1048-1059
19. Seifert M, Lubitz A, Trommer J, Könnig D, Korus G, Marx U, Volk HD, Duda G, Kasper G, Lehmann K, Stolk M, Giese C. Crosstalk between immune cells and mesenchymal stromal cells in a 3D bioreactor system. Int J Artif Organs 2012;35:986-995
20. Charras G, Yap AS. Tensile forces and mechanotransduction at cell-cell junctions. Curr Biol 2018;28:R445-R457
21. Camasão DB, Mantovani D. The mechanical characterization of blood vessels and their substitutes in the continuous quest for physiological-relevant performances. A critical review. Mater Today Bio 2021;10:100106
22. Castillo AB, Jacobs CR. Mesenchymal stem cell mechanobiology. Curr Osteoporos Rep 2010;8:98-104
23. Falcones B, Söderlund Z, Ibáñez-Fonseca A, Almendros I, Otero J, Farré R, Rolandsson Enes S, Elowsson Rendin L, Westergren-Thorsson G. hLMSC secretome affects macrophage activity differentially depending on lung-mimetic environments. Cells 2022;11:1866
24. Sumanasinghe RD, Pfeiler TW, Monteiro-Riviere NA, Loboa EG. Expression of proinflammatory cytokines by human mesenchymal stem cells in response to cyclic tensile strain. J Cell Physiol 2009;219:77-83
25. Yang M, Xiao LW, Liao EY, Wang QJ, Wang BB, Lei JX. The role of integrin-β/FAK in cyclic mechanical stimulation in MG-63 cells. Int J Clin Exp Pathol 2014;7:7451-7459
26. Wu Y, van der Schaft DWJ, Baaijens FP, Oomens CWJ. Cell death induced by mechanical compression on engineered muscle results from a gradual physiological mechanism. J Biomech 2016;49:1071-1077
27. Stover J, Nagatomi J. Cyclic pressure stimulates DNA synthesis through the PI3K/Akt signaling pathway in rat bladder smooth muscle cells. Ann Biomed Eng 2007;35:1585-1594
28. Zimmerman AM, Marsland D. Cell division: effects of pressure on the mitotic mechanisms of marine eggs (Arbacia punctulata). Exp Cell Res 1964;35:293-302
29. Park J, Jia S, Salter D, Bagnaninchi P, Hansen CG. The Hippo pathway drives the cellular response to hydrostatic pressure. EMBO J 2022;41:e108719
30. Sugimoto A, Miyazaki A, Kawarabayashi K, Shono M, Akazawa Y, Hasegawa T, Ueda-Yamaguchi K, Kitamura T, Yoshizaki K, Fukumoto S, Iwamoto T. Piezo type mechanosensitive ion channel component 1 functions as a regulator of the cell fate determination of mesenchymal stem cells. Sci Rep 2017;7:17696
31. Stavenschi E, Corrigan MA, Johnson GP, Riffault M, Hoey DA. Physiological cyclic hydrostatic pressure induces osteogenic lineage commitment of human bone marrow stem cells: a systematic study. Stem Cell Res Ther 2018;9:276
32. Yu W, Chen C, Kou X, Sui B, Yu T, Liu D, Wang R, Wang J, Shi S. Mechanical force-driven TNFα endocytosis governs stem cell homeostasis. Bone Res 2021;8:44
33. Ghaemi SR, Harding FJ, Delalat B, Gronthos S, Voelcker NH. Exploring the mesenchymal stem cell niche using high throughput screening. Biomaterials 2013;34:7601-7615
34. Novoseletskaya E, Grigorieva O, Nimiritsky P, Basalova N, Eremichev R, Milovskaya I, Kulebyakin K, Kulebyakina M,

Rodionov S, Omelyanenko N, Efimenko A. Mesenchymal stromal cell-produced components of extracellular matrix potentiate multipotent stem cell response to differentiation stimuli. Front Cell Dev Biol 2020;8:555378
35. Bartosh TJ, Ylöstalo JH, Mohammadipoor A, Bazhanov N, Coble K, Claypool K, Lee RH, Choi H, Prockop DJ. Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. Proc Natl Acad Sci U S A 2010;107:13724-13729
36. Xie L, Mao M, Zhou L, Zhang L, Jiang B. Signal factors secreted by 2D and spheroid mesenchymal stem cells and by cocultures of mesenchymal stem cells derived microvesicles and retinal photoreceptor neurons. Stem Cells Int 2017;2017:2730472
37. Ylostalo JH, Bazhanov N, Mohammadipoor A, Bartosh TJ. Production and administration of therapeutic mesenchymal stem/stromal cell (MSC) spheroids primed in 3-D cultures under xeno-free conditions. J Vis Exp 2017;(121):55126
38. Camões SP, Bulut O, Yazar V, Gaspar MM, Simões S, Ferreira R, Vitorino R, Santos JM, Gursel I, Miranda JP. 3D-MSCs A151 ODN-loaded exosomes are immunomodulatory and reveal a proteomic cargo that sustains wound resolution. J Adv Res 2022;41:113-128
39. von der Mark K, Park J, Bauer S, Schmuki P. Nanoscale engineering of biomimetic surfaces: cues from the extracellular matrix. Cell Tissue Res 2010;339:131-153
40. Li Y, Wu Q, Wang Y, Li L, Chen F, Shi Y, Bu H, Bao J. Immunogenicity of hepatic differentiated human umbilical cord mesenchymal stem cells promoted by porcine decellularized liver scaffolds. Xenotransplantation 2017;24: e12287
41. Mk MV, Krishna B, Sampath S. Secular trends in an Indian intensive care unit-database derived epidemiology: the stride study. Indian J Crit Care Med 2019;23:251-257
42. Kim OH, Park JH, Son JI, Yoon OJ, Lee HJ. Bone marrow mesenchymal stromal cells on silk fibroin scaffolds to attenuate polymicrobial sepsis induced by cecal ligation and puncture. Polymers (Basel) 2021;13:1433
43. Kim OH, Yoon OJ, Lee HJ. Silk fibroin scaffolds potentiate immunomodulatory function of human mesenchymal stromal cells. Biochem Biophys Res Commun 2019;519:323-329
44. Dong L, Li L, Song Y, Fang Y, Liu J, Chen P, Wang S, Wang C, Xia T, Liu W, Yang L. MSC-derived immunomodulatory extracellular matrix functionalized electrospun fibers for mitigating foreign-body reaction and tendon adhesion. Acta Biomater 2021;133:280-296
45. Su N, Gao PL, Wang K, Wang JY, Zhong Y, Luo Y. Fibrous scaffolds potentiate the paracrine function of mesenchymal stem cells: a new dimension in cell-material interaction. Biomaterials 2017;141:74-85
46. Vallés G, Bensiamar F, Crespo L, Arruebo M, Vilaboa N, Saldaña L. Topographical cues regulate the crosstalk between MSCs and macrophages. Biomaterials 2015;37:124-133
47. Wan S, Fu X, Ji Y, Li M, Shi X, Wang Y. FAK- and YAP/TAZ dependent mechanotransduction pathways are required for enhanced immunomodulatory properties of adipose-derived mesenchymal stem cells induced by aligned fibrous scaffolds. Biomaterials 2018;171:107-117
48. Li J, Chen T, Huang X, Zhao Y, Wang B, Yin Y, Cui Y, Zhao Y, Zhang R, Wang X, Wang Y, Dai J. Substrate-independent immunomodulatory characteristics of mesenchymal stem cells in three-dimensional culture. PLoS One 2018;13:e0206811
49. Wong SW, Lenzini S, Cooper MH, Mooney DJ, Shin JW. Soft extracellular matrix enhances inflammatory activation of mesenchymal stromal cells to induce monocyte production and trafficking. Sci Adv 2020;6:eaaw0158
50. Darnell M, Gu L, Mooney D. RNA-seq reveals diverse effects of substrate stiffness on mesenchymal stem cells. Biomaterials 2018;181:182-188
51. Murphy KC, Whitehead J, Zhou D, Ho SS, Leach JK. Engineering fibrin hydrogels to promote the wound healing potential of mesenchymal stem cell spheroids. Acta Biomater 2017;64:176-186
52. Roger Y, Schäck LM, Koroleva A, Noack S, Kurselis K, Krettek C, Chichkov B, Lenarz T, Warnecke A, Hoffmann A. Grid-like surface structures in thermoplastic polyurethane induce anti-inflammatory and anti-fibrotic processes in bone marrow-derived mesenchymal stem cells. Colloids Surf B Biointerfaces 2016;148:104-115
53. Li M, Wei F, Yin X, Xiao L, Yang L, Su J, Weng J, Feng B, Xiao Y, Zhou Y. Synergistic regulation of osteoimmune microenvironment by IL-4 and RGD to accelerate osteogenesis. Mater Sci Eng C Mater Biol Appl 2020;109:110508